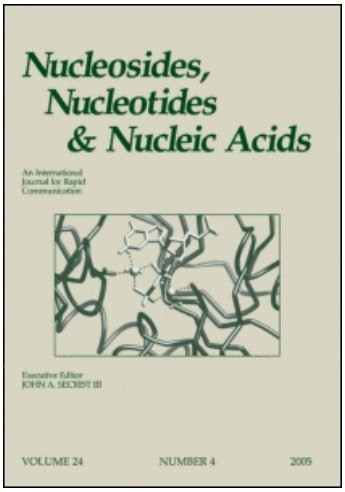
This article was downloaded by:
On: *26 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Oligonucleotides and Nucleotide-Peptides. LV. Synthesis and Some Properties of Triesters of Hydroxy Aming Acids and Thymidilyl-(3' → 5')-Thymidine[1]

B. Juodka[a]; L. Bagdoniene[a]; R. Plaipa[a]
[a] Department of Biochemistry and Biophysics, Vilnius University, Vilnius, Lithuania

To cite this Article Juodka, B. , Bagdoniene, L. and Plaipa, R.(1995) 'Oligonucleotides and Nucleotide-Peptides. LV. Synthesis and Some Properties of Triesters of Hydroxy Aming Acids and Thymidilyl-(3' → 5')-Thymidine[1]', Nucleosides, Nucleotides and Nucleic Acids, 14: 1, 229 — 241
To link to this Article: DOI: 10.1080/15257779508014666
URL: http://dx.doi.org/10.1080/15257779508014666

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

NUCLEOSIDES & NUCLEOTIDES, 14(1&2), 229-241 (1995)

OLIGONUCLEOTIDES AND NUCLEOTIDE-PEPTIDES. LV. SYNTHESIS AND SOME PROPERTIES OF TRIESTERS OF HYDROXY AMINO ACIDS AND THYMIDILYL-(3'→5')-THYMIDINE[1]

B.Juodka*, L.Bagdonienė, R.Plaipa
Department of Biochemistry and Biophysics, Vilnius University,
Vilnius 2009, Čiurlionio 21, Lithuania

Downloaded At: 17:37 26 January 2011

Abstract: N-Cbz-Ser(OMe)-(P_i→O)-(TpT) and N-Bz-Tyr(OEt)-(P_i→O)-(TpT) were synthesized as diastereomeric mixtures of approximately (1 : 1) by reacting (TpT) with 2,4,6-triisopropylbenzenesulphonyl chloride and hydroxy amino acids. Their stability to the action of chemical agents and phosphodiesterases was investigated.

Nucleic acid-protein complexes with a covalent bond between the components have recently been isolated from various sources[2-6] The functions of these complexes are of great importance. It has been shown, that in most cases protein and nucleic acid components are covalently linked via a phosphodiesteric bond between the terminal phosphate of nucleic acids and the hydroxy group of amino acid residues of proteins [2,7,8]. However, the lysis of cells and isolation of DNA in alkaline conditions yields decomposition of internucleotidic bonds in covalent DNA-protein complexes [4,9,10].

Prefix d(deoxy) is omited

Copyright © 1995 by Marcel Dekker, Inc.

Lability of these complexes in alkaline medium allows one to think about the phosphotriesteric bond between DNA and hydroxy amino acids[9]. Recently this type of DNA-protein complex was found in human placenta and Ehrlich ascites tumor cells[11].

It is difficult to determine the type of covalent bond between DNA and proteins because the molecules themselves are complex. Functional groups of proteins can influence NA-protein bond properties under various conditions[2].

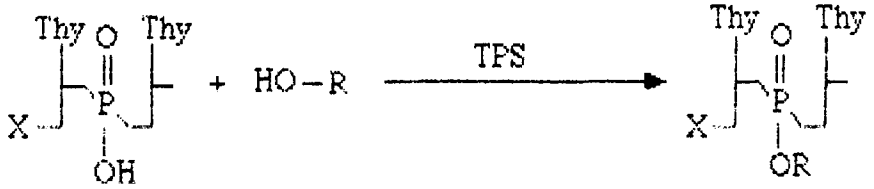
The investigation of the properties of synthetic model compounds in which nucleotide or oligonucleotide is linked to the amino acid/peptide by a covalent bond is of great importance in solving these problems. Nucleotide-peptides with a phosphoamide or phosphoester bond between the amino acid and terminal phosphate of nucleotide(oligonucleotide) have been studied in detail [reviews 2,12]. However, there is no data about the phosphotriesteric nucleotide-peptide type,in which hydroxy amino acids are linked to an internucleotide phosphate of oligonucleotides by a phosphoester bond.

The object of this research was to synthesize the analogues of serine and tyrosine of dinucleoside monophosphate and investigate the hydrolysis of phosphotriester bonds by chemical agents and phosphodiesterases.

Downloaded At: 17:37 26 January 2011

Results and discussions

For the synthesis of 5'-O-tritylthymidilyl-(3'→5')-thymidine-(P_1→O)-N-Cbz-L-serine methyl ester (I) and 5'-O-tritylthymidilyl-(3'→5')-thymidine-(P_1→O)-N-Bz-L-tyrosine ethyl ester (II) we have used triisopropylbenzenesulphonylchloride for the activation of the internucleotide phosphate as previously described[13].



I. R = $-CH_2-\underset{\displaystyle NH-Cbz}{\underset{|}{CH}}-COOMe$;
X = $-C(C_6H_5)_3$ N - Cbz - Ser(OMe) - ($P_i \rightarrow O$) - (TrTpT)

II. R = $-C_6H_4-CH_2-\underset{\displaystyle NH-Bz}{\underset{|}{CH}}-COOEt$;
X = $-C(C_6H_5)_3$ N-Bz-Tyr(OEt)-($P_i \rightarrow O$) - (TrTpT)

III. R = $-CH_2-\underset{\displaystyle NH-Cbz}{\underset{|}{CH}}-COOMe$;
X = H N - Cbz - Ser(OMe) - ($P_i \rightarrow O$) - (TpT)

IV. R = $-C_6H_4-CH_2-\underset{\displaystyle NH-Bz}{\underset{|}{CH}}-COOEt$;
X = H N-Bz-Tyr(OEt)-($P_i \rightarrow O$) - (TpT)

Downloaded At: 17:37 26 January 2011

Tritylated oligonucleotide-hydroxy amino acid derivatives (I, II) were isolated by chromatography on a silica gel column. The gradient elution by ethanol in chloroform (0-20%) was used. Reaction products were obtained as a diastereomeric mixture. 4-5 times chromatography on the silica gel column enriched separate fractions of one of the diastereomers only in part, although we could not separate them one from another completely.

After detritylation and rechromatography on the silica gel column, N-Cbz-Ser(OMe)-($P_i \rightarrow O$)-(TpT) (III) and N-Bz-Tyr(OEt)-($P_i \rightarrow O$)-(TpT) (IV) as a mixture of diastereomers of approximately 1:1 were obtained. HPLC chromatography on an Ultrasphere ODS column was most effective for the separation of the diastereomers of compounds (III,IV). Analytical separation of the diastereomers of compounds (III,IV) is shown in FIGURE 1.

The structure of compounds (III,IV) was proved by determination of ratio of amino acid: phosphorus : thymine after complete acidic hydrolysis[14] and by H^1NMR.

Some characteristics of obtained diastereomers of phosphotriesters (III,IV) are shown in TABLE 1.

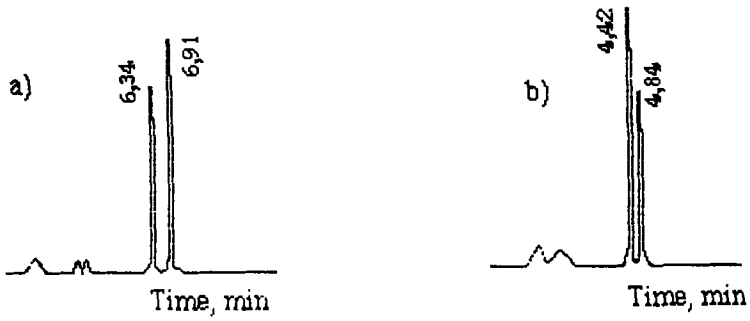


FIGURE 1: The separation of the diastereomers of N-Cbz-Ser(OMe)-(P_i→O)-(TpT) (a) and N-Bz-Tyr(OEt)-(P_i→O)-(TpT) (b). Elution with 30% (a) and 20% (b) of acetonitrile in water was used.

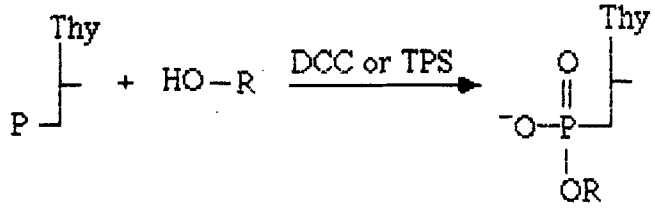
Downloaded At: 17:37 26 January 2011

TABLE 1: HPLC analysis and TLC characteristics of the diastereomers of serine and tyrosine dinucleoside monophosphates.

Compound	HPLC conditions		R_f	
	retention time, min	concentration of acetonitrile in water, %	A	B
N-Cbz-Ser(OMe)-(P_i → O)-(TpT) (IIIa)	6.34	30	0.34	0.42
(IIIb)	6.91	30	0.38	0.45
N-Bz-Tyr(OEt)-(P_i → O)-(TpT) (IVa)	4.57	20	0.4	0.48
(IVb)	4.98	20	0.43	0.51

R_f in solvent systems: A - chloroform . ethanol (9:1), B - chloroform: ethyl acetate : ethanol (75 : 25 : 2).

For comparison of the hydrolytic stability of serine and tyrosine triesters of TpT and diesters of TMP, we synthesized thymidilyl-(5'→0)-N-Cbz-L-serine methyl ester(V) and thymidilyl-(5'→0)-N-Bz-L-tyrosine ethyl ester (VI). Synthesis was carried out using carbodiimide[14] and triisopropylbenzenesulphonylchloride[13] methods.



V. R = $-CH_2-CH(NH-Cbz)-COOMe$ N - Cbz - Ser(OMe) - pT

VI. R = $-C_6H_4-CH_2-CH(NH-Bz)-COOEt$ N - Bz - Tyr (OEt) - pT

Thymidilyl-(5'→O)-hydroxy amino acids (V, VI) were isolated by chromatography on a DEAE-cellulose column and by rechromatography on paper in solvent system D. Some characteristics and proof of the structure of the compounds (III-VI) are given in TABLE 2.

From the molecular biological point of view it is important to know the hydrolytic stability of model nucleotide-peptides and their resistance to different phosphodiesterases. These approaches are often used in determining the structure of covalent nucleic acid-protein complexes.

Serine and tyrosine dinucleoside monophosphate analogues were investigated (III, IV), and their properties were compared with the properties of corresponding phosphodiesters N-Cbz-Ser(OMe)-pT (V) and N-Bz-Tyr(OEt)-pT (VI).

It is known, that diesteric nucleotide-peptide derivatives are stable in acidic and weak alkaline medium (pH 0-11, 37°C, 1h) and decompose only in strong alkali[14]. Our investigation showed that N-Cbz-Ser(OMe)-pT and N-Bz-Tyr(OEt)-pT are stable in acidic medium as well (1N HCl, 37°C,

Downloaded At: 17:37 26 January 2011

TABLE 2: Yields and some characteristics of hydroxy amino acid compounds of TMP and TpT

Compound	Yield, %	R_f a)		U_{pT} b)	Ratio thymine: phosphorus : amino acid
		C	D		
N-Cbz-Ser(OMe)- -($P_i \rightarrow O$)-(TpT) (III)	48	0.92	0.9	0	2 : 1 : 0.9
N-Bz-Tyr(OEt)- -($P_i \rightarrow O$)-(TpT) (IV)	32	0.93	0.92	0	1.67 : 0.9 : 1
N-Cbz-Ser(OMe)-pT (V)	22	0.72	0.68	0.46	0.9 : 1 : 1
N-Bz-Tyr(OEt)-pT (VI)	11	-	0.88	0.45	1.1 : 1 : 1

a) R_f in solvent systems: C - 2 - propanol : conc. NH_4OH : water (7 : 1 : 2); D - tert.butyl alcohol : water (7 : 3); b) electrophoretic mobility relative to thymidine - 5'-phosphate (0.05M triethylammonium bicarbonate buffer, pH 7.5, 0.5h, 1200 V).

Downloaded At: 17:37 26 January 2011

1h). There is a great difference of stability in alkaline medium between serine and tyrosine phosphodiesters. It may be inferred from the experimental data (TABLE 3) that the rate of the hydrolysis of phosphodiesteric bond of nucleotidyl-(P→O)-amino acids depends on the structure of the amino acid component. 30% of the diester of serine and thymidylic acid in the alkaline medium (0.5 N NaOH, 37°C, 1h) decomposes to a nucleotide and amino acid derivative. The tyrosine analogue in these conditions is stable.

The different behaviour of these compounds in the alkaline medium is consistent with the different mechanism of their decomposition. It was shown earlier[14], that nucleotidyl-(5'→0)-N-Cbz-amino-β-hydroxy acid esters decompose in alkali by the β-elimination mechanism which is impossible in the case of tyrosine analogue. Hydrolysis of N-Bz-Tyr(OEt)-pT may proceed analogously as in the case of aryl diesters of phosphoric acid[15].

TABLE 3: Hydrolytic stability of nucleotidyl-(5'→O)- and oligonucleotidyl-(P_i→O)-serine and tyrosine derivatives (37°C, 1h)

Compound	Products of hydrolysis (pT or TpT) %			
	0.1N NaOH	0.5N NaOH	1N NaOH	1N HCl
N-Cbz-Ser(OMe)-pT	28	28	32	0
N-Bz-Tyr(OEt)-pT	0	0	0	0
N-Cbz-Ser(OMe)-(-(P_i→ O)-(TpT)	68	75	93	0
N-Bz-Tyr(OEt)-(-(P_i→ O)-(TpT)	85	87	90	0

Downloaded At: 17:37 26 January 2011

It may be concluded that the tyrosine derivative of thymidylic acid is stable under strong acidic and alkaline conditions (1N HCl, 1N NaOH, 37°C, 1h) (TABLE 3). Under very strong alkaline conditions (1N NaOH, 2h, 75°C) 53% of N-Bz-Tyr(OEt)-pT decomposes to thymidylic acid. The phosphotriester derivatives N-Cbz-Ser(OMe)-(P_i→O)-(TpT) and N-Bz-Tyr(OEt)-(P_i→O)-(TpT) are also stable in acidic medium but in alkali they are much more unstable than phosphodiester analogues (TABLE 3). While N-Bz-Tyr(OEt)-pT in 1N NaOH (37°C, 1h) is stable, 90% of N-Bz-Tyr(OEt)-(P_i→O)-(TpT) decomposes under these conditions. In a strong alkaline medium there is no difference between the hydrolytic stability of serine (III) and tyrosine (IV) analogues. It should be mentioned that in the case of the serine derivative (III) only the bond between serine and TpT in alkaline medium (0.5N NaOH, 37°C, 1h) is splitted of. In the case of N-Bz-Tyr(OEt)-(P_i→O)-(TpT) the linkage between the hydroxy group of tyrosine and dinucleoside phosphate decomposes mainly too. Only a little cleavage (3-5%) of internucleotide bonds was obtained (0.5N, 1N NaOH, 37°C, 1h). The triesteric analogue of serine N-Cbz-Ser(OMe)-(P_i→O)-(TpT) hydrolyzes to TpT (20%) even in mild neutral conditions (pH 7, 3h, 37°C). N-Bz-

Tyr(OEt)-(P_i→O)-(TpT) is stable in these conditions. One of the explanations concerning the lability of the serine triesteric derivative N-Cbz-Ser(OMe)-(P_i→O)-(TpT) is the ability of this compound to undergo β- elimination easily, as in[14].

In some investigations 10% piperidine in water was used as a reagent for determining the phosphoester linkage between nucleic acid and protein[4,16]. We have found that the 50% piperidine in water (75°C, 2h) does not cleave the phosphoester bond in N-Bz-Tyr(OEt)-pT and N-Bz-Tyr(OEt)-(P_i→O)-(TpT)

It may be concluded that tyrosine analogues of TpT are stable in acidic medium, piperidine,and they decompose in alkaline medium more easily than their diester analogues.

Phosphodiesterases are often used for the determination of the presence of phosphodiester linkages in naturally occuring nucleotide-peptides[2,3]. It has previously[14] been found, that nucleotidyl-(5'→O)-amino acids were degradated by snake venom phosphodiesterase. In our experiments N-Cbz-Ser(OMe)-pT and N-Bz-Tyr(OEt)-pT were hydrolyzed by snake venom phosphodiesterase (65% and 50% of nucleotide was obtained respectively in conditions where TpT was hydrolyzed completely, 37°C, 3h). Having in mind that snake venom and spleen phosphodiesterases are often used to determine the type of bond between nucleic acid and protein in nucleotide-peptides, it was important to find out whether commercial preparations of these enzymes had impurities of phosphotriesterases.

The problem is that synthetic oligonucleotide-peptides (III, IV) are insoluble in water. Before experimenting with phosphodiesterases we performed a number of testing experiments and found conditions in which enzymes fully decompose their natural substrate TpT with organic solvents in the reaction medium. It was detected, that 85%,15% and 100% of TpT was decomposed with snake venom phosphodiesterase (3h, 37°C) in ethanol-water (1:1), dioxane-water (1:1) and in dimethylsulphoxide-water (1:10) respectively. Spleen phosphodiesterase is inactive in ethanol and dioxane, and splits TpT completely only in dimethylsulphoxide-water (1 : 10) (3h, 37°C).

Downloaded At: 17:37 26 January 2011

So, the hydrolysis of oligonucleotide-peptides was carried out in the mixture of dimethylsulphoxide-water (1:10). It was determined, that N-Bz-Tyr(OEt)-(P_i→O)-(TpT) is stable to the action of snake venom and spleen phosphodiesterases. During the reaction of N-Cbz-Ser(OMe)-(P_i→O)-(TpT) with snake venom and spleen phosphodiesterases, small quantities (~20%) of nucleotide and nucleoside were found in the reaction mixture. But it has been shown that N-Cbz-Ser(OMe)-(P_i→O)-(TpT) decomposes a little in such conditions without any enzymes to TpT, which is cleaved by phosphodiesterases to nucleotide and nucleoside.

It may be concluded that neither snake venom phosphodiesterase nor spleen phosphodiesterase split phosphotriester type oligonucleotide-peptides.

Downloaded At: 17:37 26 January 2011

Experimental

The following reagents were used: thymidine, TPS, triethylamine (Merck, Germany), snake venom and spleen phosphodiesterases (Worthington,USA), 1-N-methylimidazole, Cbz-Ser-OMe, N-Bz-Tyr-OEt (Fluka,Switzerland), silica gel (Pharmacia, Sweden). ^{1}H NMR spectra were recorded on a spectrometer WM-360 (Brucker). Tetramethylsilane was used as an internal standart and cited chemical shifts (δ) are given in ppm downfield to this standart. UV - VIS spectra were obtained on DU-50 (Beckman, USA) spectrophotometer. For flash column chromatography Kieselgel 60 (Merck, Germany) was used. Silica gel TLC was carried out on Kieselgel 60 254 (Merck, Germany) plates, using solvent systems: A - chloroform - ethanol (1 : 1, V/V), B - chloroform - ethyl acetate - ethanol (75: 25 : 2, V/V). Paper chromatography was performed on FN- 1 and FN-14 papers (Filtrak, Germany) (the paper was washed with 2N HCl and water before use, until neutral pH was obtained). The following solvent systems were used: C - 2-propyl alcohol - conc. NH_4OH - water (7 : 1 : 2 , V/V); D - tert.butyl alcohol - water (7 : 3, V/V). HPLC analysis was carried out on an Ultrasphere ODS column (Beckman,USA) (4.6 × 250 mm), rate of elution 1 ml/min. Pyridine, acetonitrile were dried by refluxing over CaH_2, distilled and stored over CaH_2. Chloroform, ethyl acetate, ethanol were dried with $MgSO_4$ and

redistilled. TpT was synthesized according to[17], N-Cbz-Ser(OMe)-pT - according to[14].

The structure of compounds (III-VI) was partly proved by determining the ratio of base to phosphorus and to amino acid after complete acid hydrolysis[14].

N-Cbz-Ser(OMe)-(P_i→O)-(TrTpT): TrTpT (0.1 mmol, 100 mg) and N-Cbz-Ser-OMe (0.5 mmol) was dissolved in 5 ml of anhydrous pyridine and dried by evaporation with anhydrous pyridine (5 × 5 ml). The residue was dissolved in 0.5 ml of acetonitrile, TPS (0.4 mmol, 130 mg) and immediately 1-N-methylimidazole (0.4 mmol, 32 µl) and triethylamine (0.4 mmol, 28 µl) were added. The reaction mixture was allowed to stand for 0.5h at room temperature, the reaction was quenched with a mixture of pyridine - water (1 : 1) (2 ml) and the solvent was removed under reduced pressure; chloroform was added and it was dried by evaporation (3 × 10 ml). The resulting oil was dissolved in 0.5 ml of chloroform and purified by flash column chromatography on silica gel (2.5 × 7 cm). Elution with the gradient of ethanol in chloroform (0- 20%) (200 ml) gave an impure product N-Cbz-Ser(OMe)-(P_i→O)-(TrTpT) which was rechromatographed, using first chloroform (100 ml) and then the gradient of ethanol in chloroform (0-20%) (200 ml). Evaporation of appropriate fractions produced a yellow oil. It was dissolved in 0.3 ml of chloroform, and 0.5 ml of 10% trichloroacetic acid was added. After 10 min, the reaction mixture was transferred to an analogous silica gel column and chromatography was repeated under the same conditions. 0.048 mmol of N-Cbz-Ser(OMe)-(P_i→O)-(TpT) as a diastereomeric mixture was obtained in yield 48%. Some characteristics and proof of the structure of compound (III) are given in TABLE 1 and TABLE 2.

^{1}H NMR (DMSO) δ ppm: 8.6 (d 1H, NH_α); 7.82 (S 1H, H-6); 7.62 (S 1H, H-6); 7.31-7.4 (m 5H, C_6H_5); 6.13 (t 1H, H-1'); 6.2 (t 1H, H-1'); 4.1-4.6 (m 8H, H-3', H-4', H-5'); 3.6 (t 3H, CH_3); 2.08 (S 3H, H-CH_3, Thy); 2.1 (S 3H, H-CH_3, Thy); 2.35-2.45 (m 2H, H-2'); 5 (S 1H, $C_\alpha H$); 5.1 (S 2H, CH_2 β).

N-Bz-Tyr(OEt)-(P_i→O)-(TrTpT): This nucleotide-peptide was obtained analogously as N-Cbz--Ser-(OMe)-(P_i→O)-(TrTpT). After chromatography

Downloaded At: 17:37 26 January 2011

on the silica gel column two fractions were obtained with similar characteristics (R_f in solvent systems C and D and U_{pN} were identical). Complete acidic hydrolysis (6N HCl, 105°C, 24h) indicated that the product of II fraction contains no tyrosine and obviously may be a symmetric pyrophosphate of TrTpT. N-Bz-Tyr(OEt)-(P_i→O)-(TpT) (IV) was obtained in 32% yield after detritylation and rechromatography of compound (II) first fraction.

Some characteristics and proof of the structure of compound (IV) are given in TABLE 1 and TABLE 2.

^{1}H NMR (DMSO) δ ppm 8.78 (d 1H, NH_α); 7.82 (S 1H, H-6); 7.62 (S 1H, H-6), 7.15 (d 2H, H-3, H-5, C_6H_4); 7.05 (d 2H, H-2, H-6, C_6H_4); 7.3-7.4 (m 5H C_6H_5); 3.82 (t, 3H, CH_3); 4.1-4.6 (m 8H, H-3', H-4', H-5'); 2.08 (S 3H, H-CH_3, Thy); 2.1 (S 3H, H-CH_3, Thy); 2.35-2.45 (m 2H, H-2').

N-Bz-Tyr(OEt)-pT: Tri-n-octylammonium salt of thymidine-5'-phosphate (0.1 mmol, 32 mg) and N-Bz-Tyr-OEt (0.5 mmol, 160 mg) were dissolved in 2 ml of anhydrous pyridine and dried by evaporation with anhydrous pyridine (5 × 5 ml). The residue was dissolved in 1 ml of pyridine and TPS (0.3 mmol, 7 mg) was added. The reaction mixture was allowed to stand at room temperature for 3h, quenched with mixture of pyridine - water (1 : 3). This solution was placed on the column (1.5 × 16 cm) with DEAE - cellulose, 200 ml of 0.02 M triethylammonium bicarbonate buffer (TEAB), pH 7.5 and a gradient elution with 400 ml of 0.02 M - 0.2 M TEAB buffer was used for chromatography. The rate of elution was 0.5 ml/min. The impure fraction of N-Bz-Tyr(OEt)-pT was concentrated and subjected to paper chromatography in solvent system D. The band with Rf 0.88 was eluted with water. N-Bz-Tyr(OEt)-pT (0.011 mmol) was obtained in 11% yield.

Some characteristics of N-Bz-Tyr(OEt)-pT are given in TABLE 2.

^{1}H NMR (DMSO) δ ppm 8.78 (d 1H, NH_α); 7.7 (s 1H, H-6), 7.15 (d 2H, H-3, H-5, C_6H_4); 7.05 (d 2H, H-2, H-6, C_6H_4); 7.3-7.4 (m 5H C_6H_5); 4.1-4.6 (m 4H, H-3', H-4', H-5'); 3.82 (t, 3H, H-CH_3); 4.35 (SH, P-OH); 2.05 (s 3H, H-CH_3, Thy); 2.45 (mH, H-2').

Investigation of the stability of synthesized compounds : 20 µl acidic or alkaline solution were added to 20 µl (0.2 µmol) of investigated compounds

Downloaded At: 17:37 26 January 2011

in dioxane, and the reaction mixture was incubated at an appropriate temperature. It was then subjected to paper chromatography in solvent system D, and aliquots (2 µl) were taken for analysis on HPLC (Ultrasphere ODS column).

The hydrolysis of oligonucleotide-peptides with snake venom and spleen phosphodiesterases: 0.2 µmol of investigated compounds was dissolved in 10 µl of an appropriate mixture of solvents (water, water - dioxane (1 : 1, V/V), water - ethanol (1 : 1, V/V), water - dimethylsulphoxide (10 : 1, V/V)), and 20 µl of solution of snake venom phosphodiesterase (0.2 mg of enzyme dissolved in 0.5 ml of 0.03 M Tris-HCl, pH 8.1 with 0.06 M $MgCl_2$) or 20 µl solution of spleen phosphodiesterase (0.4 mg of enzyme was dissolved in 0.2 ml of 0.4 M Tris-HCl buffer, pH 7) were added. The reaction mixture was incubated at 37°C for 3h, and the products were identified by paper chromatography and HPLC analysis.

Downloaded At: 17:37 26 January 2011

Acknowledgement

The authors are grateful to Dr J.A.Maurins for ^{1}H NMR spectra data (Institute of Organic Synthesis, Latvian Academy of Sciences).

REFERENCES

1. Part LIV : Juodka B., Sasnauskienė S., Petniūnaitė R., Nucleosides & Nucleotides, 11(8), 1445-1453 (1992).
2. Juodka B.A., Nucleosides & Nucleotides, 3(8), 445-483 (1984).
3. Juodka B.A., Bioorgan. Khim., 6(10), 1445-1465 (1980).
4. Neuer B., Plagens U., Werner D., J.Mol.Biol., 164, 213-235 (1983).
5. Drigin J.F., Vartapetian A.B., Chumakov K.M., Mol.Biol.(Russia), 16(13), 777-788 (1979).
6. Wang J.S., Liu L.F., In Molecular Genetics, Part III, New York, Academic Press, Inc., 65-88 (1979).
7. Zenk D.W., Ginder G.D., Brotherton T.W., Biochemistry, 29(22), 5221-5226 (1990).

8 Worland C.T , Wang J.C., J.Biol. Chem., 264, 4412-4416 (1989).
9. Tsanev R., Avramova Z., Tasheva B., In: Progress in Non Histone Protein Research,I. Bekhor (ed.), CRC Press, Inc ,Boca Raton 3, 39-58 (1989).
10 Werner D., Neuer-Nitche B., In: Progressin Non Histone Protein Research, I.Bekhor (ed.).,CRC Press, Inc.,Boca Raton 3, 99-118 (1989).
11. Juodka B., Pfütz M., Werner D., Nucl. Acids Res., 19 (23), 6391-6398(1991).
12 Shabarova Z.A, In: Progr. Nucl. Acids Res. and Mol. Biol., J.N.Davidson, W.E. Cohn(ed). Acad. Press, 10, 145-182 (1970).
13 Efimov V.A.,Reverdatto S.V.,Chakhmakhcheva O.G., Bioorgan. Khim., 8 (2), 231-239 (1982).
14 Juodka B., Kirveliene V., Povilionis P., Nucleosides & Nucleotides, 1(2), 111-125 (1982).
15. Cox J.R.,Ramsay O.B., Chem. Rev., 64, 317-352 (1964).
16. Stillman B.W., Lewis J.B., Chow L.T., Mathews M.B., Smart J.E., Cell, 23, 497-508 (1981).
17. Khorana H.G.,Moffat J.G., J.Amer. Chem. Soc., 83 (3), 649-655 (1981).

Received July 10, 1992
Accepted October 31, 1994

Downloaded At: 17:37 26 January 2011