

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>

## New Photoreactive mRNA Analogues for the Affinity Labeling of Ribosomes

A. G. Venyaminova<sup>a</sup>; M. N. Repkova<sup>a</sup>; T. M. Ivanova<sup>a</sup>; M. I. Dobrikov<sup>a</sup>; K. N. Bulygin<sup>a</sup>; D. M. Graifer<sup>a</sup>; G. G. Karpova<sup>a</sup>; V. F. Zarytova<sup>a</sup>

<sup>a</sup> Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia

**To cite this Article** Venyaminova, A. G. , Repkova, M. N. , Ivanova, T. M. , Dobrikov, M. I. , Bulygin, K. N. , Graifer, D. M. , Karpova, G. G. and Zarytova, V. F.(1995) 'New Photoreactive mRNA Analogues for the Affinity Labeling of Ribosomes', *Nucleosides, Nucleotides and Nucleic Acids*, 14: 3, 1069 – 1072

**To link to this Article:** DOI: 10.1080/15257779508012536

**URL:** <http://dx.doi.org/10.1080/15257779508012536>

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.

## BIOLOGICAL ACTIVITY

### NEW PHOTOREACTIVE mRNA ANALOGUES FOR THE AFFINITY LABELING OF RIBOSOMES

A.G.Veniaminova, M.N.Repkova, T.M.Ivanova, M.I.Dobrikov, K.N.Bulygin,  
D.M.Graifer, G.G.Karpova, V.F.Zarytova\*.

Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Novosibirsk 630090, Russia

**Abstract.** Chemical synthesis of the model mRNA analogues (AUGU<sub>3</sub>, (pU)<sub>n</sub>) 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 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<sup>1,2</sup>. 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 excellent 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<sup>3,4</sup>.

The present work is devoted to the chemical synthesis of an artificial short mRNA analogues (AUGU<sub>3</sub> and (pU)<sub>n</sub>) bearing p-azidotetrafluorobenzoic (R<sub>1</sub>), p-azidobenzoic (R<sub>2</sub>) or 2-nitro-5-azidobenzoic (R<sub>3</sub>) 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<sup>5,6</sup>.

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<sup>7</sup>. The attachment of short aliphatic diamines to the deprotected oligoribonucleotides was performed with triphenylphosphine (Ph<sub>3</sub>P) and 2,2'-dipyridyldisulfide (PyS)<sub>2</sub>

mixture in the presence of 4-N,N-dimethylaminopyridine (DMAP) as nucleophilic catalyst<sup>8</sup>. The active zwitterionic derivatives **2** formed are readily converted to the 5'-amino containing oligouridyates **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%).

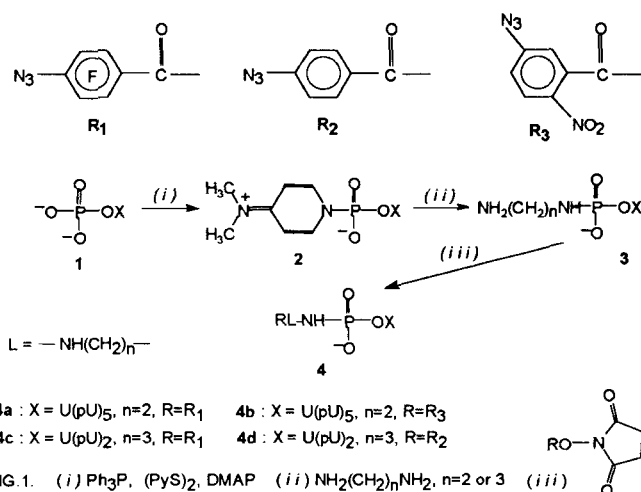


FIG. 1. (i)  $Ph_3P$ ,  $(PyS)_2$ , DMAP (ii)  $NH_2(CH_2)_nNH_2$ , n=2 or 3 (iii)

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-(tetraisopropylidisiloxane-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

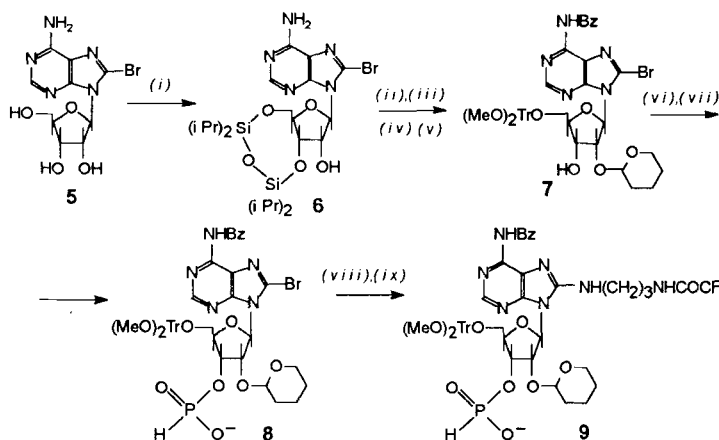
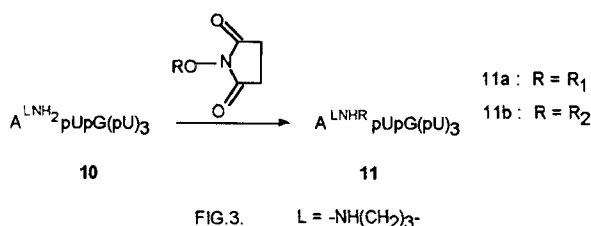


FIG. 2 (i):  $(iPr)_2(Cl)Si-O-Si(Cl)(iPr)_2$ ; (ii): 2,3-dihydropyran,  $H^+$ ; (iii):  $BzCl$ ; (iv):  $(C_2H_5)_4NF$ ; (v): dimethoxytrityl chloride; (vi): salicylchlorophosphine; (vii):  $H_2O$ ; (viii):  $NH_2(CH_2)_3NH_2$ ; (ix):  $CF_3CO_2C_2H_5$ .

protected uridine and guanosine was carried out using the monofunctional phosphitylating reagent salicylchlorophosphine<sup>9</sup>. The replacement of 8-Br-substitute in **8** was achieved by interaction with 1,3-diaminopropane at elevated temperature, followed by the protection of the outer amino group. The structure of the intermediate products

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



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<sup>7</sup> and opening

this group simultaneously with the standard deprotection of oligoribonucleotides allowed us to synthesize C-8-aminoadenosine containing  $\text{A}^*\text{pUpG(pU)}_3$  (**10**). FIG.3 depicts the transformation of this modified oligoribonucleotide into oligomers **11a,b** with arylazide groups ( $\text{R}_1$  and  $\text{R}_2$ ) 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 time, min	Spectral ratio			
		250/260	270/260	280/260	290/260
$\text{ApUpG(pU)}_3$	8.4	0.839	0.799	0.403	0.108
$\text{A}^{\text{LNHR}^2}\text{pUpG(pU)}_3$ ( <b>10</b> )	9.8	0.792	0.932	0.651	0.303
$\text{A}^{\text{LNHR}^1}\text{pUpG(pU)}_3$ ( <b>11a</b> )	12.6	0.807	0.904	0.615	0.300
$\text{A}^{\text{LNHR}^2}\text{pUpG(pU)}_3$ ( <b>11b</b> )	11.2	0.742	0.998	0.742	0.407
$(\text{pU})_6$	7.0	0.774	0.836	0.395	0.060
$\text{R}_1\text{LNHpU(pU)}_5$ ( <b>4a</b> )	10.4	0.785	0.818	0.403	0.085
$\text{R}_3\text{LNHpU(pU)}_5$ ( <b>4b</b> )	9.6	0.812	0.853	0.459	0.174
$[\text{}^{32}\text{P}]\text{U(pU)}_2$	7.8				
$\text{R}_1\text{LNH}[\text{}^{32}\text{P}]\text{U(pU)}_2$ ( <b>4c</b> )	16.1				
$\text{R}_2\text{LNH}[\text{}^{32}\text{P}]\text{U(pU)}_2$ ( <b>4d</b> )	14.3				
$\text{A}^{\text{LNHR}^2}(n=3)^{**}$	14.2	0.489	1.500	1.680	1.102
$\text{A}^{\text{LNHR}^1}(n=3)^{**}$	18.6	0.752	1.000	0.863	0.532
$\text{A}^{\text{LNHR}^2}(n=3)^{**}$	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 <sup>LNHR2</sup> **	A <sup>LNHR</sup> ***	U	G
ApUpG(pU) <sub>3</sub>	1		3.9	1.1
pUpG(pU) <sub>3</sub> ( <b>10</b> )	1.1		3.9	1
A <sup>LNHR1</sup> pUpG(pU) <sub>3</sub> ( <b>11a</b> )		0.7	3.7	1
A <sup>LNHR2</sup> pUpG(pU) <sub>3</sub> ( <b>11b</b> )		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

\*\*\*  $\epsilon_{260} A^{LNHR2} = \epsilon_{260} A = 15400 \text{ M}^{-1} \text{ cm}^{-1}$ ;  $\epsilon_{260} A^{LNHR1} = 38700 \text{ M}^{-1} \text{ cm}^{-1}$ ;  
 $\epsilon_{260} A^{LNHR2} = 27800 \text{ M}^{-1} \text{ cm}^{-1}$ , as the sum of molar extinction coefficients of the unmodified adenosine and corresponding azidoarylamide  $\text{RNH}(\text{CH}_2)_3 \text{OH}$  at  $260\text{nm}^6$

The composition of modified oligoribonucleotides **11a,b** was confirmed by the digestion of snake venom phosphodiesterase 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

This investigation was supported by the Russian Foundation of Fundamental Research.

#### REFERENCES

1. Mitchell,P.; Stade,K.; Oßwald,M.; Brimacombe,R. *Nucleic Acids Res.* **1993**, *21*, 887-896. and ref. therein.
2. Hanna,M.M.; Zhang,Y.; Reiding,J.C.; Thomas,M.J.; Jou,J. *Nucleic Acids Res.* **1993**, *21*, 2073-2079 and ref. therein.
3. Lewis, R.V.; Roberts,M.F.; Dennis,E.A.; Allison,W.S. *Biochemistry* **1977**, *16*, 5650-5654.
4. Schnapp,K.A.;Poe,R.; Leyva, E.;Soundararajan.N.; Platz,M.S. *Bioconjugate Chem.***1993**, *4*, 172-177 and ref. therein.
5. Levina,A.S.; Berezovskii,M.V.;Venyaminova,A.G.; Dobrikov,M.I.; Repkova,M.N.; Zarytova,V.F. *Biochimie* **1993**, *75*, 25-27.
6. Levina,A.S.; Tabatadse,D.R.; Khalimskaya,L.M.; Prichodko,T.A.; Shishkin,G.V.; Alexandrova, L.A.; Zarytova,V.F. *Bioconjugate Chem.* **1993**, *4*, 319-325.
7. Venyaminova,A.G.;Repkova,M.N.;Maltseva,T.V.*Nucleic Acids Symp.Ser.***1991**,*24*, 263.
8. Knorre,D.G.; Vlassov,V.V.; Zarytova,V.F.; Lebedev,A.V.; Fedorova, O.S. *Design and Targeted Reactions of Oligonucleotide Derivatives*, CRC Press, Inc., Boca Raton, **1994**, p.123-124.
9. Venyaminova,A.G.; Komarova,N.I.; Levina,A.S.; Repkova,M.N. *Bioorgan.Khim.* (Russia) **1988**, *14*, 484-489.