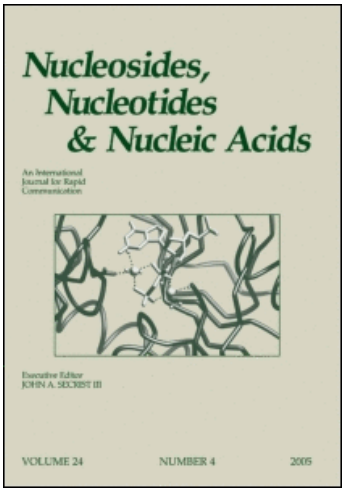

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SYNTHESIS OF RNA BY THE RAPID PHOSPHOTRIESTER METHOD USING AZIDO-BASED 2′-O-PROTECTING GROUPS

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□ *The azidomethyl and 2-(azidomethyl)benzoyl as 2′-OH protecting groups are reported for preparation of oligoribonucleotides by the phosphotriester solid-phase method using O-nucleophilic intramolecular catalysis. The procedures for the synthesis of the corresponding monomer synthons were developed and the usefulness of the application of 2′-O-azidomethyl and 2′-O-2-(azidomethyl)benzoyl groups was examined in the synthesis of different RNA fragments with a chain length of 15*–22 nucleotides. The azidomethyl group was found to be more preferable for effective synthesis of oligoribonucleotides. Hybridization properties of RNAs toward their complementary oligonucleotides were examined before and after the removal of 2′-O-azidomethyl groups.

Keywords Oligoribonucleotides; synthesis; phosphotriester method; azidomethyl and 2-(azidomethyl)benzoyl groups

INTRODUCTION

Natural and modified oligonucleotides are universal tools in a variety of studies in molecular biology, biotechnology, and medicine. They are widely used in chip technology, gene diagnostics and therapy, functional genomics, and nanotechnology because of their hybridization affinity for target DNA or RNA molecules. Thus, synthetic RNA fragments such as ribozymes and small interfering RNAs (siRNAs) as well as chemically modified oligonucleotides have attracted increasing interest for molecular biologists and medical researchers. In response to the current increased demand for natural RNA fragments and their analogues with predetermined properties,

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much attention is paid to the development of new strategies for their chemical synthesis.

Recently, the phosphoramidite method is routinely used for the synthesis of oligoribonucleotides.[1] However, by virtue of its chemical peculiarities, it cannot be used for the synthesis of some modified oligonucleotides, particularly derivatives containing strong electrophilic centers (e.g., azide and N-oxide groups) due to the proceeding side reactions. Also, it cannot be used for the synthesis of stereospecific phosphorothioate analogues of nucleic acids.

As ribonucleotides contain an additional 2′-hydroxyl compared with deoxyribonucleotides, the chemical synthesis of RNA requires an additional protecting group for this function. The selection of suitable protecting groups for the 2′-OH function of the ribose is one of the most demanding problems in RNA synthesis. A protective group for 2′-hydroxyl must be stable both in the process of chain elongation and during the removal of other blocking groups, and it must be readily removable at the last step under mild conditions preventing degradation of the resulting oligoribonucleotide. With this connection, several problems have been pointed out, including the necessity of prolonged coupling times due to the steric hindrance of the 2′-O-protecting group, difficulties in its deprotection, 3′-2′ migration of internucleotidic phosphate groups, the necessity of purification of 2′-O-protected oligonucleotide intermediates, and instability of RNA fragments. Various kinds of 2′-OH protecting groups have been reported for the phosphoramidite method, particularly a tert-butyldimethylsilyl (TBDMS),[2] triisopropylsilyloxymethyl (TOM),[3] and bis(2-acetoxyethoxy)methyl (ACE),[4] t-butyldithiomethyl (DTM),[5] 2-(4-tolylsulfonyl)ethoxymethyl (TEM),[6] cyanoethyl,[7] 2-cyanoethoxymethyl (CEM),[8] and monomers containing some of these blocking groups are commercially available. Although known protecting groups represent improvements in the synthesis of oligoribonucleotides, there are still many points to be resolved in the field of RNA synthesis.

Earlier, we reported the rapid phosphotriester method involving O-nucleophilic intramolecular catalysis at the stage of internucleotide bond formation as an alternative approach to the phosphoramidite oligonucleotide synthesis.[9] It was successfully applied for the synthesis of natural oligodeoxyribonucleotides as well as modified oligonucleotides containing 2′-azido- and 2′-amino-2′-deoxyuridine residues.[10] Moreover, the potential of this method for preparation of stereospecific oligonucleotide phosphorothioate analogues has been demonstrated.[11,12] In the process of the further improvement of this approach to the synthesis of natural and modified RNA fragments, we screened various blocking groups for 2′-hydroxyls of ribonucleotides.[12] In the present study, we have examined the usefulness of azido-based protecting groups, particularly 2-(azidomethyl)benzoyl

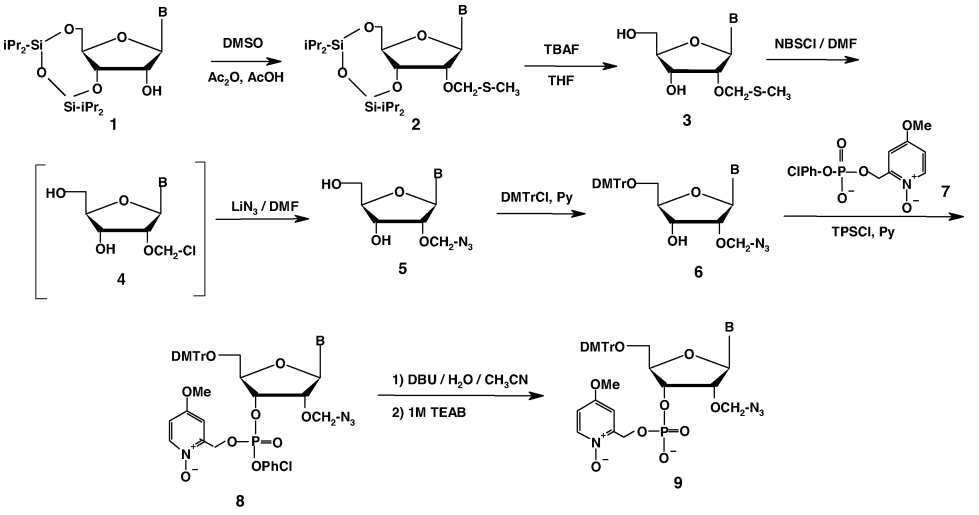
(AZMB) and azidomethyl (AZM) groups, as 2′-O-protective in the synthesis of oligonucleotides by the phosphotriester method under intramolecular O-nucleophilic catalysis.

RESULTS AND DISCUSSION

Synthesis of Monomer Building Blocks

The preparation and properties of several types of nucleoside derivatives containing azido-based protecting groups were described earlier,[13–16] but they were not applied to the phosphoramidite method of oligonucleotide synthesis. It was shown that the azido function had a negative influence on the coupling behavior of the 3′-*O*-phosphoramidites, presumably due to iminophosphorane formation by Staudinger reaction.[17,18]

At first, we developed a synthetic route for the ribonucleotide monomeric synthons **9**, which contained 2′-O-AZM group, 5′-O-4,4′-dimethoxytrityl (DMTr) group, and O-nucleophilic catalytic 4-methoxy-1-oxido-2-picolyl phosphate protective group. In this route, shown in Scheme 1, we introduce O-methylthiomethyl group (MTM) into the 2′-position of a 3′,5′-protected nucleoside **1** by the reaction with a mixture of dimethylsulfoxide, acetic anhydride and acetic acid.[19] Then, the MTM derivative of the nucleoside **2** was converted into the azidomethyl compound **5**. The earlier described procedure included the treatment of MTM derivative **2** with sulfuryl chloride (or Br_2) followed by the action of lithium azide solution in dimethylformamide.[16,20]

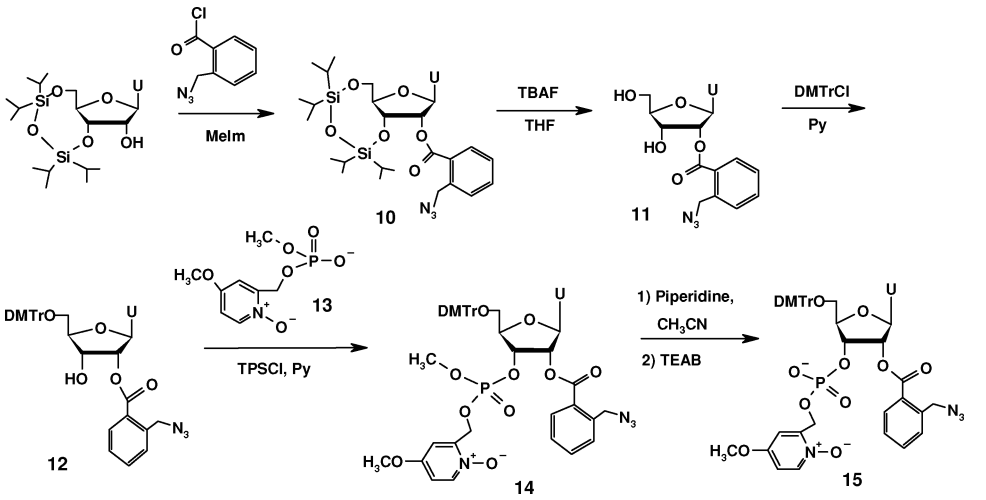


SCHEME 1

However, we observed that a side reaction of halogenation of uracil and cytosine *C*-5 positions took place in the presence of Br_2, or sulfuryl chloride, and the yields of undesired halogenation products achieved up to 50%. We found a solution to this problem, when a milder reagent, 2-nitrobenzenesulfenyl chloride (NBSCl), was used at this stage.[21] As NBSCl cannot interact with free hydroxyl groups in the absence of bases, a nucleoside 3′,5′-protective group was removed from the derivative **2** before the treatment with NBSCl. The following in situ addition of lithium azide allowed us to obtain the pyrimidine nucleoside derivatives **5** with high yields. For preparation of the purine derivatives **5**, NBSC1 was added in the presence of trifluoromethanesulfonic acid to avoid the side reaction of the interaction of the 2′-O-chloromethyl intermediate **4** with the *N*-3 atom of a purine nucleobase with the formation of a cyclonucleoside.[21] After the introduction of 5′-OH protective dimethoxytrityl group, the preparation of 3′-phosphodiester **9** containing the catalytic 4-methoxy-1-oxido-2-picolyl group was performed using a modified procedure.

Earlier, we described a two-step preparation of the nucleotide phosphotriesters of type **8**, which included the phosphorylation of the 3′-hydroxyl of a protected nucleoside with bis(triazolyl)arylphosphate followed by the treatment with an aqueous triethylammonium bicarbonate solution (TEAB) to obtain the corresponding nucleoside phosphodiester, which was converted into the fully protected nucleoside phosphotriester by the action of 1-oxido-4-methoxy-2-pyridinemethanol in the presence of TPSCl and MeIm.[21] In this study, we developed a simplified procedure based on the conversion of the 2′,5′-protected nucleoside **6** into the phosphotriester **8** by the action of the preliminary prepared phosphodiester **7** in the presence of a condensing agent. The following removal of the chlorophenyl protecting group by the action of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in aqueous acetonitrile gave the desired synthon **9**.

The other group tested by us for the protection of ribonucleotide 2′-hydroxyls was the AZMB group. In contrast to the usual protecting groups of acyl type, which require basic conditions for their removal, AZMB group can be removed from nucleosides in neutral conditions.[13–15] The introduction of an AZMB group into 5′- and 3′-hydroxyls of deoxyribonucleosides by the action of (2-azidomethyl)benzoyl chloride was described earlier.[13] Using a similar procedure, we have synthesized the 2′-O-AZMB uridine derivative **11** as shown in Scheme 2. After protection of the 5′-OH group, phosphorylation of the compound **12** was performed by the action of the phosphodiester **13** in the presence of a condensing agent. The removal of the methyl protecting group from the phosphotriester **14** by treatment with piperidine gave the monomeric synthon **15**. Chemical structures of all monomers obtained in this study were confirmed by mass spectrometry and NMR spectroscopy data.



SCHEME 2

Automated Phosphotriester RNA Synthesis and Postsynthetic Deprotection Treatment

To verify the potential of 2′-O-azido-based protective groups for automated solid phase synthesis, we have undertaken the preparation of oligoribonucleotides with chain lengths of 15–22 units. At first, we synthesized a set of U_{15} oligomers to examine the effect of 2′-O-protecting groups on the kinetics of the internucleotide bond formation. The synthesis was performed on a 1 μmol scale using the 1000 Å CPG support containing uridine derivatives **6**, or **12**. For coupling reactions, monomer synthons were used as 0.05 M solutions in acetonitrile-pyridine (3:1, v/v), and TPSCl (0.15 M) was used as the condensing reagent. The average stepwise yield, obtained by a detritylation assay, was used to evaluate the synthesis efficiency. It was found that in the case of monomers with an AZM 2′-O-blocking group practically quantitative yields were achieved in 2 min, whereas in the case of monomers with a bulky AZMB blocking group a longer coupling time was needed (up to 5 minutes; see Figure 1). The conditions of the optimized synthetic cycle are shown in Table 1.

After the chain elongation was completed, oligonucleotide-anchored solid supports were treated to remove phosphate protecting groups. Earlier, we described the use of piperidine (10 hours, 20°C), or thiophenol-dioxane-triethylamine (1:2:2, v/v, 3–5 hours, 20°C) for this purpose. In this study, we used 1 M lithium iodide solution in acetonitrile (3 hours, room temperature) for the removal of 1-oxido-4-methoxy-2-picolyl groups from internucleotide phosphate residues and found that it is a very effective and convenient reagent. Cleavage from the solid support was then carried out by the treatment with a mixture of 28% ammonia water solution and ethanol

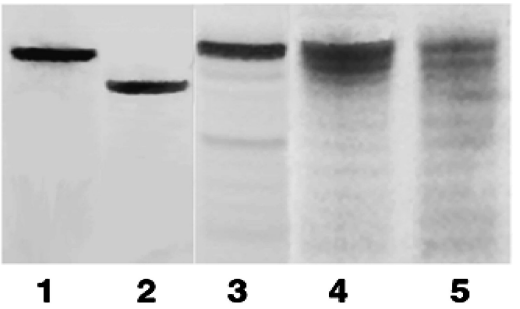


FIGURE 1 Mobility shift analysis of crude U_{15} oligonucleotides by a 15% denaturing PAGE. Deprotection of oligonucleotides was carried out by the treatment with 1 M lithium iodide solution in acetonitrile for 3 hours to remove phosphate blocking groups. Then, oligonucleotides were cleaved from the support by the treatment with a mixture of 28% ammonia water solution and ethanol (3:1, v/v) for 2 hours. 2′-O-AZM blocking groups were removed by the action of 0.1 M triphenylphosphine in acetonitrile-water (9:1, v/v) for 2 hours, and 2′-O-AZMB groups were removed in the same conditions for 5 hours. Lane 1 shows the oligonucleotide before the removal of 2′-O-AZM blocking groups (coupling time in internucleotide condensations was 2 minutes). Lane 2 shows the same fully deprotected oligonucleotide. Lanes 3–5 show crude oligonucleotides before the removal of 2′-O-AZMB blocking groups synthesized using 5 minutes (lane 3), 2 minutes (lane 4), and 1 minute (lane 5) coupling times in the reactions of the internucleotide bond formation. Visualization was by UV-shadowing at 254 nm.

(3:1, v/v) at room temperature for 1.5–2 hours. As it can be expected from the literature data,[16] 2′-O-AZM group remained intact in an aqueous ammonia solution for a long time at room temperature. The preliminary examination of the 2′-O-AZMB group stability to the action of ammonia using the monomer **15** and control by TLC revealed that it was fully stable under these conditions for more than 5 hours (data not shown). The resulting homo-U oligonucleotides carrying 2′-O-protective groups were isolated by gel filtration on a NAP-10 column, and the crude products were subjected to PAGE analysis. As it followed from HPLC data and PAGE analysis (e.g., see Figure 1, lane 3), the treatment with ammonia did not influence the stability of the oligonucleotide 2′-O-AZMB groups and the stability of an

TABLE 1 Elongation cycle for the solid phase phosphotriester synthesis of oligoribonucleotides using 2′-O-AZM and 2′-O-AZMB blocking groups

Step	Solvents and reagents	Time (min)
1. Detritylation	2% Trichloroacetic acid in dichloromethane	1.0
2. Wash	Acetonitrile	1.0
3. Wash	Acetonitrile—pyridine (3:1, v/v)	0.5
4. Coupling	0.05 M P-component; 0.15 M TPSCl in acetonitrile—pyridine (3:1, v/v)	2.0(5.0*)
5. Wash	Acetonitrile-pyridine	0.5
6. Capping	Ac_2O - 1-methylimidazole - pyridine - acetonitrile (1:1:2:6, v/v/v/v)	0.5
7. Wash	Acetonitrile	1.5

*The reaction time for internucleotide condensations with monomers containing 2′-O-AZMB groups.

oligonucleotide itself during 2 hours. The next step was removal of the 2′-O-blocking groups. In model experiments, the efficiency of the removal of 2′-O–azido-based groups by the action of 0.1 M triphenylphosphine in acetonitrile-water (9:1, v/v), or methyldiphenylphosphine in dioxane-water (4:1, v/v) was checked using all four monomers and the control by TLC. It was found that 2′-O-AZM group can be completely removed from the monomers during 5–10 min, whereas the removal of 2′-O-AZMB group from the monomer **15** required about 30 min under the same conditions. Then, the aliquots of homo-U oligonucleotides carrying 2′-O-protective groups were subjected to the treatment with the same reagents to remove azido-based 2′-O-blocking groups for 1, 2, 3, and 5 hours with the following analysis by PAGE. The results obtained have shown that the removal of 2′-O-AZM groups from oligonucleotide by 0.1 M triphenylphosphine in acetonitrile-water (9:1, v/v), or by methyldiphenylphosphine in dioxane-water (4:1, v/v) is complete in 2 hours and 30 minutes, respectively. The removal of 2′-O-AZMB groups from oligonucleotides required more time (1 hour with methyldiphenylphosphine and 3–5 hours with triphenylphosphine), probably due to spatial hindrances, and some degradation of the oligonucleotide chain was observed during this deprotection step (data not shown).

So, we concluded that 2′-O-AZM blocking group is more preferable for the effective synthesis of oligoribonucleotides, and further demonstration of the efficacy of the automatic solid phase synthesis using our strategy was carried out on the examples of preparation of several RNAs with mixed nucleobase sequences using 2′-O-AZM protected monomer synthons. The average yields at the coupling steps exceeded 98%. The resulting oligonucleotides were deprotected as described above. Cleavage from the solid support and removal of blocking groups from heterocycles were carried out by the treatment with ammonia. In all cases, reverse-phase and anion-exchange HPLC as well as PAGE analysis of the final oligomers were used to confirm the purity of the RNA obtained (Figure 2). For comparison, oligoribonucleotides with the same sequences were obtained by the phosphoramidite method using commercially available monomers and the standard procedure.

Enzymatic Digestion and Hybridization Properties of Synthetic Oligoribonucleotides

Examination of synthetic RNAs obtained by the phosphotriester method has shown that they exhibited the same properties as those obtained by the phosphoramidite method. They demonstrated similar cleavage results when subjected to RNase H and phosphodiesterase digestion. Figure 3 shows the analysis of cleavage efficiency with RNase H of oligoribonucleotides. The results obtained allow us to conclude that oligoribonucleotides

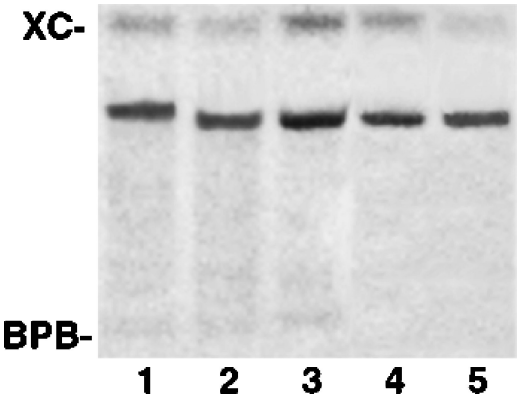


FIGURE 2 Analysis of an oligoribonucleotide CAGACAAAAGAAAGCGCA by a 15% denaturing PAGE. Visualization was by UV-shadowing at 254 nm. Lane 1 shows crude oligonucleotide synthesized by the phosphotriester method before the removal of 2′-O-AZM blocking group; lane 2 shows the same fully deprotected crude oligonucleotide; lane 3 shows the control iso-sequential fully deprotected crude oligoribonucleotide obtained by the phosphoramidite method; lanes 4 and 5 show the oligonucleotides from lanes 2 and 3 after purification by HPLC.

synthesized by our procedure are pure enough for biological studies.

At the same time, oligonucleotides containing 2′-O-AZM groups were more resistant to the action of nucleases than fully deprotected oligonucleotides. We have tested the stability of oligonucleotides containing 2′-O-AZM groups towards RNA-specific nucleases, particularly RNase A and RNase T1, and found that 2′-O-AZM RNA is stable to the action of these enzymes in concentrations that produced total digestion of natural oligoribonucleotides. Moreover, S1 nuclease, mung bean nuclease and exonuclease III were also unable to cleave 2′-O-AZM RNA (data not shown). At the same time, snake venom phosphodiesterase and bovine spleen phosphodiesterase were able to cleave 2′-O-modified oligoribonucleotides, but the resistance of 2′-O-AZM RNA to these enzymes was higher then the resistance of natural oligonucleotides (Figure 4). The data on the human plasma stability and other biological properties of 2′-O-AZM RNA will be presented in detail elsewhere.

Also, we observed a high stability of duplexes formed by 2′-O-AZM RNAs with the complementary targets. A comparison of melting temperatures (T_m) of duplexes formed by fully deprotected RNAs and those containing 2′-O-AZM groups with complementary synthetic oligonucleotides has shown that the T_m values of both types of duplexes are very close. Thus, the presence of 2′-O-AZM groups in oligoribonucleotides did not significantly affect the stability of duplexes, although it slightly destabilized them ($\sim$0.25°C per base pair; see Table 2).

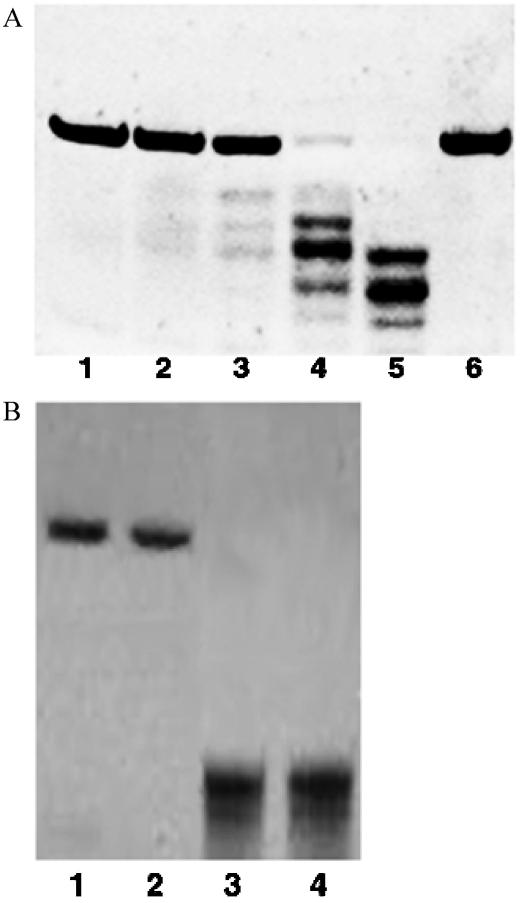


FIGURE 3 Analysis of the RNase H digestion of ^{32}P-labeled oligoribonucleotides by denaturing PAGE. A) RNase H cleavage of oligonucleotide r(CAUGGAGAGCGACGAGAGC)dTT obtained by the phosphotriester method. Lanes 1–5 represent digestion of RNA after 2, 5, 10, 30, and 60 minutes of incubation with the enzyme. Lane 6 shows the oligonucleotide before the treatment. B) RNase H cleavage of RNA CGCUCUCGUCGCUCUCCAUGU. Lanes 1 shows oligonucleotide synthesized by phosphotriester method before the addition of the nuclease; lane 2 shows the control iso-sequential oligonucleotide synthesized by the phosphoramidite method before the addition of the nuclease; lanes 3 and 4 represent digestion of the same oligonucleotides after the incubation with the enzyme for 2 hours. Visualization by autoradiography.

CONCLUSIONS

In this study, we developed a highly effective rapid phosphotriester-based method for the synthesis of oligoribonucleotides using the AZM group as a protecting for the 2′-hydroxyl of ribonucleotides. With this methodology, RNA synthesis can be carried out on a standard solid-phase synthesizer with high coupling yields and a coupling time of only 2 minutes. As the 2′-O-AZM protective group is stable with ammonia treatment, standard acyl N-blocking groups and supports can be used. The postsynthesis

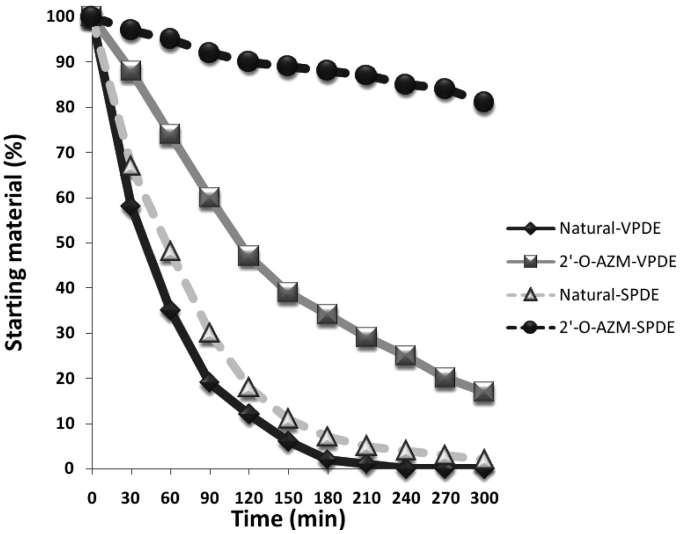


FIGURE 4 Nuclease stability of 2′-O-AZM oligouridylate. Time course of degradation of 2′-O-AZM homo-U_{15} and natural homo-U_{15} by venom (VPDE) and spleen (SPDE) phosphodiesterases.

deprotection procedure is simple and gives products of high purity. The developed method opens prospects for the effective solid phase synthesis of stereospecific phosphorothioate oligoribonucleotide analogues.[12] Also, our results demonstrated that the 2′-O-AZM RNA can be used as a synthetic intermediate for natural-type RNA as well as a 2′-O-modified functional RNA. As the azido group can be converted to other functional groups by a variety of chemical reactions, single incorporations of 2′-O-AZM monomers into oligoribonucleotides may be useful precursors for further postsynthetic RNA modification by the introduction of various labels and functional groups via the azido function. Further development of the strategy using azido-based protection groups for RNA synthesis will be reported in the near future.

EXPERIMENTAL

Solvents and reagents were obtained from commercial suppliers and were used without further purification. ^{1}H spectra were recorded on a Avance III-800 (Bruker, USA) and DPX-300 (Bruker, Germany) spectrometers, and ^{31}P NMR spectra were recorded on a Bruker DPX-300 spectrometer. Chemical shifts are given in p.p.m. relative to tetramethylsilane (^{1}H), or H_3PO_4 (^{31}P). Mass spectra were recorded using Ultraflex II MALDI-TOF (Bruker Daltonics, Germany) mass spectrometer. TLC was carried out on Merck Silica Gel 60 F_{254} plates in $CHCl_3$-CH_3OH (3:1, v/v; solvent A), or $CHCl_3$-CH_3OH (9:1, v/v; solvent B). Silica gel column chromatography was performed using Merck silica gel 60. Synthesis of oligonucleotides was

TABLE 2 Sequences, yields and hybridization properties of synthesized RNAs

RNA sequence	Coupling yield,%[a]	Overall yield,%[b]	Complementary oligodeoxyribonucleotide	T_m,°C[c]
UUUUUUUUUUUUUUU	99.2	56	AAAAAAAAAAAAAAA	39 (−3)
AUGGUCACCGACGCCA	98.5	51	TGGCGTCGGTGACCAT	56 (−4)
CGCUCUCGUCGCUCUCCAUGU	97.4	47	ACATGGAGAGCGACGAGAGCG	71 (−5)
AAGAAGAGCCUGGAGCCCAUCU	98.1	50	AGATGGGCTCCAGGCTCTTCTT	71 (−6)
AGAUGGGCUCCAGGCUCUUCUU	97.6	48	AAGAAGAGCCTGGAGCCCATCT	69 (−5)
r(GCUCUCGUCGCUCUCCAUG)dTT	98.3	49	CATGGAGAGCGACGAGAGC	70 (−5)
r(CAUGGAGAGCGACGAGAGC)dTT	97.7	55	GCTCTCGTCGCTCTCCATG	68 (−4)
CAGACAAAAGAAAGCGCA	99.1	60	TGCGCTTTCTTTTGTCTG	64 (−4)
UCUCCUGUGUACAAGUGAGUGC	98.9	57	GCTCTCACTTGTACTCAGGAGA	72 (−6)

[a]Average stepwise coupling yield.

[b]Overall yields of crude products were measured on the base of UV absorption at 260 nm.

[c]The value in brackets shows the difference between the melting temperature of duplexes formed by a native oligoribonucleotide and the same compound containing 2′-O-AZM groups.

performed on a1 μmol scale using Applied Biosystems Synthesizer 381A. Electrophoresis of oligomers was performed using polyacrylamide gel/7 M urea with 0.1 M Tris–borate/EDTA buffer (pH 8.3).

General Procedure for the Synthesis of 5′-O-(4,4′-Dimethoxytrityl)-2′-O-(azidomethyl)nucleosides (6)

The 2′-O-methylthiomethyl derivatives **3** were synthesized starting from the 3′,5′-protected nucleosides **1** essentially as it was described by Pechenov et al.[19] Then, the compound **3** (1 mmol) was treated with 1.5 mmol of 2-nitrobenzenesulfenyl chloride in 5 ml of dry DMF at 0°C. For the 2′-O-MTM derivatives of adenosine and guanosine, trifluoromethane sulfonic acid (1.2 mmol) was added to the reaction mixture also. After 5 minutes, 5 ml of 1 M solution of lithium azide in DMF was added. After the incubation during 5 minutes, the reaction was terminated by the addition of 0.25 M TEAB (25 ml). In the case of the uridine derivative **5**, the mixture obtained was washed with chloroform (2 × 20 ml), and the aqueous solution containing 2′-azidomethyluridine was evaporated to dryness. In all other cases, the reaction mixture was extracted with chloroform—1-butanol (7:3, v/v, 3 × 20 ml), and combined organic fractions were evaporated. The residue containing the crude product was dried by co-evaporation with toluene, and a 2′-O-AZM nucleoside (**5**) was isolated by silica gel column chromatography using a gradient of methanol (0–10%) in chloroform. Fractions containing the desired product were combined, concentrated to a gum and dried in vacuo. The analytical data obtained for compounds **5** were in accordance with the literature data.[16] The following 5′-protection of 2′O-azidomethyl nucleosides was performed by the action 4,4′-dimethoxytritylchloride (1.2 equiv.) in pyridine during 1.5 hours. The reaction was stopped by the addition of 0.5 M TEAB (20 ml), the crude product was extracted with chloroform (2 × 20 ml). The combined organic fractions were evaporated to a gum. The residue was dissolved in chloroform, and a 5′-O-dimethoxytrityl -2′-O-azidomethyl nucleoside **6** was isolated by silica gel column chromatography using a gradient of methanol (0–5%) in chloroform containing 0.1% of triethylamine (TEA). The fractions containing the desired derivative (**6**) were evaporated to a gum and dried in vacuo.

5′-O-(4,4′-Dimethoxytrityl)-2′-O-(azidomethyl)uridine. Yield 85%, R_f (solvent B) = 0.45. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.01 (1H, d, J = 8.1 Hz, Ura-*H*-6), 7.82–7.43 (13H, m, DMTr-*H*-Ar), 5.98 (1H, d, J = 5.4 Hz, *H*-1′), 5.33 (1H, d, J = 8.1 Hz, Ura-*H*-5), 5.03 (1H, d, J = 9.0 Hz, $OCH_\alpha N$), 4.99 (1H, d, J = 9.0 Hz, $OCH_\beta N$), 4.56–4.52 (1H, m, *H*-2′), 4.33–4.28 (1H, m, *H*-3′), 4.10–4.08 (1H, m, *H*-4′), 3.81 (6H, s, DMTr-OCH_3), 3.64–3.52 (2H, m, *H*-5′). MS: 602.20, calc. for $C_{31}H_{31}N_5O_8$ ($M+H^+$) 602.22.

N^4-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(azidomethyl)cytidine. Yield 77%, R_f (solvent B) = 0.55. ^{1}H-NMR (800 MHz, $CDCl_3$): δ 8.58 (1H, d, J = 7.3

Hz, Cyt-*H*-6), 7.94–7.50 (5H, m, Bz-*H*-Ar), 7.48–6.89 (14H, m, DMTr-*H*-Ar, Cyt-*H*-5), 5.99 (1H, s, *H*-1′), 5.20 (1H, d, $J = 8.7$ Hz, OCH_αN), 5.15 (1H, d, $J = 8.7$ Hz, OCH_βN), 4.58–4.54 (1H, m, *H*-3′), 4.33 (1H, d, $J = 5.6$ Hz, *H*-2′), 4.15 (1H, dt, $J = 8.8$ Hz, $J = 2.3$ Hz, *H*-4′), 3.85 (6H, d, $J = 1.6$ Hz, DMTr-OCH_3), 3.66 (1H, dd, $J = 10.8$ Hz, $J = 2.3$ Hz, H_α-5′), 3.61 (1H, dd, $J = 10.8$ Hz, $J = 2.3$ Hz, H_β-5′). MS: 727.30, calc. for $C_{38}H_{36}N_6O_8$ (M $+Na^+$) 727.25.

***N*^{6}-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(azidomethyl)adenosine.** Yield 63%, R_f (solvent B) = 0.55. ^{1}H-NMR (800 MHz, $CDCl_3$): δ 8.82 (1H, s, Ade-*H*-8), 8.09 (1H, s, Ade-*H*-2), 8.06–7.54 (5H, m, Bz-*H*-Ar), 7.35–6.84 (13H, m, DMTr-*H*-Ar), 6.05 (1H, d, $J = 7.6$ Hz, *H*-1′), 5.05–5.03 (1H, m, *H*-3′), 4.73 (1H, d, $J = 9.1$ Hz, OCH_αN), 4.69 (1H, d, $J = 9.1$ Hz, OCH_βN), 4.64–4.63 (1H m, *H*-2′), 4.41 (1H, m, *H*-4′), 4.03–4.00 (1H, m, H_α-5′), 3.82 (6H, s, DMTr-OCH_3), 3.81–3.80 (1H, m, H_β-5′). MS: 729.20, calc. for $C_{39}H_{36}N_8O_7$ (M $+H^+$) 729.28.

***N*^{2}-Isobutyryl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-(azidomethyl)guanosine.** Yield 57%, R_f (solvent B) = 0.40. ^{1}H-NMR (800 MHz, $CDCl_3$): δ 7.87 (1H, s, Gua-*H*-8), 7.38–6.84 (13H, m, DMTr-*H*-Ar), 5.99 (1H, d, $J = 5.9$ Hz, *H*-1′), 4.89 (1H, d, $J = 9.1$ Hz, OCH_αN), 4.85 (1H, d, $J = 9.1$ Hz, OCH_βN), 4.72–4.69 (1H, m, *H*-3′), 4.28–4.25 (1H, m, *H*-2′), 3.97–3.94 (1H, m, *H*-4′), 3.82 (6H, s, DMTr-OCH_3), 3.56–3.53 (1H, m, H_α-5′), 3.26–3.23 (1H, m, H_β-5′), 2.61 (1H, m, *i*Bu-C*H*), 1.10, 1.06 (6H, d, $J = 7.2$ Hz, *i*Bu-CH_3). MS: 711.30, calc. for $C_{36}H_{38}N_8O_8$ (M $+H^+$) 711.29.

Triethylammonium (4-Chlorophenyl)-(1-oxido-4-methoxy-2-picolyl) phosphate (7)

1-Oxido-4-methoxy-2-pyridinemethanol (3.1 g, 20 mmol) was dissolved in CH_2Cl_2 (100 ml), TEA (3.5 ml, 25 mmol) was added, and the solution was cooled to 0°C. Then, bis(2-chlorophenyl) chlorophosphate (5.28 ml, 22 mmol) was slowly added under stirring. After 20 minutes, CH_2Cl_2 (200 ml) was added, and the reaction mixture was washed with 0.5 M TEAB (2 × 100 ml). The organic fraction was evaporated, and the phosphotriester obtained was isolated by silica gel column chromatography in a gradient of methanol (1–2%) in chloroform. Fractions containing the desired product [R_f (solvent B) = 0.7] were evaporated. The residue was dissolved in 75 ml of acetonitrile, and 11 ml of water and 14 ml of TEA were added. The solution obtained was incubated at 50°C for 2 hours. Then, water (100 ml) was added, and the mixture was washed with CH_2Cl_2 (2 × 100 ml). The water solution was evaporated to dryness, and the residue was co-evaporated with pyridine. The gum obtained was dissolved in 50 ml of pyridine and precipitated with dry ether (500 ml). The precipitate of the compound **7** [R_f (solvent A) = 0.3] was collected by centrifugation. The yield was 8.85 g (81%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.05 (1H, d, $J = 7.2$ Hz, picolyl-*H*-6),

7.71–7.10 (4H, m, *H*-Ar), 7.25 (1H, d, $J = 3.4$ Hz, picolyl-*H*-3), 6.69 (1H, dd, $J = 7.2$ Hz, $J = 3.4$ Hz, picolyl-*H*-5), 5.27 (2H, d, $J = 7.4$ Hz, P-OCH_2), 3.73 (3H, s, picolyl-OCH_3), 3.05 (6H, dq, $J = 7.3$ Hz, $J = 3.5$ Hz, $^{+}$HNEt$_3$-CH$_2$), 1.31 (9H, t, $J = 7.3$ Hz, $^{+}$HNEt$_3$-CH$_3$). ^{31}P NMR (121.5 MHz, $CDCl_3$) $\delta = 4.92$. MS: 346.05, calc. for $C_{13}H_{13}ClNO_6P$ (M $+H^+$) 346.02.

General Method for the Synthesis of Ribonucleotide Monomers (9) Containing 2′-*O*-AZM Protecting Groups

To introduce a phosphate residue bearing the catalytic protective group, the compound **6** (1 mmol) was allowed to react with (4-chlorophenyl)-(1-oxido-4-methoxy-2-picolyl) phosphate **7** (2 mmol) in 10 ml of dry pyridine in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) (3 mmol) for 15 minutes. The reaction was terminated by the addition of 1 M TEAB (20 ml). The solution obtained was extracted with chloroform (2 × 20 ml), and the combined organic fractions were evaporated to dryness. To remove the traces of pyridine, the residue was evaporated with toluene. The gum obtained was treated with 10 ml of 0.25 M solution of DBU in CH_3CN-H_2O (9:1, v/v) for 16 hours at room temperature. After the following treatment with 0.5 M TEAB (10 ml), the reaction mixture was extracted with chloroform (2 × 15 ml). The combined organic fractions were washed with 0.5 M TEAB (20 ml), evaporated to dryness and co-evaporated with toluene. The product was isolated by silica gel column chromatography using 0–15% gradient of methanol in chloroform with 1% of TEA. The fractions containing the desired compound **9** were concentrated by evaporation to a gum, which was dissolved in chloroform (20 ml), washed with 20 ml of 0.5 M 1,8-Diazabicyclo[5.4.0]undec-7-enium hydrogen carbonate. After the concentration of chloroform solution under reduced pressure, foam obtained was additionally dried in vacuo.

1,8-Diazabicyclo[5.4.0]undec-7-enium 5′-O-(4,4′-dimethoxytrityl)-2′-O-(azidomethyl)uridine 3′-O-(1-oxido-4-methoxy-2-picolyl)phosphate. Yield 67.5%, R_f (solvent A) = 0.25. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.10 (1H, d, J = 7.2 Hz, picolyl-H-6), 7.74 (1H, d, J = 8.1 Hz, Ura-H-6), 7.37–6.77 (14H, m, DMTr-H-Ar, picolyl-H-3), 6.71 (1H, dd, J = 7.2 Hz, J = 3.4 Hz, picolyl-H-5), 6.12 (1H, d, J = 5.4 Hz, H-1′), 5.20–5.07 (2H, m, P-OCH$_2$), 5.16 (1H, d, J = 8.1 Hz, Ura-H-5), 5.05–4.99 (2H, m, OCH$_\alpha$N, H-3′), 4.77 (1H, d, J = 9.0 Hz, OCH$_\beta$N), 4.65–4.59 (1H, m, H-2′), 4.41–4.35 (1H, m, H-4′), 3.79 (3H, s, picolyl-OCH$_3$), 3.77 (6H, d, J = 1.8 Hz, DMTr-OCH$_3$), 3.57–3.36 (8H, m, H-5′, DBU-CH$_2$), 2.83–2.86 (2H, m, DBU-CH$_2$), 1.96 (2H, q, J = 5.6 Hz, DBU-CH$_2$), 1.71–1.64 (6H, m, DBU-CH$_2$). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ = 0.35. MS: 993.40, calc. for $C_{47}H_{55}N_8O_{13}P$ (M $+Na^+$) 993.35.

1,8-Diazabicyclo[5.4.0]undec-7-enium N^4-benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(azidomethyl)cytidine 3′-O-(1-oxido-4-methoxy-2-picolyl)phosphate. Yield 64%, R_f (solvent A) = 0.3. ^{1}H-NMR (800 MHz, $CDCl_3$): 8.06 (1H, d,

J = 7.0 Hz, picolyl-H-6), 7.93 (1H, m, Cyt-H-6), 7.61–7.41 (5H, m, Bz-H-Ar), 7.34–6.85 (15H, m, DMTr-H-Ar, Cyt-H-6, picolyl-H-3), 6.71 (1H, dd, J = 7.0 Hz, J = 3.4 Hz, picolyl-H-5), 6.15 (1H, d, J = 3.2 Hz, H-1′), 5.17 (1H, dd, J = 16.7 Hz, J = 7.3 Hz, P-OCH$_\alpha$), 5.11 (1H, d, J = 8.8 Hz, OCH$_\alpha$N), 5.06 (1H, dd, J = 16.7 Hz, J = 7.3 Hz, P-OCH$_\beta$), 5.01–4.98 (1H, m, H-3′), 4.98 (1H, d, J = 8.8 Hz, OCH$_\beta$N), 4.62–4.60 (1H, m, H-2′), 4.46–4.44 (1H, m, H-4′), 3.81 (6H, d, J = 2.3 Hz, DMTr-OCH$_3$), 3.79 (3H, s, picolyl-OCH$_3$), 3.51–3.35 (8H, m, H-5′, DBU-CH$_2$), 2.81 (2H, m, DBU-CH$_2$), 1.97 (2H, q, J = 5.6 Hz, DBU-CH$_2$), 1.71 (4H, m, DBU-CH$_2$), 1.66 (2H, m, DBU-CH$_2$). ^{31}P NMR (121.5 MHz, CDCl$_3$) $\delta = 0.08$. MS: 1074.40, calc. for $C_{54}H_{60}N_9O_{13}P$ (M +H$^+$) 1074.41.

1,8-Diazabicyclo[5.4.0]undec-7-enium N^6-benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(azidomethyl)adenosine 3′-O-(1-oxido-4-methoxy-2-picolyl)phosphate. Yield 62%, R$_f$ (solvent A) = 0.3. ^{1}H-NMR (800 MHz, CDCl$_3$): δ 8.72 (1H, s, Ade-H-8), 8.18 (1H, s, Ade-H-2), 8.08 (1H, d, J = 7.3 Hz, picolyl-H-6), 8.06–7.52 (5H, m, Bz-H-Ar), 7.43–6.77 (14H, m, DMTr-H-Ar, picolyl-H-3), 6.74 (1H, dd, J = 7.3 Hz, J = 3.4 Hz, picolyl-H-5), 6.34 (1H, d, J = 6.4 Hz, H-1′), 5.32–5.30 (1H, m, H-3′), 5.20 (1H, dd, J = 17.0 Hz, J = 7.0 Hz, P-OCH$_\alpha$), 5.16–5.12 (2H, m, P-OCH$_\beta$, H-2′), 5.01 (1H, d, J = 9.1 Hz, OCH$_\alpha$N), 4.80 (1H, d, J = 9.1 Hz, OCH$_\beta$N), 4.60–4.58 (1H, m, H-4′), 3.81 (3H, s, picolyl-OCH$_3$), 3.78 (6H, s, DMTr-OCH$_3$), 3.53 (1H, dd, J = 10.8 Hz, J = 2.6 Hz, H$_\alpha$-5′) 3.48 (1H, dd, J = 10.8 Hz, J = 2.6 Hz, H$_\beta$-5′), 3.44–3.36 (6H, m, DBU-CH$_2$), 2.83 (2H, m, DBU-CH$_2$), 1.97 (2H, q, J = 5.6 Hz, DBU-CH$_2$), 1.73 (4H, m, DBU-CH$_2$), 1.66 (2H, m, DBU-CH$_2$). ^{31}P NMR (121.5 MHz, CDCl$_3$) $\delta = 0.01$. MS: 1098.40, calc. for $C_{55}H_{60}N_{11}O_{12}P$ (M +H$^+$) 1098.42.

1,8-Diazabicyclo[5.4.0]undec-7-enium N^2-isobutyryl-5′-O-(4,4′-dimethoxytrityl)-2′-O-(azidomethyl)guanosine 3′-O-(1-oxido-4-methoxy-2-picolyl)phosphate. Yield 60%, R$_f$ (solvent A) = 0.2. ^{1}H-NMR (800 MHz, CDCl$_3$): δ 8.03 (1H, d, J = 7.0 Hz, picolyl-H-6), 7.76 (1H, s, Gua-H-8), 7.38–6.71 (14H, m, DMTr-H-Ar, picolyl-H-3), 6.73 (1H, dd, J = 7.0 Hz, J = 3.4 Hz, picolyl-H-5), 6.01 (1H, d, J = 5.6 Hz, H-1′), 5.42–5.39 (1H, m, H-3′), 5.22–5.16 (2H, m, P-OCH$_2$), 5.14–5.11 (1H, m, H-2′), 4.91 (1H, d, J = 9.1 Hz, OCH$_\alpha$N), 4.72 (1H, d, J = 9.1 Hz, OCH$_\beta$N), 4.48–4.45 (1H, m, H-4′), 3.79 (3H, s, picolyl-OCH$_3$), 3.77 (6H, d, J = 3.2 Hz, DMTr-OCH$_3$), 3.48–3.26 (8H, m, H-5′, DBU-CH$_2$), 2.78–2.62 (2H, m, DBU-CH$_2$), 2.51–2.44 (1H, m, iBu-CH), 1.95 (2H, q, J = 5.6 Hz, DBU-CH$_2$), 1.78–1.58 (6H, m, DBU-CH$_2$), 1.11, 1.03 (6H, d, J = 7.3 Hz, iBu-CH$_3$). ^{31}P NMR (121.5 MHz, CDCl$_3$) $\delta = 0.76$. MS: 1080.40, calc. for $C_{52}H_{62}N_{11}O_{13}P$ (M +H$^+$) 1080.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-(azidomethyl)benzoyl]uridine (12)

5′,3′-*O*-(1,1,3,3-tetraisopropyldisilox-1,3-diyl)uridine (0.98 g, 2 mmol) was dissolved in 10 ml of dichloromethane and allowed to react with

2-(azidomethyl)benzoyl chloride (0.59 g, 3 mmol) in the presence of 0.5 ml of 1-methylimidazole for 30 minutes. The reaction was terminated by the addition of 15 ml of 0.5 M TEAB. Then, the organic fraction was isolated and washed with 10% solution of citric acid (15 ml). Dichloromethane solution of 2′-*O*-2-(azidomethyl)benzoyl)-5′,3′-*O*-(1,1,3,3-tetraisopropyldisiloxy-1,3-diyl)uridine **10** was evaporated and co-evaporated with acetonitrile. The residue was dissolved in THF (20 ml) and treated with 1 M solution of TBAF (5 ml) in THF for 2 hours. After the evaporation, crude 2′-*O*-(2-azidomethyl)benzoyl uridine **11** [R_f (solvent B) = 0.65] was dissolved in chloroform and purified by silica gel column chromatography in a gradient of methanol (0–10%) in chloroform. The dimethoxytritylation of compound **11** was performed as described for the derivative **6**. The product **12** [R_f (solvent B) = 0.8] was obtained with the yield 0.93 g (66%). ^{1}H-NMR (800 MHz, $CDCl_3$): δ 8.01 (1H, d, J = 7.3 Hz, Ura-*H*-6), 7.94–7.45 (17H, m, DMTr-*H*-Ar, AZMB-*H*-Ar), 6.12 (1H, d, J = 5.4 Hz, *H*-1′), 5.56–5.54 (1H, m, *H*-2′), 5.35 (1H, d, J = 7.3 Hz, Ura-*H*-5), 4.91(1H, d, J = 15.0 Hz, AZMB-CH_αN), 4.79 (1H,d, J = 15.0 Hz, AZMB-CH_βN), 4.37–4.35 (1H,m, *H*-3′), 4.12–4.09 (1H, m, *H*-4′), 3.81 (6H,s, DMTr-O-CH_3), 3.66–3.57 (2H, m, *H*-5′). MS: 728.20, calc. for $C_{38}H_{35}N_5O_9$ ($M + Na^+$) 728.23.

Triethylammonium Methyl-(1-oxido-4-methoxy-2-picolyl)-phosphate (13)

1-Oxido-4-methoxy-2-pyridinemethanol (1.55 g, 10 mmol) was dried by co-evaporation with pyridine. Then, CH_2Cl_2 (25 ml) and TEA (3.5 ml, 25 mmol) were added, and the suspension obtained was cooled to 0°C. Dimethylchlorophosphate (11 ml of 1 M solution in CH_2Cl_2) was slowly added to the suspension under the stirring. After 30 minutes, the reaction was terminated by the addition of 1 M solution of TEAB (30 ml). The crude product was extracted with 1-butanol-chloroform (3:7, v/v, 2 × 30 ml). The organic fractions were combined and evaporated to a gum. It was dissolved in acetonitrile (20 ml) and treated with piperidine (4 ml) for 16 hours at room temperature to remove a methyl group. After evaporation, the crude compound 13 was dissolved in 20 ml of chloroform, and TEA (5 ml) was added. After 10 minutes, the solution was evaporated to dryness. The residue was dissolved in pyridine (10 ml) and the product 13 [R_f (solvent A) = 0.22] was precipitated with dry ether (100 ml). The precipitate was collected by centrifugation and dried in vacuo. The yield was 1.97 g (56%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.12 (1H, d, J = 7.2 Hz, picolyl-*H*-6), 7.37 (1H, d, J = 3.4 Hz, picolyl-*H*-3), 6.81 (1H, dd, J = 7.2 Hz, J = 3.4 Hz, picolyl-*H*-5), 5.28 (2H, d, J = 7.4 Hz, P-OCH_2), 3.81 (3H, s, picolyl-OCH_3), 3.65 (3H, s, P-OCH_3), 3.05 (6H, q, J = 7.3 Hz, $^+$HNEt$_3$-CH_2), 1.31 (9H, t, J = 7.3 Hz, $^+$HNEt$_3$-CH_3). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ = 0.1. MS: 252.10, calc. for $C_8H_{14}NO_6P$ ($M + H^+$) 252.06.

1,8-Diazabicyclo[5.4.0]undec-7-enium 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[2-(azidomethyl)benzoyl]uridine 3′-*O*-(1-oxido-4-methoxy-2-picolyl)phosphate (15)

The synthesis and isolation of the compound **14** was accomplished starting from the derivative **12** (0.71 g, 1 mmol) and methyl-(1-oxido-4-methoxy-2-picolyl) phosphate **13** (0.7 g, 2 mmol) according the method described above for **8**. The removal of methyl group from the phosphate residue of the nucleoside phosphotriester **14** was performed by the action of piperidine (4 ml) in acetonitrile (10 ml) during 3 hours at room temperature. The yield of compound **15** was 0.59 g (55%), R_f (solvent A) = 0.2. ^{1}H-NMR (800 MHz, $CDCl_3$): δ 8.03 (1H, d, J = 7.2, picolyl-*H*-6), 7.76 (1H, m, Ura-*H*-6), 7.56–6.78 (18H, m, DMTr-*H*-Ar, AZMB-*H*-Ar, picolyl-*H*-3), 6.71 (1H, dd, J = 7.2 and 3.4 Hz, picolyl-*H*-5), 6.27 (1H, d, J = 4.3 Hz, *H*-1′), 5.68–5.64 (1H, m, *H*-2′), 5.37 (1H, d, J = 8.1 Hz, Ura-*H*-5), 5.25–5.20 (1H,m, *H*-3′), 5.11–5.03 (2H, m, P-CH_2), 4.87 (1H, d, J = 15.0 Hz, AZMB-CH_α-N), 4.74 (1H,d, J = 15.0 Hz, AZMB-CH_βN), 4.44–4.39 (1H, m, *H*-4′), 3.77 (6H,d, J = 7.0 Hz, DMTr-O-CH_3), 3.72 (3H, s, picolyl-OCH_3), 3.57–3.37 (8H, m, *H*-5′, DBU-CH_2), 2.83–2.86 (2H, m, DBU-CH_2), 1.96 (2H, q, J = 5.6 Hz, DBU-CH_2), 1.71–1.65 (6H, m, DBU-CH_2). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ = 0.309. MS: 1075.35, calc. for $C_{54}H_{59}N_8O_{14}P$ (M $+H^+$) 1075.40.

Synthesis and Deprotection of Oligonucleotides

The synthesis of oligodeoxyribonucleotides by the phosphoramidite method was performed using commercially available monomers and standard protocols. Synthesis of oligoribonucleotides was carried out by the automated phosphoramidite method using 1-phenyl-1*H*-tetrazole-5-thiol as the activation agent as described.[22] Chain elongation in the solid phase synthesis of oligoribonucleotides by the phosphotriester method was carried out at 1 μmol scale according to conditions given in Table 1. Solid supports derivatized with nucleosides (**6**) and (**12**) were prepared from LCAA CPG using the procedure described earlier.[23] The deprotection of oligonucleotides included the removal of catalytic P-protective groups by the action of 1 M solution of LiI in acetonitrile (1 ml) at room temperature for 3 hours with the following washing the support-bound oligonucleotide with acetonitrile. The removal of N-protecting groups from heterocycles and cleavage from the support were achieved by the treatment with concentrated aqueous ammonia for 48–62 hours at room temperature. Then, oligonucleotides were desalted by gel filtration using Pharmacia NAP-10 columns, and the fractions containing oligonucleotides were lyophilized. The powder of 2′-O-protected oligonucleotide obtained was treated with 0.5 ml of 0.1 M solution of triphenylphosphine in acetonitrile-water (9:1, v/v)

containing 25 mM Tris-HCl (pH 7.0) for 2 hours, or with 0.1 M solution of methyldiphenylphosphine in dioxane-water (4:1, v/v) containing 25 mM Tris-HCl (pH 7.0) for 20–30 minutes at room temperature. The mixture was diluted with 0.5 ml of 0.1 M TEAB (pH 7) and washed with 1 ml of the ether. The water fraction was purified by anion-exchange HPLC (high performance liquid chromatography) and/or gel-electrophoresis as described.[9,22] The identity of the oligomers obtained was confirmed by MALDI-TOF MS.

Thermal Denaturation and Enzyme Degradation Experiments

Absorbance (260 nm) versus temperature curves of duplexes formed by RNA and DNA oligomers were measured using a Pharmacia Ultrospec 2000 (Pharmacia, Sweden) UV-VIS spectrophotometer equipped with a heated sample holder and a Peltier temperature control accessory. Solutions contained 3–5 μM of each oligomer in 150 mMNaCl/10 mM Tris–HCl (pH 7–8)/5 mM EDTA/10 mM $MgCl_2$. Data were collected with the help of the corresponding software from 20 to 95°C using the heating rate 0.5°C/min with an equilibration time 0.2 minute at each temperature.

In assays for enzymatic stability, each RNA sample (5–10 pmol) was 5′-end labeled with ^{32}P using T4 polynucleotide kinase and [γ-^{32}P]ATP by standard procedure and mixed with the corresponding unlabeled RNA (1 OD unit at 260 nm). The digestions of synthetic oligonucleotides with phosphodiesterase I (0.5 U) were performed in 20 mM Tris-HCl (pH 8.0)/50 mM NaCl/10 mM $MgCl_2$ with a total reaction volume of 100 μl. Assays for bovine spleen phosphodiesterase stability were performed in 30 mM NaOAc (pH 6.0) (pH 7.5)/10mM supplemented with the enzyme (0.4 U). A reaction mixture was incubated at 37°C. At appropriate periods, 5 μl aliquots of the reaction mixture were taken, and the enzyme was deactivated by heating at 100°C for 2 minutes. The mixtures were then analyzed by 20% denaturing PAGE, and radioactive densities of the gel were visualized by a bio-imaging analyzer.

RNase H digestion of RNAs was carried out according to the following procedure: oligonucleotide (0.1 μM, 25 μl), which was preliminary 5′-labeled using T4 polynucleotide kinase and [γ-^{32}P]ATP by the standard procedure, and the complementary DNA (0.5 μM, 25 μl) in 20 mM Tris-HCl(pH 8)/0.1 M KCl/0.1M EDTA/10 mM $MgCl_2$ were annealed by heating at 90°C for 5 minutes with the following slow cooling to 20°C. After the addition of DTT to 0.1 mM, the solution was incubated with 1 U RNase H (Gibco BRL) at 37°C. Aliquots (5 μl) were taken after 2, 5, 10, 30, and 60 minutes, and the reactions were terminated by mixing with 10 μl of 0.05 M EDTA, 0.05% (w/v) xylene cyanole FF and 0.05% (w/v) bromophenol blue in 80% formamide. The digestion products were analyzed by a 15% denaturing PAGE.

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