Oligodeoxynucleotides Containing 2′-Deoxy-1-methyladenosine and *Dimroth* Rearrangement

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2′-Deoxy-1-methyladenosine was incorporated into synthetic oligonucleotides by phosphoramidite chemistry. Chloroacetyl protecting group and controlled anhydrous deprotection conditions were used to avoid *Dimroth* rearrangement. Hybridization studies of intramolecular duplexes showed that introduction of a modified residue into the loop region of the oligonucleotide hairpin increases the melting temperature. It was shown that modified oligonucleotides may be easily transformed into oligonucleotides containing 2′-deoxy-N^6-methyladenosine.

1. Introduction. – Naturally occurring 1-methyladenosine (m^1A) attracted much attention due to its role in building of tRNA secondary structure [1–5]. This modified nucleoside has shown to be responsible for cloverleaf conformation of tRNA [3]. Post-transcriptional methylation of adenosine in tRNA with methyl-1-adenosine transferase dramatically changes charge and hydrophobic properties of this heterocyclic base. The consequences of such a modification influence both stacking and pairing properties of m^1A. Inability of the base to form a *Watson–Crick* (*WC*) duplex favors the loop formation which may be additionally stabilized by the positive charge of m^1A. Indeed, some stabilization effect was observed in synthetic oligonucleotide hairpins due to the presence of m^1A in the loop [6].

Recently, the synthesis of protected phosphoramidite of m^1A and hybridization properties of short modified synthetic oligoribonucleotides have been reported [6][7]. The selection of appropriate N^6-protecting group and mild ammonolysis conditions allowed us to avoid complications associated with incomplete deprotection and *Dimroth* rearrangement.

Oligodeoxynucleotides containing 2′-deoxy-1-methyladenosine (m^1dA) possess a great potential in studies of DNA alkylation and repair mechanism [8–10] and in construction of deoxyribozymes [11][12]. Substitution of adenosine (p*K* 3.6–3.8) with 1-methyladenosine (p*K* 8.2–8.8) [13][14] may have considerable effect on the catalytic properties of deoxyribozymes. Particular interest may arise with respect to non-canonical DNA studies due to the feature of m^1A to form reverse-*Hoogsteen* base pairs [15].

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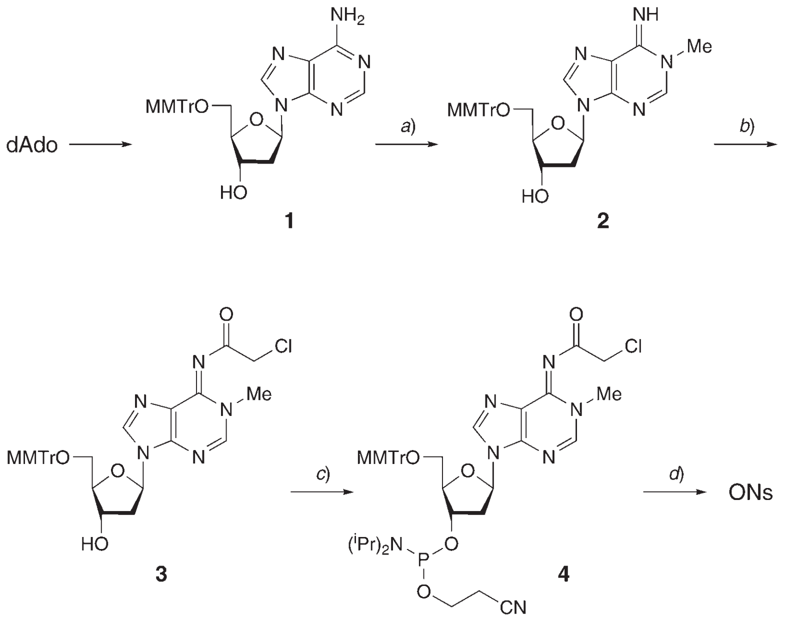
Here, we report the preparation of a m^{1}dA synthon suitable for standard oligonucleotide synthesis, its incorporation into oligodeoxynucleotides, and their hybridization behavior in short DNA hairpins. *Dimroth* rearrangement in concentrated aqueous ammonia solutions have been also studied in detail.

2. Results and Discussion. – 2.1. *Synthesis of m^{1}dA Phosphoramidite and Modified Oligonucleotides.* The synthetic route to the protected m^{1}dA cyanoethyl phosphoramidite (**4**) is shown in the *Scheme*. We followed the synthetic strategy that was successfully implemented for the preparation of m^{1}A phosphoramidite [6]. However, some modifications were introduced in the present strategy. Prior the methylation of 2′-deoxyadenosine, we introduced 5′-*O*-MMTr (MMTr = monomethoxytrityl) protecting group. This derivative, **1**, was further reacted with MeI in *N*,*N*-dimethylacetamide according to the procedure described for adenosine [16] to give **2** in 72% yield. If the methylation was performed first, as described for m^{1}A [6], introduction of MMTr took up to 7 d, and the yield of **2** was not more than 50% due to the low solubility of m^{1}dA in pyridine.

An increase of the acid stability of the 5′-*O*-protecting group (MMTr *vs.* DMTr = dimethoxytrityl) is preferred in view of the formation of hydroiodide salt of **2** during alkylation with MeI. We have found that such a replacement did not affect the efficiency of the deblocking step in oligonucleotide synthesis.

Scheme



a) MeI. *b*) $(ClCH_2CO)_2O$/Py, NH_3/MeOH. *c*) $(^iPr_2N)_2P(OCH_2CH_2CN)$, 1*H*-tetrazole, Py. *d*) Automated oligonucleotide synthesis.

Reaction of MMTr derivative **2** with chloroacetic anhydride provided protected **3** in 78% yield. Preparation of phosphoramidite **4** was performed with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite. Unlike the adenosine derivative bearing a bulky 2′-*O*-^{t}BuMe$_2$Si protecting group and requiring the use of 2-cyanoethyl chloro-*N*,*N*-diisopropylphosphoramidite, 2′-deoxynucleoside **3** easily reacted with bis-amidite in the presence of pyridinium tetrazolide. We found that purification of m^{1}dA phosphoramidite **4** may be carried out with high efficiency in AcOEt/hexane or hexane/toluene mixtures, allowing easy separation of the product.

Incorporation of modified deoxyadenosines during automated oligonucleotide synthesis was carried out without modifications of the standard protocol. The m^{1}dA amidite **4** coupled very well using the standard DNA coupling time (30 s) and with 0.45M 1*H*-tetrazole as activator. Efficiency of coupling for **4** was found to be at least 99% as estimated by comparing DMTr cation absorbance at 495 nm for $n-1$ and $n+1$ base. Complete removal of the MMTr protecting group was achieved when using deblock parameters for thymidine. Reverse-phase (RP) HPLC of crude oligodeoxynucleotides was also used to determine the percentage of full-length product and deduce overall coupling efficiency specific monomers. This technique gave consistent results.

It was demonstrated for m^{1}A-modified oligoribonucleotides [6][7] that cleavage from solid support and further deprotection with 2M NH$_3$ in MeOH for 60 h at room temperature does not induce *Dimroth* rearrangement. According to this procedure, we synthesized six oligodeoxynucleotide hairpins, ON1–ON7, modified with m^{1}dA (*Table*). In a separate study, we found that the use of labile protecting groups for dA (PAC = phenylacetyl), dC (Ac), and dG (^{i}Pr-PAC) may reduce deprotection time in 2M NH$_3$ in MeOH or in 0.05M K$_2$CO$_3$ in MeOH to 24 h at room temperature.

Table. *Thermal Stability of DNA Hairpin Oligodeoxynucleotides and Mass-Spectrometric Results*

No.	Sequence[a])	M_{calc}	M_{exper}	T_m [°]
ON1	5′-GGACGTAATAGTCC	4287.9	4286.8	53.3
ON2	5′-GGACGTAAT**A**GTCC	4301.9	4302.2	53.8
ON3	5′-GGACGTA**A**TAGTCC	4301.9	4304.7	55.6
ON4	5′-GGACGT**A**ATAGTCC	4301.9	4301.4	56.9
ON5	5′-GG**A**CGTAATAGTCC	4301.9	4303.2	n/d
ON6	5′-GGACGT**A**AATAGTCC	4615.1	4613.8	48.5
ON7	5′-GGACGT**A**AA**A**TAGTCC	4942.3	4942.2	45.7
ON8	5′-GG$\underline{\mathbf{A}}$CGTAATAGTCC	4301.9	4304.2	46.6
ON9	5′-GGACGT$\underline{\mathbf{A}}$ATAGTCC	4301.9	4304.5	56.7

[a]) m^{1}dA Residues are shown in bold, m^{6}dA residues are underlined.

MALDI Mass spectra of synthesized oligonucleotides confirmed incorporation of modified residues. A representative spectrum of the oligodeoxynucleotide ON6 is shown in *Fig. 1*.

Convincing proofs of the presence of m^{1}dA and the absence of a rearrangement product were obtained from the HPLC analysis of nucleoside mixtures resulting from venom phosphodiesterase and phosphatase digest of modified oligodeoxynucleotides.

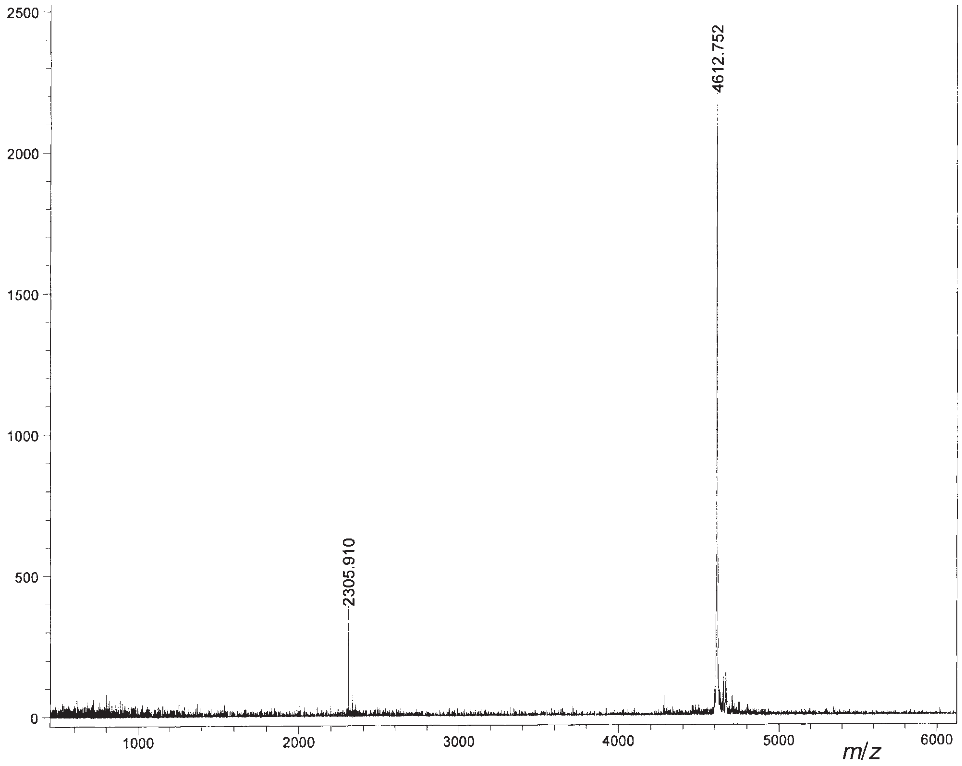


Fig. 1. *MALDI-MS Analysis* (in negative-ion mode) *of modified oligodeoxynucleotide ON6*

We observed formation of m^{1}dA along with four natural 2′-deoxynucleosides (*Fig. 2*). Due to the polar nature of this nucleoside, its retention time in RP-HPLC was shorter than for natural deoxynucleosides. In contrast, the product of *Dimroth* rearrangement, 2′-deoxy-*N*^{6}-methyladenosine (m^{6}dA), is more hydrophobic as compared with dA, dC, dG, and T, and has a considerably increased retention time. It should be mentioned, however, that minor amount of m^{6}dA was formed when using prolonged incubation time (2 h) at pH 8.5 during phosphatase treatment. This product did not appear when a shorter incubation time (30 min) was used.

The later circumstance clearly indicates the possibility of *Dimroth* rearrangement at higher pH in aqueous solutions [13]. For example, in concentrated aqueous NH_3, the half-time of *Dimroth* rearrangement of m^{1}A to m^{6}A was 36 h at room temperature [7]. Under these conditions, nearly quantitative conversion of m^{1}dA into m^{6}dA was observed after 7 d at room temperature (*cf. Exper. Part*). In spite of these results, in two recent studies, for the deprotection of oligodeoxynucleotides containing m^{1}dA, concentrated aqueous NH_3 at elevated temperatures was used [9][10]. It was reported that deprotection of m^{1}dA-modified oligodeoxynucleotides with aqueous NH_3 at 37° for 4 h resulted in 8% *Dimroth* rearrangement [10]. We verified the compatibility of the standard deprotection protocol (conc. aq. NH_3, 55°) with the synthesis of m^{1}dA-

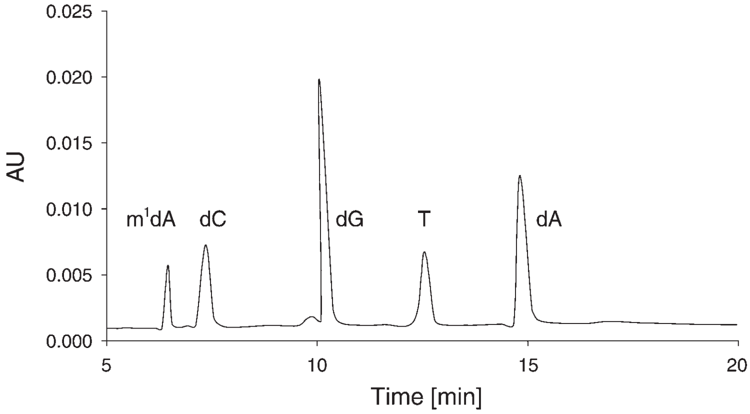


Fig. 2. *Enzymatic digest of m^1dA-modified oligonucleotide ON4.* Retention times for nucleosides: 6.3 (m^1dA), 7.4 (dC), 10.2 (dG), 12.4 (T), and 14.8 min (dA). Analysis was performed on a *Hypersil ODS* column (5 μm), 4.6 × 250 mm using 0.05M triethylammonium acetate (TEAA; pH 7.0) and linear gradient of MeCN (0–25%) for 30 min. Flow rate was 1 ml/min.

modified oligodeoxynucleotides. Treatment of m^1dA-modified oligonucleotides with concentrated NH_3 at 55° for 10 h resulted in *ca.* 80% conversion to oligodeoxynucleotide containing 2′-deoxy-N^6-methyladenosine. When the treatment time was increased to 16 h, virtually complete *Dimroth* rearrangement was observed (*Fig. 3*). Enzymatic hydrolysis of the new oligonucleotide product gave a mixture of natural nucleosides and m^6dA. There was no m^1dA found in the digest (*Fig. 4*). As can be seen from *Fig. 3*, *Dimroth* rearrangement proceeded cleanly without any visible degradation. Thus, synthon **4** may be used successfully for the preparation of oligodeoxynucleotides containing either m^1dA or m^6dA, depending on deprotection conditions.

Modified m^1dA residues are expected to affect total charge of oligonucleotides. We expected to find differences in mobility between all-natural and modified hairpins in non-denaturing gel electrophoresis. Examples of reduced migration rate in polyacrylamide for amine-modified oligonucleotides have been reported [17]. Given this consideration, we failed to observe the effect of m^1dA addition. Instead, only minor irregular variations of mobility were observed in denaturing gel electrophoresis at pH 8.5 or 7.0 (data not shown).

2.2. *Hybridization Properties of Modified Hairpins.* We have studied thermal denaturation of short oligodeoxynucleotide hairpins modified with m^1dA (*Table*). Due to the inability of m^1dA to participate in *Watson–Crick* base pairing, its incorporation into oligodeoxynucleotide forces the formation of unpaired, single-stranded fragments. When introduced into the loop of a hairpin, m^1dA is expected to provide some stabilization effect as was observed for the m^1A analog [7]. To compare the effect of modification with previously reported data, we used the same set of oligonucleotides used in that study. Two hairpins with enlarged loop were added to this set to follow the influence of loop size and multiple m^1dA residues on the hairpin stability.

As shown in the *Table*, the T_m values of modified hairpins with a single m^1dA in the loop are higher than that for the unmodified oligonucleotide. The stabilization effect

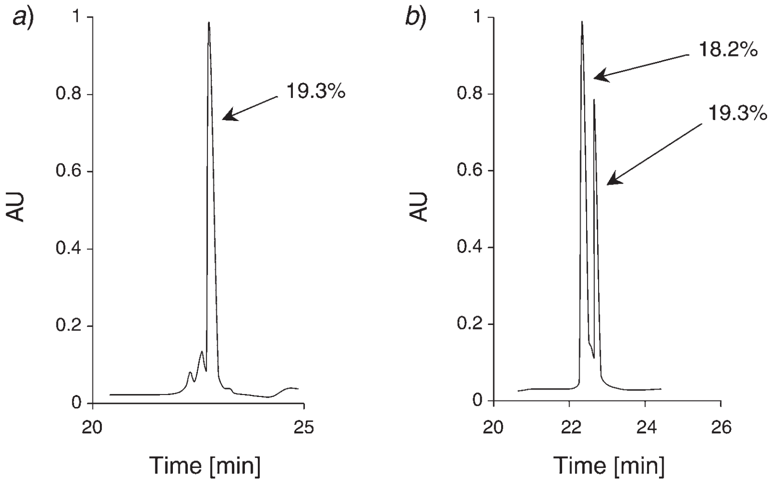


Fig. 3. a) *Oligonucleotide ON9* (m^6dA) *derived from oligonucleotide ON4* (m^1dA) *by ammonia treatment for 16 h at 55°.* b) *Coinjection of oligonucleotides ON4 and ON9.* Percentages of MeCN in the mobile phase are shown for each peak. Analysis was performed on a *Hypersil ODS* column (5 µm; 4.6 × 250 mm) using 0.05M TEAA (pH 7.0) and linear gradient of MeCN (0–25%) for 30 min. Flow rate was 1 ml/min.

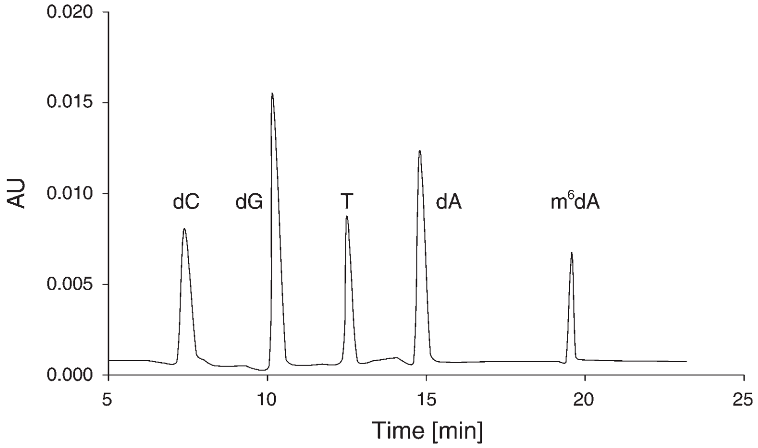


Fig. 4. *Enzymatic digest of* m^6dA *modified oligonucleotide ON9.* Retention times for nucleosides: 7.4 (dC), 10.2 (dG), 12.4 (T), 14.8 (dA), and 19.6 min (m^6dA). Analysis was performed on a *Hypersil ODS* column (5 µm; 4.6 × 250 mm) using 0.05M TEAA (pH 7.0) and linear gradient of MeCN (0–25%) for 30 min. Flow rate was 1 ml/min.

observed is very similar to that found for the m^1A analog [6] and shows the same order of stabilities for selected positions of modification. When placed in the duplex region, m^1dA prevented base pairing, and the T_m value for this hairpin could not be determined as expected (ON5 in the *Table*).

Increasing the loop size and introduction of an extra m^{1}dA residue into the loop had a negative effect on the duplex stability. A hairpin with a one-base (dA) larger loop (ON6) decreased the T_m value by 4.5°, while introduction of two bases (dA and m^{1}dA) induced destabilization by 7.6° (ON7). Thus, the negative effect associated with larger loop size considerably exceeded stabilization induced by introduction of m^{1}dA.

Transformation of 1-methyl oligodeoxynucleotides to N^6-methyl oligodeoxynucleotides *via Dimroth* rearrangement induced different changes in hybridization behavior of hairpins, depending on the location of modified base. Hairpin ON8, derived from oligonucleotide ON5, contained a m^{6}dA residue in the stem region and was able to form an intramolecular duplex with a T_m value of 46.6°. A similar destabilization effect was observed for DNA duplexes containing m^{6}dA [18]. When present in the loop (ON9, originally ON4), the m^{6}dA residue had a minor effect on hairpin stability (T_m 56.7° *vs.* 56.9° in ON4; *Table*).

3. Conclusions. – We have developed an efficient method to introduce the m^{1}dA residue into synthetic oligodeoxynucleotides *via* the corresponding protected phosphoramidite **4**. Anhydrous deprotection conditions should be used for m^{1}dA-modified oligonucleotides to avoid *Dimroth* rearrangement. The synthon **4** may be used successfully for the preparation of oligodeoxynucleotides containing m^{1}dA or m^{6}dA.

Experimental Part

General. Column chromatography (CC): silica gel (0.06–0.20 mm). TLC: *Kieselgel 260 F* (*Merck*); eluents: CH_2Cl_2/MeOH 9:1 (*A*); CH_2Cl_2/MeOH 1:1 (*B*); CH_2Cl_2/MeOH/hexane 9:1:10 (*C*); AcOEt/hexane 2:1 (*D*); detection by UV light. UV measurements and thermal denaturation studies were conducted with a *Shimazu UV-160A* spectrophotometer. NMR Spectra: *Bruker AMX-400* spectrometer; at 300 K; chemical shifts δ in ppm were recorded relative to the solvent signals (^{1}H and ^{13}C) and relative external ref. = H_4PO_3 (capil.) (^{31}P); coupling constants *J* in Hz. The signals were assigned using double-resonance techniques. MALDI-MS of oligonucleotides were acquired on a *Bruker Reflex IV* mass spectrometer in negative-ion mode with hydroxypicolinic acid as a matrix.

Hydroiodide Salt of 2′-Deoxy-1-methyladenosine (m^{1}dA) was obtained as monohydrate [16]. ^{1}H-NMR (D_2O): 8.47 (*s*, H–C(2,8)); 6.49 (*t*, *J*(1′,2′a) = *J*(1′,2′b) = 6.7, H–C(1′)); 4.64 (*ddd*, *J*(3′,2′a) = 6.3, *J*(3′,2′b) = 4.2, *J*(3′,4′) = 3.5, H–C(3′)); 4.13 (*ddd*, *J*(4′,5′b) = 5.0, *J*(4′,5′a) = 3.7, *J*(4′,3′) = 3.5, H–C(4′)); 3.89 (*s*, MeN); 3.88 (*dd*, *J*(5′a,5′b) = –12.5, *J*(5′a,4′) = 3.7, H–C(5′a)); 3.75 (*dd*, *J*(5′b,5′a) = –12.5, *J*(5′b,4′) = 5.0, H–C(5′b)); 2.85 (*ddd*, *J*(2′a,2′b) = –14.0, *J*(2′a,1′) = 6.7, *J*(2′a,3′) = 6.3, H–C(2′a)); 2.60 (*ddd*, *J*(2′b,2′a) = –14.0, *J*(2′b,1′) = 6.7, *J*(2′b,3′) = 4.2, H–C(2′b)). ^{13}C-NMR (D_2O): 151.77 (C(6)); 148.22 (C(2)); 147.17 (C(4)); 143.54 (C(8)); 123.02 (C(5)); 88.17 (C(1′)); 85.46 (C(4′)); 71.45 (C(3′)); 62.02 (C(5′)); 39.77 (C(2′)); 38.21 (MeN).

*2′-Deoxy-*N^6*-methyladenosine* (m^{6}dA). A soln. of 411 mg (1 mmol) of HI salt of m^{1}dA in 10 ml of conc. aq. NH_3 was kept at 20°. According to TLC in system *B*, the reaction was complete after 7 d. The starting compound disappeared for 10 or 16 h at 55°. The product in system *B* moved faster (R_f 0.65) than the starting compound (R_f 0.06). The solvent was evaporated, and the residue was purified on a column with 100 ml of *Dowex-1* (OH^- form). The column was washed consequently with 200 ml of H_2O and 200 ml of 10% aq. EtOH, and eluted with 20% of aq. EtOH. Product-containing fractions were combined, evaporated to dryness, and the residue was lyophilized from H_2O to give the product as a powder. Yield: 180 mg (68%). UV Spectra at different pH values were identical with those published in [16]. ^{1}H-NMR (D_2O): 8.22 (*s*, H–C(8)); 8.10 (*s*, H–C(2)); 6.42 (*t*, *J*(1′,2′a) = *J*(1′,2′b) = 6.7, H–C(1′)); 4.72 (*ddd*, *J*(3′,2′a) = 6.7, *J*(3′,2′b) = 3.4, *J*(3′,4′) = 2.0, H–C(3′)); 4.27 (*ddd*, *J*(4′,5′b) = 4.4, *J*(4′,5′a) = 3.1, *J*(4′,3′) = 2.0, H–C(4′)); 3.95 (*dd*, *J*(5′a,5′b) = –12.6, *J*(5′a,4′) = 3.1, H–C(5′a)); 3.88 (*dd*, *J*(5′b,5′a) =

− 12.6, *J*(5′b,4′) = 4.4, H−C(5′b)); 3.08 (*s*, MeN); 2.85 (*ddd*, *J*(2′a,2′b) = − 14.0, *J*(2′a,3′) = 6.7, *J*(2′a,1′) = 6.7, H−C(2′a)); 2.64 (*ddd*, *J*(2′b,2′a) = − 14.0, *J*(2′b,1′) = 6.7, *J*(2′b,3′) = 3.4, H−C(2′b)). ^{13}C-NMR (D_2O): 155.28 (C(6)); 152.77 (C(2)); 147.73 (C(4)); 139.82 (C(8)); 119.53 (C(5)); 88.00 (C(1′)); 85.12 (C(4′)); 71.84 (C(3′)); 62.32 (C(5′)); 39.80 (C(2′)); 27.89 (MeN).

2′-Deoxy-5-O-*[(4-methoxyphenyl)(diphenyl)methyl]adenosine* (**1**). To 2′-deoxyadenosine (2.5 g, 10 mmol), dried two times by evaporation with anh. pyridine (2 × 20 ml) and suspended in dry pyridine (20 ml), monomethoxytrityl (MMTr) chloride (3.7 g, 12 mmol) was added, and the mixture was stirred for 16 h at 20°. Then, CH_2Cl_2 (100 ml) and H_2O (50 ml) were added, and the org. layer was washed consequently with 10% aq. $NaHCO_3$ (30 ml) and H_2O (20 ml). The org. layer was dried (Na_2SO_4), evaporated *in vacuo* and co-evaporated with toluene (3 × 30 ml). The residue was purified by CC on silica gel (50 g). The column was washed with CH_2Cl_2 (200 ml) and CH_2Cl_2/MeOH 97 : 3 (200 ml), and then eluted with CH_2Cl_2/MeOH 95 : 5. The corresponding fractions were evaporated to dryness *in vacuo* to give **6** (3.5 g, 67%). Foam. R_f 0.35 (*A*). ^{1}H-NMR (400 MHz, $CDCl_3$): 8.25 (*s*, H−C(8)); 7.94 (*s*, H−C(2)); 7.40 – 7.19 (*m*, 12 arom. H); 6.79 (*d*, *J* = 8.8, 2 H, *Ph*OMe); 6.43 (*t*, *J*(1′,2′a) = *J*(1′,2′b) = 6.3, H−C(1′)); 6.02 (br. *s*, NH_2); 4.66 (*ddd*, *J*(3′,2′a) = 6.5, *J*(3′,2′b) = 4.0, *J*(3′,4′) = 2.9, H−C(3′)); 4.17 (*ddd*, *J*(4′,5′b) = 5.0, *J*(4′,5′a) = 4.7, *J*(4′,3′) = 2.9, H−C(4′)); 3.76 (*s*, MeO); 3.41 (*dd*, *J*(5′a,5′b) = − 10.3, *J*(5′a,4′) = 4.7, H−C(5′a)); 3.38 (*dd*, *J*(5′b,5′a) = − 10.3, *J*(5′b,4′) = 5.0, H−C(5′b)); 2.78 (*ddd*, *J*(2′a,2′b) = − 13.4, *J*(2′a,3′) = 6.5, *J*(2′a,1′) = 6.3, H−C(2′a)); 2.52 (*ddd*, *J*(2′b,2′a) = − 13.4, *J*(2′b,1′) = 6.3, *J*(2′b,3′) = 4.0, H−C(2′b)). ^{13}C-NMR ($CDCl_3$): 158.85 (Ph); 155.63 (C(2)); 153.03 (C(6)); 149.62 (C(4)); 144.20 (Ph); 139.00 (C(8)); 135.33, 130.45, 128.50, 128.02, 127.22 (Ph); 120.08 (C(5)); 113.37 (Ph); 87.04 (C−O); 86.25 (C(1′)); 84.51 (C(4′)); 72.47 (C(3′)); 63.98 (C(5′)); 55.35 (OMe); 40.51 (C(2′)).

2′-Deoxy-5-O-*[(4-methoxyphenyl)(diphenyl)methyl]-1-methyladenosine* (**2**). A mixture of 2.62 g (5 mmol) of **1** and 1.25 ml (20 mmol) of MeI in *N*,*N*-dimethylacetamide (8 ml) was stirred in the dark for 16 h at 20°. A slightly yellow soln. was diluted with 100 ml of CH_2Cl_2 and was washed consequently with H_2O (20 ml), 10% aq. $NaHCO_3$ (20 ml), 5% aq. $Na_2S_2O_3$ (30 ml), and H_2O (2 × 10 ml). The org. layer was dried (Na_2SO_4), evaporated *in vacuo* (bath temp. < 30°) to a volume of *ca.* 5 ml, and powdered into hexane/Et_2O 2 : 1 (200 ml), and the mixture was allowed to stand at 0° overnight. The hygroscopic precipitate was filtered, washed with the same mixture (10 ml), and dried in the vacuum to give **2** (1.94 g, 72%). Slightly yellow powder. R_f 0.20 (*B*). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.70 (*s*, H−C(8)); 7.56 (*s*, H−C(2)); 7.41 – 7.21 (*m*, 12 arom. H); 6.81 (*d*, *J* = 8.7, 2 H, *Ph*OMe); 6.26 (*t*, *J*(1′,2′a) = *J*(1′,2′b) = 6.5, H−C(1′)); 4.61 (*ddd*, *J*(3′,2′a) = 6.5, *J*(3′,2′b) = 4.3, *J*(3′,4′) = 2.8, H−C(3′)); 4.11 (*ddd*, *J*(4′,5′b) = 5.3, *J*(4′,5′a) = 5.0, *J*(3′,4′) = 2.8, H−C(4′)); 3.78 (*s*, MeO); 3.54 (*s*, MeN); 3.42 (*dd*, *J*(5′a,5′b) = − 10.0, *J*(5′a,4′) = 5.0, H−C(5′a)); 3.32 (*dd*, *J*(5′b,5′a) = − 10.0, *J*(5′b,4′) = 5.3, H−C(5′b)); 2.72 (*ddd*, *J*(2′a,2′b) = − 13.4, *J*(2′a,3′) = 6.5, *J*(2′a,1′) = 6.5, H−C(2′a)); 2.46 (*ddd*, *J*(2′b,2′a) = − 13.4, *J*(2′b,1′) = 6.5, *J*(2′b,3′) = 4.3, H−C(2′b)). ^{13}C-NMR ($CDCl_3$): 158.71 (Ph); 155.28 (C(6)); 147.08 (C(2)); 144.12 (Ph); 144.00 (C(4)); 141.72 (C(8)); 136.68, 135.17, 130.36, 128.34, 127.89, 127.07 (Ph); 123.68 (C(5)); 113.21 (Ph); 86.87 (C−O); 85.96 (C(1′)); 84.15 (C(4′)); 72.53 (C(3′)); 63.92 (C(5′)); 55.24 (MeO); 40.21 (C(2′)); 35.53 (MeN).

N^6-*(Chloroacetyl)-2′-deoxy-5*-O-*[(4-methoxyphenyl)(diphenyl)methyl]-1-methyladenosine* (**3**). To a cold (0°) soln. of 1.61 g (3 mmol) of **2** in a mixture of 3 ml of dry pyridine and 30 ml of dry CH_2Cl_2, 2.05 g (12 mmol) of $(ClCH_2CO)_2O$ was added, and the soln. was kept at 0° for 1 h. The soln. was diluted with CH_2Cl_2 (50 ml), and H_2O (30 ml) was added. The org. layer was washed consequently with 10% aq. $NaHCO_3$ (20 ml) and H_2O (20 ml), dried (Na_2SO_4), and evaporated *in vacuo* (bath temp. < 30°) to a volume of *ca.* 5 ml. To the residue, 2M NH_3 in MeOH (15 ml) was added, and the brown soln. was kept at 0° for 20 min. The mixture was evaporated *in vacuo* (bath temp. < 30°) and co-evaporated with toluene (2 × 30 ml). The residue was purified by CC on silica gel (100 g). The column was washed with CH_2Cl_2 (200 ml), CH_2Cl_2/MeOH 99 : 1 (200 ml) and 98 : 2 (200 ml), and then eluted with CH_2Cl_2/MeOH 97 : 3. The corresponding fractions were evaporated to dryness *in vacuo* to give **3** (1.44 g, 78%). Foam. R_f 0.60 (*A*). ^{1}H-NMR ($CDCl_3$): 7.82 (*s*, H−C(8)); 7.81 (*s*, H−C(2)); 7.40 – 7.21 (*m*, 12 arom. H); 6.81 (*d*, *J* = 8.7, 2 H, *Ph*OMe); 6.29 (*t*, *J*(1′,2′a) = *J*(1′,2′b) = 6.4, H−C(1′)); 4.60 (*ddd*, *J*(3′,2′a) = 6.3, *J*(3′,2′b) = 4.2, *J*(3′,4′) = 3.0, H−C(3′)); 4.40 (*s*, $ClCH_2$); 4.12 (*ddd*, *J*(4′,5′b) = 5.3, *J*(4′,5′a) = 5.0, *J*(4′,3′) = 3.0, H−C(4′)); 3.79 (*s*, MeO); 3.60 (*s*, MeN); 3.40 (*dd*, *J*(5′a,5′b) = − 10.0, *J*(5′a,4′) = 5.0, H−C(5′a)); 3.31 (*dd*, *J*(5′b,5′a) = − 10.0, *J*(5′b,4′) = 5.3, H−C(5′b)); 2.65 (*ddd*, *J*(2′a,2′b) = − 13.2, *J*(2′a,1′) = 6.4,

J(2′a,3′) = 6.3, H−C(2′a)); 2.47 (*ddd*, J(2′b,2′a) = −13.2, J(2′b,1′) = 6.4, J(2′b,3′) = 4.2, H−C(2′b)). ^{13}C-NMR ($CDCl_3$): 178.15 (C=O); 158.73 (Ph); 147.29 (C(2)); 146.52 (C(6)); 145.13 (C(4)); 143.98 (Ph); 138.74 (C(8)); 135.08, 130.36, 128.30, 127.91, 127.09 (Ph); 122.32 (C(5)); 113.24 (Ph); 86.89 (C−O); 86.02 (C(1′)); 84.21 (C(4′)); 72.41 (C(3′)); 63.80 (C(5′)); 55.28 (MeO); 45.98 ($ClCH_2$); 40.18 (C(2′)); 36.68 (MeN).

N^6-*(Chloroacetyl)-2′-deoxy-5-*O*-[(4-methoxyphenyl)(diphenyl)methyl]-1-methyladenosine-3′-(2-cyanoethyl* N,N-*diisopropylphosphoramidite)* (**4**). Compound **3** (0.5 g, 0.81 mmol) was dissolved in 2 ml of anh. MeCN. 1*H*-Tetrazole (0.06 g, 0.85 mmol), dry pyridine, (70 ml, 0.85 mmol), and molecular sieves (4 Å; *ca.* 5% by volume) were added. After 30 min, 2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphoramidite (0.25 ml, 0.83 mmol) was added to the mixture under intensive stirring. Phosphitylation was completed in 15 min as evidenced by TLC (system *C*). The reaction was quenched with cold sat. $NaHCO_3$ (100 ml), and the mixture was extracted with AcOEt (100 ml). The org. layer was filtered through Na_2SO_4, and the solvent was removed *in vacuo* to give a white foam. Phosphoramidite **4** was purified by CC on silica gel (100 g) in hexane/AcOEt mixture (500 ml; gradient from 1 : 1 to 1 : 3 in the presence of 3% Et_3N) to yield a mixture of diastereoisomers (520 mg, 79%). R_f 0.50 (*C*), 0.21, and 0.32 (*D*). ^{1}H-NMR ($CDCl_3$): selected signals: 7.85 (*s*), 7.88 (*s*) (H−C(8)); 7.78 (*s*, H−C(2)); 7.20 – 7.40 (*m*, 12 arom. H); 6.80 (*m*, 2 H, *Ph*OMe); 6.29 (*t*, J(1′,2′a) = J(1′,2′b) = 6.8, H−C(1′)); 4.39 (*s*, CH_2Cl); 3.79 (br. *s*, MeO); 3.60 (br. *s*, MeN). ^{31}P-NMR ($CDCl_3$): 150.65; 150.10.

Oligonucleotide Synthesis and Purification. Oligodeoxynucleotides were synthesized either with an *ASM-102U* DNA synthesizer (*Biosset Ltd.*, Russia) or on an *ABI 394* (*Applied Biosystems*, Forster City, CA, USA). Oligodeoxynucleotides were synthesized in DMTr-ON mode by phosphoramidite chemistry with standard protecting groups at the 0.2- or 1.0-μmol scales. We used *Glen Research* reagents (Sterling, VA) for these synthesis.

For estimation of m^{1}dA average stepwise-coupling yield (ASY), two methods have been used. First, trityl solns. for preceding and following base couplings were collected, and their absorbance at 495 nm was measured. Second, we added m^{1}dA to a T_6 oligonucleotide, or synthesized [T-T-m^{1}dA]$_3$-T_6 and analyzed the resulting oligomers by RP-HPLC on *Spherisorb ODS2* column (5 μm; 4.6 × 150 mm) using 0.1M triethylammonium acetate (TEAA; pH 7.0) and a linear gradient of MeCN (3 – 40%, 30 min for 'trityl-on' oligonucleotides and 3 – 25%, 30 min for 'trityl-off' oligonucleotides). Flow rate was 1 ml/min. Elution was monitored using a PDA detector, and UV spectra of the eluted peaks were recorded.

Modified oligodeoxynucleotides were deprotected in 2M methanolic NH_3 for 60 h at 25°. When labile protecting groups were used (PAC-dA, Ac-dC, and ^{i}Pr-PAC-dG), deprotection time could be reduced to 24 h. Alternatively, 50 mM K_2CO_3 in MeOH for 24 h was tested with success on oligomers containing T and m^{1}dA. This last deprotection protocol must also be used in combination with PAC-dA, Ac-dC, and ^{i}Pr-PAC-dG.

For deprotection experiments, we synthesized the oligo m^{1}dA-T_6 'trityl-off' using dT-Q support [19] (*Glen Research – 21-2030-10*) which can be cleaved in 60 s. Zero-time sample was cleaved by adding 0.5 ml of 50 mM K_2CO_3/MeOH (*Glen Research – 60-4600-30*) to CPG-bound oligomer. The soln. was then neutralized by adding 0.5 ml of 2M TEAA (*Glen Research – 60-4110*). Deprotection rates were determined by removing aliquots at various times, evaporating NH_3/MeOH, resuspending in 0.1M TEAA, and analyzing by RP-HPLC as described above. Progression of the hydrolysis of *N*^{6}-chloroacetyl protection of m^{1}dA resulted in a change in retention time and was easy to follow.

Purification of oligodeoxynucleotides was carried out on a *Hypersil ODS* column (5 μm; 4.6 × 250 mm) using 0.05M TEAA (pH 7.0) and linear gradient of MeCN (10 – 50%, 30 min for DMTr-protected oligodeoxynucleotides and 0 – 25%, 30 min for fully deblocked oligodeoxynucleotides). Flow rate was 1 ml/min. Removal of the 5′-*O*-DMTr group was achieved by treatment with 2% aq. TFA for 1 min, followed by Et_3N neutralization and precipitation with 2% $LiClO_4$ in acetone. Typically, 10 – 15 o.u. (260) of oligodeoxynucleotide was obtained in 0.2-μmol synthesis.

Enzymatic Digest of Modified Oligonucleotides. Oligodeoxynucleotide (0.2 o.u.) was dissolved in 100 μl of buffer containing 50 mM *Tris* · HCl (pH 7.5), 50 mM NaCl, and 7 mM $MgCl_2$. The soln. was treated with 1 unit of venom phosphodiesterase for 16 h at 37°. Then, 5 μl of 0.5M *Tris* · HCl (pH 9.0) was added. The resulting soln. (pH 8.5) was treated with 1 unit of shrimp alkaline phosphatase for 30 min at 37°. The mixture was then diluted to 1 ml, filtered, and analyzed by RP-HPLC. Analysis was performed on a *Hypersil ODS* column (5 µm; 4.6 × 250 mm) using 0.05M TEAA (pH 7.0) and linear gradient of MeCN (0–25%) for 30 min. Flow rate was 1 ml/min. Quantification of the constituents was achieved on the basis of peak areas, which were divided by the extinction coefficients of the nucleoside (260-nm values: m^1dA, 12000; dA, 15000; dC, 7500; dG, 12500; dT, 8500). Retention times for m^1dA, dC, dG, T, dA, and m^6dA were 6.3, 7.4, 10.2, 12.4, 14.8, and 19.6 min, resp.

Oligonucleotide Dimroth *Rearrangement.* Modified oligodeoxynucleotide (0.2 o.u.) was heated in 1 ml of conc. aq. NH_3 for 10 or 16 h at 55°. NH_3 soln. was then evaporated *in vacuo*, the residue was dissolved in H_2O and analyzed by RP-HPLC as described above for fully deprotected oligodeoxynucleotides. Retention times for dC, dG, T, dA, and m^6dA were 7.4, 10.2, 12.4, 14.8, and 19.6 min, resp.

Denaturing Gel Electrophoresis. Denaturing gel electrophoresis was performed in 20% polyacrylamide, containing 7M urea. Running buffer was either 0.1M *Tris* · borate (pH 8.5) or *Tris* · HCl (pH 7.0).

Thermal Denaturation Profiles. Absorbance *vs.* temp. profiles were determined with a *Shimadzu UV160A* spectrophotometer equipped with a water-jacketed cell holder. Dry N_2 gas was flushed through the cuvette chamber to prevent moisture condensation at low temp. Melting experiments were carried out at 260 nm and with the oligonucleotide concentration at 1 µM. The solns. were heated from 0 to 90° at a rate 0.2°/min. Melting temps. were calculated from ΔH and ΔS values. Thermodynamic parameters were obtained by fitting of experimental curves using two-state intramolecular model with sloping base lines.

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REFERENCES

[1] P. F. Agris, *Prog. Nucleic Acid Res. Mol. Biol.* **1996**, *53*, 79.
[2] M. Sprinzl, C. Horn, M. Brown, A. Ioudovitch, S. Steinberg, *Nucleic Acids Res.* **1998**, *26*, 148.
[3] M. Helm, R. Giegé, C. Florentz, *Biochemistry* **1999**, *38*, 13338.
[4] J. Anderson, L. Phan, A. G. Hinnebusch, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5173.
[5] H. Sierzputowska-Gracz, H. D. Gopal, P. F. Agris, *Nucleic Acids Res.* **1986**, *14*, 7783.
[6] S. N. Mikhailov, J. Rozenski, E. V. Efimtseva, R. Busson, A. Van Aerschot, P. Herdewijn, *Nucleic Acids Res.* **2002**, *30*, 1124.
[7] S. N. Mikhailov, E. V. Efimtseva, A. A. Rodionov, A. A. Shelkunova, J. Rozenski, G. Emmerechts, G. Schepers, A. Van Aerschot, P. Herdewijn, *Chem. Biodiv.* **2005**, *2*, 1153.
[8] B. Sedgwick, *Nat. Rev. Mol. Cell. Biol.* **2004**, *5*, 148.
[9] J. Ringvoll, L. M. Nordstrand, C. B. Vagbo, V. Talstad, K. Reite, P. Aas, K. H. Lauritzen, N. B. Liabakk, A. Bjork, R. W. Doughty, P. O. Falnes, H. E. Krokan, A. Klungland, *EMBO J.* **2006**, *25*, 2189.
[10] J. C. Delaney, J. M. Essigmann, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14051.
[11] L. Q. Sun, M. J. Cairns, E. G. Saravolac, A. Baker, W. L. Gerlach, *Pharmacol. Rev.* **2000**, *52*, 325.
[12] S. K. Silverman, *Nucleic Acids Res.* **2005**, *33*, 6151.
[13] J. B. Macon, R. Wolfenden, *Biochemistry* **1968**, *7*, 3453.
[14] Y. Takeuchi, I. Tazawa, Y. Inoue, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3598.
[15] P. F. Agris, H. Sierzputowska-Gracz, C. Smith, *Biochemistry* **1986**, *25*, 5126.
[16] J. W. Jones, R. K. Robins, *J. Am. Chem. Soc.* **1963**, *85*, 193.
[17] S. V. Kochetkova, E. N. Timofeev, E. A. Korobeinikova, N. A. Kolganova, V. L. Florentiev, *Tetrahedron* **2001**, *57*, 10287.
[18] A. Ono, T. Ueda, *Nucleic Acids Res.* **1987**, *15*, 219.
[19] R. T. Pon, S. Y. Yu, *Tetrahedron Lett.* **1997**, *38*, 3327.

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