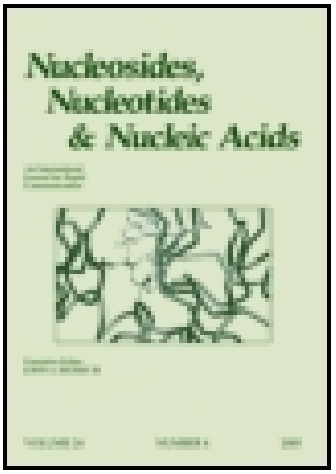

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New Photoreactive mRNA Analogues for the Affinity Labeling of Ribosomes

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BIOLOGICAL ACTIVITY

NEW PHOTOREACTIVE mRNA ANALOGUES FOR THE AFFINITY LABELING OF RIBOSOMES

A.G.Venyaminova, M.N.Repkova, T.M.Ivanova, M.I.Dobrikov, K.N.Bulygin, D.M.Graifer, G.G.Karpova, V.F.Zarytova*.
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Abstract. Chemical synthesis of the model mRNA analogues ($AUGU_3$, $(pU)_n$) bearing p-azidotetrafluorobenzamido, p-azidobenzamido or 2-nitro-5-azidobenzamido groups coupled to the 5'-terminal phosphate or to the C-8-position of adenosine is described. The first results of the photoaffinity labeling study of human placenta ribosomes are presented.

Photochemical crosslinking is a powerful tool for the analysis of protein-nucleic acid or nucleic acid-nucleic acid interactions[1,2]. Arylazide-tagged oligonucleotides are now under scrutiny for the ability of the intermediates generated under UV-light irradiation to insert into adjacent molecules very rapidly and relatively nonspecifically to result in covalent attachment of the azide-tagged molecule to other molecules in the vicinity. This makes these reagents exellent probes for the environment of a molecule.

It was shown earlier that introduction of the nitro group into the aromatic ring in *para*-position to the azido group or the use of polyfluorinated aryl azides allow photoreactive reagents with superior spectral and photochemical properties [3,4].

The present work is devoted to the chemical synthesis of an artificial short mRNA analogues ($AUGU_3$ and $(pU)_n$) bearing p-azidotetrafluorobenzoic (R_1), p-azidobenzoic (R_2) or 2-nitro-5- azidobenzoic (R_3) acid residues coupled either to the 5'-terminal phosphate group of the oligoribonucleotide or to the C-8 position of adenosine.

The most suitable for the synthesis of such types of conjugates is the general strategy based upon the introduction of an aliphatic amino spacer into an oligonucleotide for the following attachment of the photoreagent by some mild procedure in an aqueous media. We have applied recently this approach for the synthesis of oligodeoxyribonucleotide derivatives carrying arylazido groups at the 5'- end or C5 of deoxyuridine[5,6].

Illustrated in FIG.1 is a synthetic route to the 5'-azidobenzamido derivatives of oligouridylates (**4a-c**). Oligouridylates and their 5'-phosphates were synthesized by the solid phase H-phosphonate method[7]. The attachment of short aliphatic diamines to the deprotected oligoribonucleotides was performed with triphenylphosphine (Ph_3P) and 2,2'-dipyridyldisulfide $(PyS)_2$

mixture in the presence of 4-N,N-dimethylaminopyridine (DMAP) as nucleophilic catalyst[8]. The active zwitterionic derivatives **2** formed are readily converted to the 5'-amino containing oligouridylates **3** with high yield. Selective acylation of the amino groups was achieved using N-hydroxysuccinimide esters of azidobenzoic acids (the degree of conversion was about 80%).

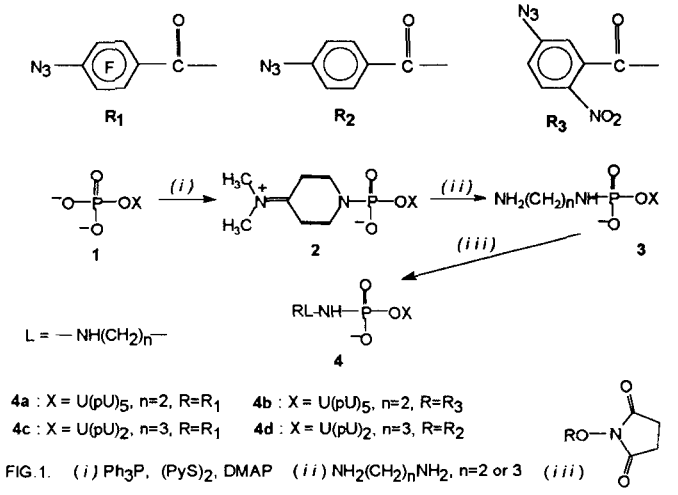


FIG.1. (*i*) Ph_3P, $(PyS)_2$, DMAP (*ii*) $NH_2(CH_2)_nNH_2$, n=2 or 3 (*iii*)

Another route based on the use of premodified monomeric synthon was chosen for the synthesis of C-8-aminoadenosine containing oligoribonucleotide **10**. The synthesis of 5'-O-dimethoxytrityl-2'-O-tetrahydropyran-2-yl-N^6-benzoyl-8-[N-(3-trifluoroacetamidopropyl)]amino-adenosine-3'-H-phosphonate (**9**) is shown (FIG.2).

We have employed the transient protecting 3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)group for the selective introduction of 2'-O-tetrahydropyran-2-yl group in **6**.

The synthesis of 3'-H-phosphonates of C-8-Br-modified adenosine **8** as well as standard protected uridine and guanosine was carried out using the monofunctional phosphitylating reagent salicylchlorohosphine[9]. The replacement of 8-Br-substi-tute in **8** was achiev-ed by interaction with 1,3-diaminopropane at elevated temperature, followed by the protection of the outer amino group. The structure of the intermediate products

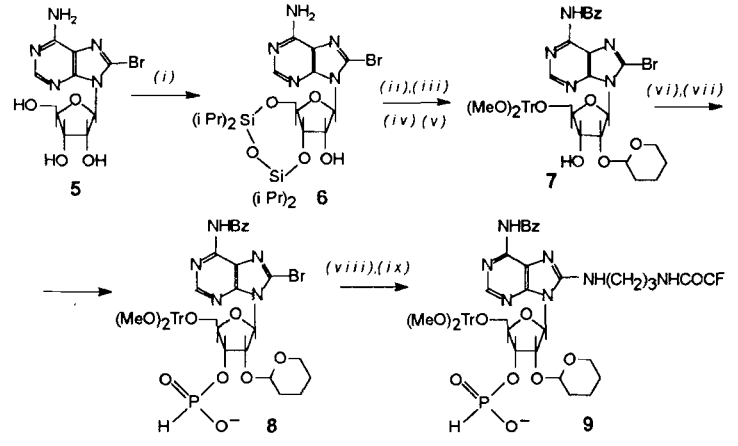
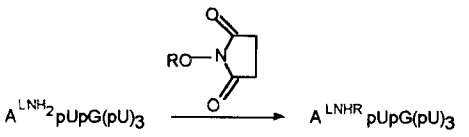


FIG.2 (i): $(iPr)_2(Cl)Si\text{-}O\text{-}Si(Cl)(iPr)_2$; (ii): 2,3-dihydropyran, H^+; (iii): BzCl; (iv): $(C_2H_5)_4NF$; (v): dimethoxytrityl chloride; (vi): salicylchlorophosphine; (vii): H_2O; (viii): $NH_2(CH_2)_3NH_2$; (ix): $CF_3CO_2C_2H_5$.

and final one in the synthesis of **9** was confirmed by means of elemental analysis, UV-, ^{1}H- and ^{31}P-NMR-spectra.



10 **11**

FIG.3. L = -NH(CH$_2$)$_3$-

Using 3'-H-phosphonate of adenosine **9** with masked C-8-aliphatic amino group at the last step of the manual solid phase H-phosphonate synthesis[7] and opening this group simultaneously with the standard deprotection of oligoribonucleotides allowed us to synthesize C-8-aminoadenosine containing A*pUpG(pU)$_3$ (**10**). FIG.3 depicts the transformation of this modified oligoribonucleotide into oligomers **11a,b** with arylazide groups (R_1 and R_2) introduced *via* an amino spacer in C-8-adenosine.

All modified oligoribonucleotides (Table 1) obtained were purified by RP HPLC and isolated as lithium salts.

The retention times for these compounds on the reverse phase C-18-column showed that aryl azide moiety notably enhances the hydrophobic character of the initial aminocontaining oligomers.

TABLE 1. Characteristics of synthesized analogues of mRNA*

Compounds	Retention	Spectral ratio			
	time, min	250/260	270/260	280/260	290/260
ApUpG(pU)$_3$	8.4	0.839	0.799	0.403	0.108
A^{LNH2}pUpG(pU)$_3$ (**10**)	9.8	0.792	0.932	0.651	0.303
A^{LNHR1}pUpG(pU)$_3$ (**11a**)	12.6	0.807	0.904	0.615	0.300
A^{LNHR2}pUpG(pU)$_3$ (**11b**)	11.2	0.742	0.998	0.742	0.407
(pU)$_6$	7.0	0.774	0.836	0.395	0.060
R$_1$LNHpU(pU)$_5$ (**4a**)	10.4	0.785	0.818	0.403	0.085
R$_3$LNHpU(pU)$_5$ (**4b**)	9.6	0.812	0.853	0.459	0.174
[^{32}P]U(pU)$_2$	7.8				
R$_1$LNH[^{32}P]U(pU)$_2$ (**4c**)	16.1				
R$_2$LNH[^{32}P]U(pU)$_2$ (**4d**)	14.3				
A^{LNH2} (n=3)**	14.2	0.489	1.500	1.680	1.102
A^{LNHR1} (n=3)**	18.6	0.752	1.000	0.863	0.532
A^{LNHR2} (n=3)**	15.5	0.529	1.346	1.282	0.897

*Microcolumn RPC, LiChrosorb RP-18, methanol gradient (0-80%)in 0.02M Tris-acetate buffer,pH 8.0.
** Microcolumn RPC, LiChrosorb RP-18, methanol gradient (0-80%) in the same buffer, pH 5.0.

TABLE 2. Molar ratio of products after enzymatic digestion* of modified oligoribonucleotides

Compounds	A or A^{LNH2} **	A^{LNHR} ***	U	G
$ApUpG(pU)_3$	1		3.9	1.1
$pUpG(pU)_3$ **(10)**	1.1		3.9	1
$A^{LNHR1}pUpG(pU)_3$ **(11a)**		0.7	3.7	1
$A^{LNHR2}pUpG(pU)_3$ **(11b)**		1	3.8	1

* Mixture of snake venom phosphodiesterase and 5'-nucleotidase.
** Microcolumn RPC, LiChrosorb RP-18, methanol gradient (0-80%) in 0,02 M Tris acetate buffer, pH5.0
*** $\varepsilon_{260}\ A^{LNH2} = \varepsilon_{260}\ A = 15400\ M^{-1}\ cm^{-1}$; $\varepsilon_{260}\ A^{LNHR1} = 38700\ M^{-1}cm^{-1}$;
$\varepsilon_{260}\ A^{LNHR2} = 27800\ M^{-1}\ cm^{-1}$, as the sum of molar extinction coefficients of the unmodified adenosine and corresponding azidoarylamide $RNH(CH_2)_3\ OH$ at 260nm[6]

The composition of modified oligoribonucleotides **11a,b** was confirmed by the digestion of snake venom phosphdiesterase and 5'-nucleotidase (Table 2).

The first promising results on the affinity labeling of human placenta ribosomes with new synthesized photoreactive analogues of mRNA were obtained.

ACKNOWLEDGMENTS

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