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N-Azidomethylbenzoyl Blocking Group in the Phosphotriester Synthesis of Oligoribonucleotides

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Abstract—An effective modification of the phosphotriester method has been developed for the automatic synthesis of DNA and RNA fragments using *O*-nucleophilic intramolecular catalysis and the 2-(azidomethyl)benzoyl group for the protection of the amino groups of nucleotide heterocyclic bases.

Key words: 2-azidomethylbenzoyl group, intramolecular catalysis, oligonucleotides synthesis, phosphotriester method

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INTRODUCTION

Some analogues and mimics of nucleic acids that are of interest as potential drugs are base-sensitive.[2] *O*-Alkyl phosphate, alkyl phosphonate, and carboxyethyl DNA analogues, as well as RNA analogues and polyester derivatives of peptide nucleic acids, belong to such compounds [1–4]. However, traditional methods of chemical oligonucleotide synthesis require the use of *N*- and *O*-protective groups that are deblocked under drastic basic conditions. Therefore, the development of the strategy of oligonucleotide synthesis where protective groups are removed under milder conditions is topical. The synthesis of deoxynucleoside analogues bearing 2-(azidomethyl)benzoyl (AZMB) groups at the hydroxyl and amino functions of heterocyclic bases was reported earlier [5, 6]. Unlike routine acyl groups removed in a basic medium, the AZMB group was shown to be removed under neutral conditions in the presence of triphenylphosphine or methyldiphenylphosphine in a water–dioxane mixture (9 : 1), as well as by the reduction with ammonium formate over Pd/C. In addition, it was demonstrated that this protective group can be used for the synthesis of boranophosphate oligonucleotide analogues [7].

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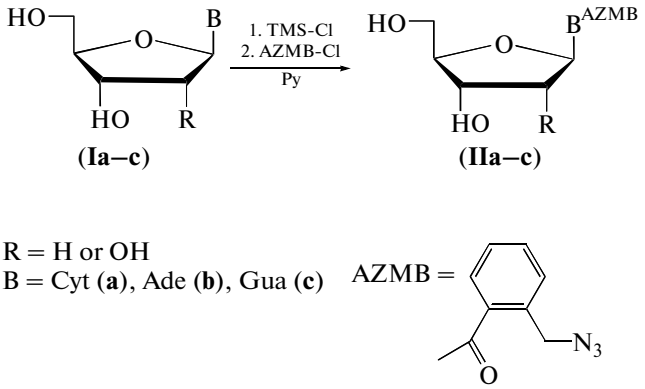
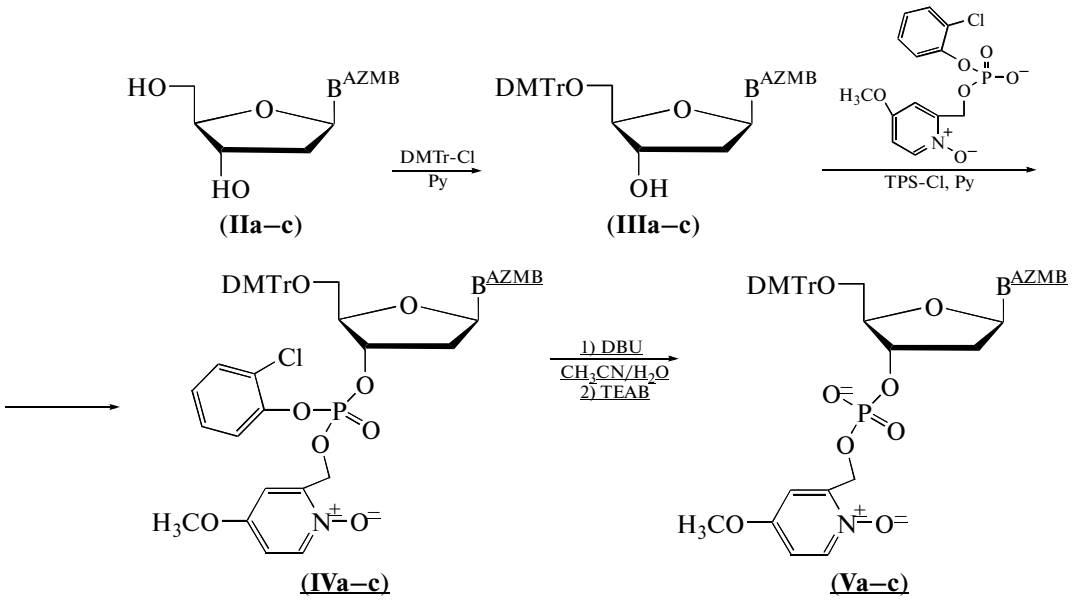
[2] Abbreviations: AZM, azidomethyl; AZMB, *o*-azidomethylbenzoyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMTr, 4,4'-dimethoxytrityl; NBS, 2-nitrobenzenesulfenyl; TEAB, triethylammonium bicarbonate; TMS, trimethylsilyl; TPS, 2,4,6-triisopropylbenzenesulfonyl; TIPDS-Cl_2, 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane; TBAF, tetrabutylammonium fluoride.

RESULTS AND DISCUSSION

Recently, we proposed the rapid and effective synthesis of oligoribonucleotides by the phosphotriester approach using *O*-nucleophilic intramolecular catalysis and monomeric syntones containing 2'-*O*-AZM or 2'-*O*-AZMB protective groups [8, 9]. Continuing our research, we studied the efficacy of the automatic solid phase synthesis of deoxyribo- and ribooligonucleotides using the AZMB group for the protection of the exocyclic amino functions of nucleotide heterocycles. The developed schemes of the synthesis of monomeric deoxyribosyntones (**V**) and ribosyntones (**XI**) containing the *N*-AZMB group in the bases and the *O*-nucleophilic catalytic 4-methoxy-1-oxido-2-picolyl protective group at the phosphate function are shown in Schemes 1–3.

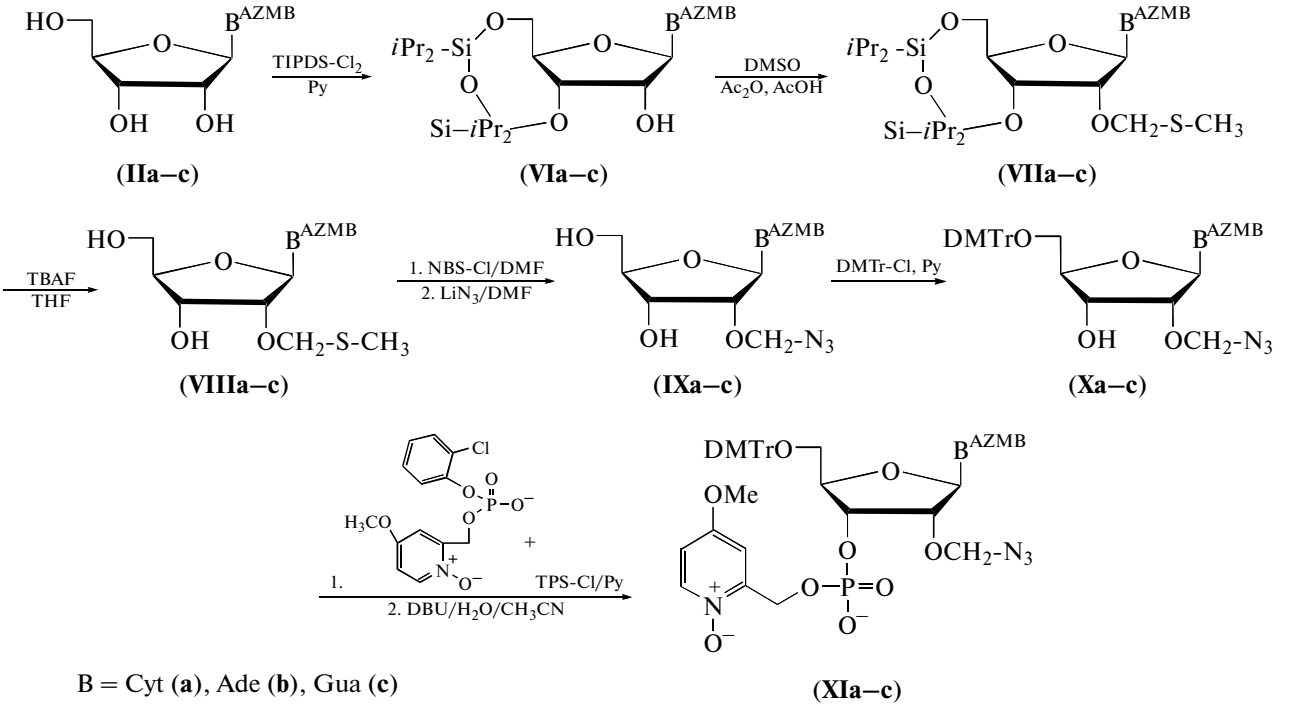


Scheme 1.



B = Cyt (**a**), Ade (**b**), Gua (**c**)

Scheme 2.



B = Cyt (**a**), Ade (**b**), Gua (**c**)

Scheme 3.

In the case of ribonucleotides, the AZM function was used for the protection of 2'-hydroxyl groups (Scheme 3). The AZMB group was introduced at the amino functions of heterocyclic bases with (2-azidomethyl)benzoyl chloride with the prior trimethylsilylation of ribose hydroxyls [5, 10]. 5'-Hydroxyl groups were blocked in a routine manner using a 4,4'-dimethoxytrityl group; 3'-phosphate derivatives containing a catalytic protective group were obtained as described earlier in [9]. The structures of the synthesized monomeric syntones were confirmed by mass spectrometry (Fig. 1) and NMR spectroscopy.

The efficacy of the automatic solid phase oligoribonucleotide synthesis by the phosphotriester approach using azido-containing protective groups was studied

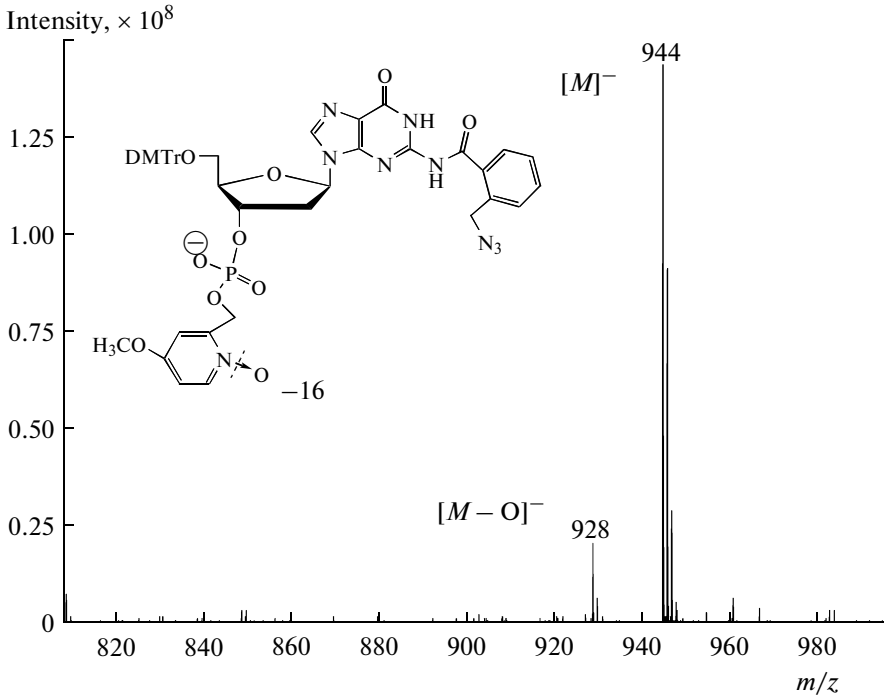


Fig. 1. Mass spectrum of monomeric synton (**V**) registered in the mode of negative ions.

with model oligonucleotides. The synthesis was performed on a standard CPG support under the conditions given in the table. The formation of the internucleotide bond proceeded in a 3 : 1 acetonitrile–pyridine mixture in the presence of TPS-C1 as a coupling agent [11]. The yields at single stages of internucleotide couplings were nearly quantitative. After the chain elongation was completed, oligonucleotides immobilized on the support were treated with 1 M lithium iodide in acetonitrile (20°C, 3 h) for the deblocking of the internucleotide phosphates [9] followed by the treatment of the polymer with a 1 : 1 mixture of ethanol and concentrated ammonia (1 h, room temperature) to cleave the oligonucleotide from the support. This treatment affected neither the *N*-AZMB nor the 2'-*O*-AZM groups [9, 12]. As was shown in the experiments with protected monomers, both groups were highly stable towards ammonia for several hours at room temperature.

It is noteworthy that the treatment of oligonucleotides with ammonia for the cleavage from the support can be avoided if chain elongation is used on a CPG support bearing alkylsulfonylethyl groups as described in [4, 13, 14]. Azido-containing protective groups were removed from the amino functions and 2'-hydroxyl groups with 0.1 M triphenylphosphine in a 9 : 1 mixture of acetonitrile–water (20°C, 5 h) or methyldiphenylphosphine in a 8 : 2 mixture of dioxane–water (20°C, 2 h). The resulting oligonucleotides were isolated by gel filtration. Their homogeneity was confirmed by electrophoresis in polyacrylamide gel and by reverse-phase HPLC (Figs. 2, 3). For compar-

A typical cycle of oligonucleotide elongation in automated phosphotriester synthesis using monomers (**V**) and (**XI**) containing *N*-AZMB groups

Stage	Solvents and reagents	Time, min
1. Detritylation	3% dichloroacetic acid in dichloromethane	1.0
2. Washing	Acetonitrile	1.0
3. Washing	Acetonitrile–pyridine (3 : 1)	0.5
4. Coupling	0.05 M monomeric synton; 0.15 M TPS-C1 in a 3 : 1 acetonitrile–pyridine mixture (3 : 1)	0.5 (3.0*)
5. Washing	Acetonitrile–pyridine (3 : 1)	0.5
6. Capping	Ac_2O–methylimidazole–acetonitrile (1 : 1 : 8)	0.5
7. Washing	Acetonitrile	1.0

* The reaction time for the synthesis of oligoribonucleotides from syntones (**XI**).

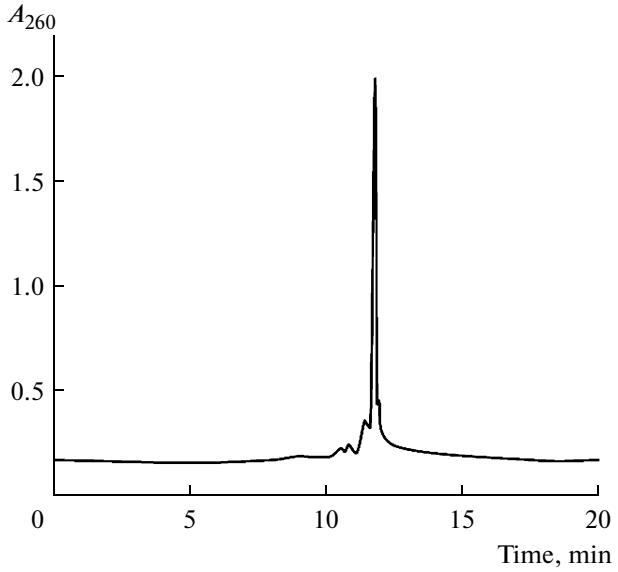


Fig. 2. The analysis of crude oligonucleotide d(CGCACTCGTCGCTCACCATGT) after the removal of the protective groups by HPLC on a Lichrosorb RP-18 column in a gradient of acetonitrile (0–35%) in 0.1 M ammonium acetate.

ison, oligonucleotides with the same base sequences were obtained by the amidophosphite method from commercially available monomers.

To conclude, we reported the effective solid phase synthesis of DNA and RNA oligonucleotides by the phosphotriester method using *O*-nucleophilic intramolecular catalysis and azido-containing *N*- and *O*-protective groups removed under neutral conditions. The developed approach opens up a prospect for the solid phase synthesis of base-sensitive oligonucleotide analogues.

EXPERIMENTAL

Commercial solvents and reagents were used without further purification. Monomeric syntones, thymidine and uridine derivatives, were prepared as described in [9, 11]. NMR spectra (δ, ppm, *J*, Hz) were registered in $CDCl_3$ at 300 (^{1}H) and 121 MHz (^{31}P) on a Bruker DPX-300 spectrometer. Chemical shifts are given relative to tetramethylsilane (^{1}H) and H_3PO_4 (^{31}P). Mass spectra were registered in the mode of negative ions on an Ultraflex II MALDI-TOF mass spectrometer (Bruker Daltonics) using 2,4,6-trihydroxyacetophenone as a template. Column chromatography was performed on silica gel 60 (Merck). Oligonucleotide synthesis was performed on a 1-μmol scale on an Applied Biosystems 381 A synthesizer. Protective groups were removed as described in [9]. Reverse-phase HPLC was performed on an Altex chromatograph (Beckman) in a gradient of acetonitrile in 0.1 M ammonium acetate. Electrophoresis was carried out in polyacrylamide gel containing 7 M urea in a 0.1 M Tris–borate buffer (pH 8.3).

Monomeric Deoxyribonucleoside Syntones (V)

Trimethylchlorosilane (6 mmol) was added dropwise under stirring to a solution of nucleoside (**I**, R = H) (1 mmol), preliminarily dried by evaporating with pyridine, and cooled to 0°C in dry pyridine (5 ml). The suspension was stirred for 1 h at 0°C and 2-(azidomethyl)benzoyl chloride (3 mmol, 0.59 g) [5] was added. The reaction mixture was stirred at room temperature for 3 h, cooled to 0°C, the reaction was stopped with water (1 ml), and pH was adjusted to 10.0 with aqueous ammonia. The mixture was evaporated to dryness and reevaporated with water to remove traces of pyridine. Water (4 ml) and ether (8 ml) were added to the residue and the mixture was shaken until the precipitate was formed. The oil-like precipitate was separated by decantation and dried in a vacuum. The crude compound (**II**, R = H) was purified by column chromatography on silica gel in a gradient of methanol (0–12%) in chloroform. The target fractions were evaporated, and the residue was dried to

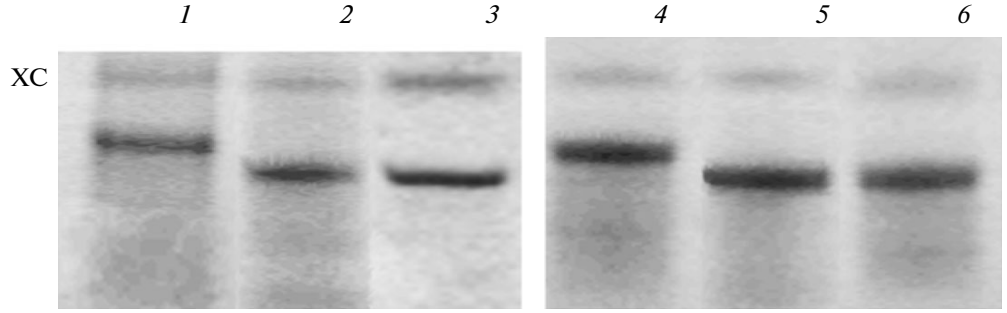


Fig. 3. The analysis of r(CGCACUCGUCGCUCACCAUGU) (lanes *1–3*) and d(CGCACTCGTCGCTCACCATGT) (lanes *4–6*) by electrophoresis in 15% denatured PAG. *3, 6*, reference oligonucleotides obtained by the phosphate amide method; *2, 5*, deblocked oligonucleotides obtained by the phosphotriester method with azido-containing protective groups; *1, 4*, oligonucleotides prior to the removal of 2'-*O*-azidomethyl and *N*-2-(azidomethyl)benzoyl protective groups. The picture was taken in reflected UV at 254 nm.

give 80, 82, and 74% of dC (**IIa**), dA (**IIb**), and dG (**IIc**) derivatives, respectively.

For the introduction of the 5'-dimethoxytrityl residue, 2'-deoxy derivatives (**IIa**)–(**IIc**) were dissolved in a minimal volume of dry pyridine and treated with 4,4'-dimethoxytrityl chloride (1.2 equiv) for 2 h at room temperature. The reaction was stopped by the addition of the same volume of 0.5 M TEAB, and the target product was extracted with chloroform. Organic fractions were evaporated to oil, and the residue was dissolved in chloroform and purified by column chromatography on silica gel in a gradient of methanol (0–5%) in chloroform containing 0.1% triethylamine to give target compounds (**IIIa**)–(**IIIc**) in 84, 82, and 73% yields, respectively. The phosphorylation of deoxyribonucleosides (**III**) and the removal of the *p*-chlorophenyl protective group from the phosphate residue of exhaustively blocked nucleotides (**IVa**)–(**IVc**) was performed as described in [9] to give (**Va**)–(**Vc**).

***N*⁴-[(2-Azidomethyl)benzoyl]-5'-*O*-(4,4'-dimethoxytrityl)deoxycytidine-3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (Va).** Yield 83%. ^{1}H NMR: 8.17 (1 H, d, *J* 7.5, H6 Cyt), 8.05 (1 H, d, *J* 7.2, H6 picolyl), 7.67–7.16 (15 H, m, H Ar, AZMB, H Ar, DMTr, H5 Cyt, H3 picolyl), 6.86–6.80 (4 H, m, H Ar, DMTr), 6.71 (1 H, dd, *J* 7.2, 3.6, H5 picolyl), 6.28 (1 H, t, *J* 6.3, H1'), 5.13 (2 H, m, P-OCH$_2$), 5.07 (1 H, m, H3'), 4.66 (2 H, s, CH$_2$-N AZMB), 4.45 (1 H, m, H4'), 3.81 (3 H, s, OCH$_3$ picolyl), 3.78 (6 H, s, OCH$_3$, DMTr), 3.48–3.39 (2 H, m, H5'), 2.92–2.87 (1 H, m, H$^{\alpha}$2'), 2.36–2.31 (1 H, m, H$^{\beta}$2'). ^{31}P NMR: 0.41. Mass (*m/z*): 904.36 [*M*$^-$]. Calc. for $C_{45}H_{43}N_7O_{12}P^-$ 904.27.

***N*⁶-[(2-Azidomethyl)benzoyl]-5'-*O*-(4,4'-dimethoxytrityl)deoxyadenosine-3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (Vb).** Yield 77%. ^{1}H NMR: 9.09 (1 H, br s, NH Ade), 8.69 (1 H, s, H8 Ade), 8.14 (1 H, s, H2 Ade), 8.08 (1 H, d, *J* 7.2, H6 picolyl), 7.79–7.13 (14 H, m, H Ar, AZMB, H Ar, DMTr; H3 picolyl), 6.78–6.69 (5 H, m, H Ar, DMTr; H5 picolyl), 6.56 (1 H, t, *J* 6.8, H1'), 5.24–5.08 (3 H, m, P-OCH$_2$, H3'), 4.76 (2 H, s, CH$_2$-N AZMB), 4.50 (1 H, m, H4'), 3.79 (3 H, s, OCH$_3$ picolyl), 3.75 (6 H, s, OCH$_3$DMTr), 3.49–3.35 (2 H, m, H5'), 2.94–2.80 (2 H, m, H2'). ^{31}P NMR: 0.27. Mass (*m/z*): 928.37 [*M*$^-$]. Calc. for $C_{46}H_{43}N_9O_{11}P^-$ 928.28.

***N*²-[(2-Azidomethyl)benzoyl]-5'-*O*-(4,4'-dimethoxytrityl)deoxyguanosine-3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (Vc).** Yield 79%. ^{1}H NMR: 12.35 (1 H, br s, NH Gua), 7.82 (1 H, C, H8 Gua), 7.77 (1 H, d, *J* 7.4, H6 picolyl), 7.61–7.16 (14 H, m, H Ar, AZMB, H-Ar DMTr, H3 picolyl), 6.84–6.79 (4 H, m, H Ar, DMTr), 6.50 (1 H, dd, *J* 7.4, 3.5, H5 picolyl), 6.34 (1 H, m, H1'), 5.45 (1 H, dd, *J* 17.3, 5.2, P-OCH$^{\alpha}$), 5.28 (1 H, dd, *J* 17.3, 7.8, P-OCH$^{\beta}$), 5.09 (1 H, m, H3'), 4.83 (1 H, d, *J* 14, CH$^{\alpha}$-N AZMB), 4.61 (1 H, d, *J*14, CH$^{\beta}$-N AZMB), 4.39 (1 H, m, H4'), 3.79 (3 H, s, OCH$_3$ picolyl), 3.78 (6 H, s, OCH$_3$ DMTr), 3.47–3.20 (2 H, m, H5'), 2.88–2.82 (1 H, m, H$^{\alpha}$2'), 2.24–2.17 (1 H, m, H$^{\beta}$2'). ^{31}P NMR: 0.91. Mass (*m/z*): 944.38 [*M*$^-$]. Calc. for $C_{46}H_{43}N_9O_{12}P^-$ 944.28.

Monomeric Ribonucleoside Syntones (XI)

Ribonucleosides bearing the AZMB protective group at the heterocyclic bases were obtained similarly to the procedure described above for deoxyribonucleosides. The yield of compounds ((**IIa**)–(**IIc**), R = OH) were 80, 85, and 83% for Cyt, Ade, and Gua derivatives, respectively.

For the preparation of 2'-*O*-azidomethyl ribonucleoside derivatives, 3'- and 5'-hydroxy groups of (**VI**) were blocked by treatment with TIPDS-Cl in pyridine, and the AZMB derivative (**II**, R = OH) (1 mmol) was treated with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.2 mmol) in dry pyridine (5 ml). The reaction mixture was stirred at room temperature for 16 h, and 1 M TEAB (5 ml) and water (10 ml) was added. The product was isolated by extraction with chloroform (2 × 15 ml). The organic fractions were evaporated to oil, dried in a vacuum to remove pyridine traces, and the residue was dissolved in chloroform. Target (**VI**) was isolated by column chromatography on silica gel in a gradient of methanol (0–5%) in chloroform. The fractions containing (**VIa**)–(**VIb**) were united and evaporated to dryness to give 89, 85, and 83% Cyt, Ade, and Gua derivatives, respectively. The 2'-*O*-AZM group was introduced in compounds (**VI**) as described in [8]. *O*-methylthiomethyl group was introduced into the nucleoside 2'-position of (**VIa**)–(**VIc**) using dimethylsulfoxide and acetic anhydride in the presence of acetic acid. The removal of the 3'- and 5'-TIPDS protective groups from (**VIIa**)–(**VIIb**) was performed with 0.5 M TBAF in tetrahydrofurane to give (**VIIIa**)–(**VIIIb**), which were treated with NBS-Cl in DMF followed by the reaction with lithium azide to give 38, 31, and 32% Cyt, Ade, and Gua derivatives (**IXa**)–(**IXc**).

In case of purine deriatives (**VIIIb**) and (**VIIIc**) transformation of methylthiomethyl group into azidomethyl groip was conducted in the presence of trifluoromethanesulfonic acid.

5'-Dimethoxytritylation of nucleosides (**IX**) was carried out as described above for deoxyribonucleoside derivatives (**II**, R = H). The yields of Cyt, Ade, and Gua derivatives (**Xa**)–(**Xc**) achieved 82, 80, and 85%, respectively. A phosphate residue containing an *O*-nucleophilic catalytic group was introduced at the 3'-hydroxyl as described in [9] to give ribonucleotide syntones (**XIa**)–(**XIc**).

***N*⁴-[2-(Azidomethyl)benzoyl]-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(azidomethyl)cytidine 3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (XIa).** Yield 82%. ^{1}H NMR: 8.77 (1 H, br s, NH Cyt), 8.35 (1 H, d, *J* 7.2, H6 Cyt), 8.06 (1 H, d, *J* 7.2, H6 picolyl), 7.70–7.43 (4 H, m, H Ar, AZMB), 7.46–6.82 (15 H, m, H Ar, DMTr; H5 Cyt; H3 picolyl), 6.71 (1 H, dd, *J* 7.2, 3.6, H5 picolyl), 6.13 (1 H, d, *J* 3.0, H1'), 5.17 (1 H, d,

J 7.5, P-OCH$^{\alpha}$), 5.10 (1H, d, *J* 8.7, OC$^{\alpha}$N), 5.02 (1 H, d, *J* 7.5, P-OCH$^{\beta}$), 4.97 (1 H, d, *J* 8.7, OCH$^{\beta}$N), 4.97–4.93 (1 H, m, H3'), 4.67 (2 H, s, CH_2-N AZMB), 4.58 (1 H, m, H2'), 4.47–4.41 (1 H, m, H4'), 3.81, 3.80 (6 H, s, OCH_3, DMTr), 3.78 (3 H, s, OCH_3 picolyl), 3.66–3.54 (2 H, m, H5'). Mass (*m/z*): 975.36 [M^-]. Calc. for $C_{46}H_{44}N_{10}O_{13}P^-$ 975.28.

[2-(Azidomethyl)benzoyl]-5'-*O*-(4,4'-dimethoxy-trityl)-2'-*O*-(azidomethyl)adenosine 3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (XIb). Yield 80%. ^{1}H NMR: 9.26 (1 H, br s, NH Ade), 8.71 (1 H, s, H8 Ade), 8.15 (1 H, s, H2 Ade), 8.05 (1 H, d, *J* 7.2, H6 picolyl), 7.81–7.42 (4 H, m, H Ar, AZMB), 7.41–6.75 (14 H, m, H Ar, DMTr; H3 picolyl), 6.72 (1 H, dd, *J* 7.2, 3.6, H5 picolyl), 6.33 (1 H, d, *J* 6.6, H1'), 5.32–5.30 (1 H, m, H3'), 5.19–4.98 (4 H, m, P-OCH_2, OCH_2N), 4.82–4.78 (1 H, m, H2'), 4.77 (2 H, s, CH_2-N AZMB), 4.60–4.55 (1 H, m, H4'), 3.79 (3 H, s, OCH_3 picolyl), 3.76 (6 H, s, OCH_3 DMTr), 3.51–3.41 (2 H, m, H5'). Mass (*m/z*): 999.36 [M^-]. Calc. for $C_{47}H_{44}N_{12}O_{12}P^-$ 999.29.

[2-(Azidomethyl)benzoyl]-5'-*O*-(4,4'-dimethoxy-trityl)-2'-*O*-(azidomethyl)guanosine 3'-*O*-(1-oxido-4-methoxy-2-picolyl) phosphate (XIc). Yield 81%. ^{1}H NMR: 11.66 (1 H, br s, NH Gua), 7.83 (1 H, s, H8 Gua), 7.54–6.71 (19 H, m, H Ar, AZMB; H Ar, DMTr; H3 picolyl, H6 picolyl), 6.59 (1 H, dd, *J* 7.2, 3.6, H5 picolyl), 6.16 (1 H, d, *J* 6.3, H1'), 5.37–5.31 (1 H, m, H3'), 5.26 (1 H, d, *J* 6.3, P-OCH$^{\alpha}$), 5.13 (1 H, d, *J* 6.3, P-OCH$^{\beta}$), 5.09–5.03 (1 H, m, H2'), 4.94 (1 H, d, *J* 9.6, OCH$^{\alpha}$N), 4.77–4.63 (3 H, m, OCH$^{\beta}$N, CH22-N AZMB), 4.53–4.47 (1 H, m, H4'), 3.75 (6 H, s, OCH_3 DMTr), 3.74 (3 H, s, OCH_3 picolyl), 3.49–3.30 (2 H, m, H5'). Mass (*m/z*): 1015.36 [M^-]. Calc. for $C_{47}H_{44}N_{12}O_{13}P^-$ 1015.29.

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REFERENCES

1. Uhlmann, E. and Peyman, A., *Chem. Rev.*, 1990, vol. 90, pp. 543–584.
2. Kurreck, J., *Eur. J. Biochem.*, 2003, vol. 270, no. 8, pp. 1628–1644.
3. Iyer, R.P., Devlin, T., Habus, I., Ho, N.-H., Yu, D., and Agrawal, S., *Tetrahedron Lett.*, 1996, vol. 37, pp. 1539–1542.
4. Efimov, V.A., Buryakova, A.A., Choob, M.V., and Chakhmakhcheva, O.G., *Nucleosides Nucleotides*, 1999, vol. 18, pp. 2533–2549.
5. Wada, T., Ohkubo, A., Mochizuki, A., and Sekine, M., *Tetrahedron Lett.*, 2001, vol. 42, pp. 1069–1072.
6. Kawanaka, T., Shimizu, M., and Wada, T., *Tetrahedron Lett.*, 2007, vol. 48, pp. 1973–1976.
7. Kawanaka, T., Shimizu, M., Shintani, N., and Wada, T., *Bioorg. Med. Chem. Lett.*, 2008, vol. 18, pp. 3783–3786.
8. Efimov, V.A., Aralov, A.V., Fedunin, S.V., Klykov, V.N., and Shakhmakhcheva, O.G., *Bioorg. Khim.*, 2009, vol. 35, pp. 270-273 [*Russ. J. Bioorg. Chem*. (Eng. Transl.), 2009, vol. 35, pp. 250–253].
9. Efimov, V.A., Aralov, A.V., Klykov, V.N., and Chakhmakhcheva, O.G., *Nucleosides Nucleotides Nucleic Acids*, 2009, vol. 28, pp. 846–865.
10. Ti, G.S., Gaffney, B.L., and Jones, R.A., *J. Am. Chem. Soc.*, 1982, vol. 104, pp. 1316–1319.
11. Efimov, V., Buryakova, A., Dubej, I., Polushin, N., Chakhmakhcheva, O., and Ovchinnikov, Yu., *Nucleic Acids Res.,* 1986, vol. 14, pp. 6526–6540.
12. Zavgorodny, S.G., Shvets, V.I., and Miroshnikov, A.I., *Nucleosides Nucleotides Nucleic Acids*, 2000, vol. 19, pp. 1977–1991.
13. Efimov, V.A., Kalinkina, A.L., and Chakhmakhcheva, O.G., *Bioorg. Khim.*, 1995, vol. 21, pp. 612–616.
14. Efimov, V., Buryakova, A., Reverdatto, S., Chakhmakhcheva, O., and Ovchinnikov, Yu., *Nucleic Acid Res.*, 1983, vol. 11, pp. 8369–8387.