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Stabilization of DNA:DNA and DNA:RNA Duplexes by Substitution of 2'-deoxyadenosine with 2'-deoxy-2-aminoadenosine

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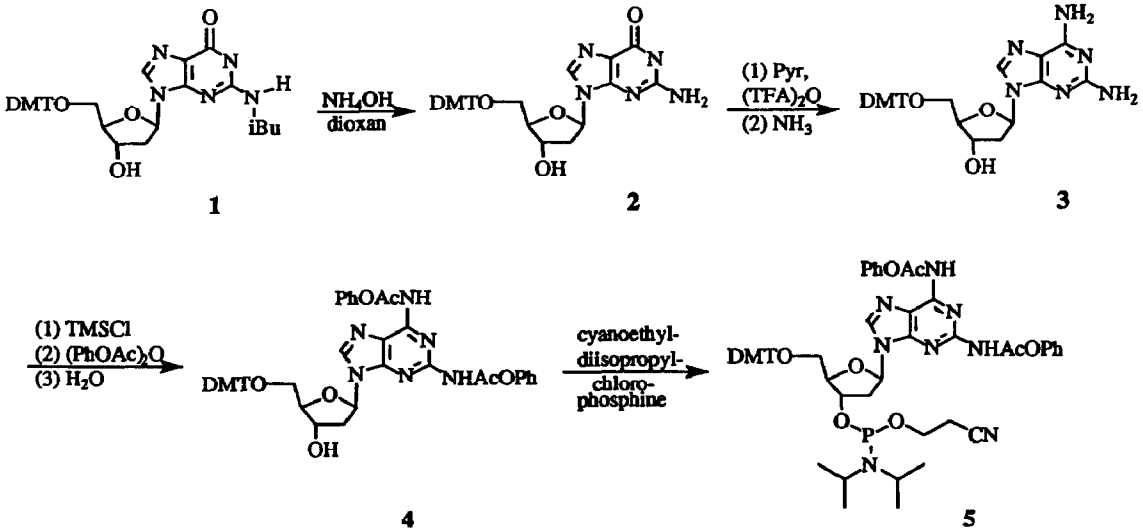
Abstract: Oligonucleotides containing either 2'-deoxyadenosine d(A) or 2'-deoxy-2-aminoadenosine d(A*) were synthesized and their relative binding affinities to complementary DNA and RNA targets compared. Substitution of dA with dA* resulted in sequence- and target-dependent changes in duplex stability.

Oligonucleotides containing 2-aminoadenosine (A*) in place of adenosine (A) are expected to bind more tightly to their complementary oligonucleotides because of the potential for 2-aminoadenine to form three hydrogen bonds with thymine or uracil bases. Chollet *et al.*[1] have reported that A*:T base pairs are intermediate in strength between A:T and G:C base pairs, while Howard and Miles[2] showed that the binding enhancement of A* over A is greater for the ribo- than deoxyribonucleic acids.

Although the synthesis of oligonucleotides incorporating 2-aminoadenosine has been reported,[1-6] the binding properties of oligomers containing this nucleoside have been limited, probably due to difficulties in preparation of monomer building blocks and oligonucleotides.[4,7] Here we report an efficient synthesis of a fully protected deoxy-2-aminoadenosine phosphoramidite and its incorporation into homo- and mixed-base oligonucleotides for studies of their binding affinities to complementary DNA and RNA.



1 2 3 4 5

Scheme 1

Fully protected 2'-deoxy-2-aminoadenosine phosphoramidite (**5**) was prepared from 5'-O- and N-protected 2'-deoxyguanosine in four steps (Scheme 1). 5'-DMT-N^6-isobutyryl-deoxyguanosine (**1**) (16.3 g) was hydrolyzed 3 days in 400 mL dioxane:conc. ammonia, 1:1 v/v. The dioxane was removed *in vacuo* and 14.2 g 5'-DMT-deoxyguanosine (**2**) recovered by filtration (98% yield). 11.4 g of dry **2** was dissolved in 300 mL dry pyridine and the mixture cooled to 0 °C and covered to protect from light. Triflouroacetic anhydride (20 mL) was added slowly and after 15 min, anhydrous ammonia bubbled in for 2 h and the reaction mixture kept at -20 °C for 2 days.[7] The mixture was then partitioned between 250 mL dichloromethane and 250 mL saturated aqueous sodium bicarbonate, and the organic layer washed with aqueous $NaHCO_3$ and concentrated *in vacuo* to a dark solid. Silica gel flash chromatography (2-6% methanol in CH_2Cl_2) gave 10.2 g DMT-deoxy-2-aminoadenosine (**3**) (90% yield). **3** (2.0 g) was dissolved in 50 mL dry pyridine and cooled to 0 °C. Trimethylsilyl chloride (3.1 mL) was added slowly and the mixture stirred 30 min while warming to room temperature, then phenoxyacetic anhydride (5.0 g) added and the mixture stirred overnight. Excess anhydride was hydrolyzed with 2 mL water and after 4 h the mixture diluted with 50 mL dichloromethane, washed 2 times with 200 mL saturated aqueous $NaHCO_3$, then washed with 100 mL saturated aqueous NaCl. The organic layer was evaporated to dryness and the residue purified on 4 mm Chromatotron^R plates to give 1.35 g (46% yield) DMT-N^2,N^6-bisphenoxyacetyl-2'-deoxy-2-aminoadenosine (**4**).[8] **4** was quantitatively converted to phosphoramidite **5** by standard procedure.[9]

Oligonucleotides containing dA* were prepared on an Applied Biosystems 380B synthesizer according to standard protocol, except that **5** was dissolved in dry dichloromethane instead of acetonitrile and phenoxyacetic acetic anhydride in THF:lutidine 1:1 v/v was used as the capping reagent. Oligomers with terminal 3'-dA or dA* were built on 2-[2-(dimethoxytrityl)ethylsulfonyl]ethyl-succinoyl-lcaa-CPG, resulting in 3'-phosphorylated compounds.[10] Several dA- and corresponding dA*-containing oligomers were synthesized, with sequences listed in Table 1. All oligomers were purified and analyzed by reversed-phase and ion-exchange HPLC (see Fig. 1a-b).[11] In addition, to prove the incorporation of dA* into oligomers, $d(A^*_{10})p$ and $d(A_{10})p$ were hydrolyzed with phosphodiesterase I and alkaline phosphatase to nucleosides, and the hydrolysis products analyzed by reversed-phase HPLC (fig. 1c-d).

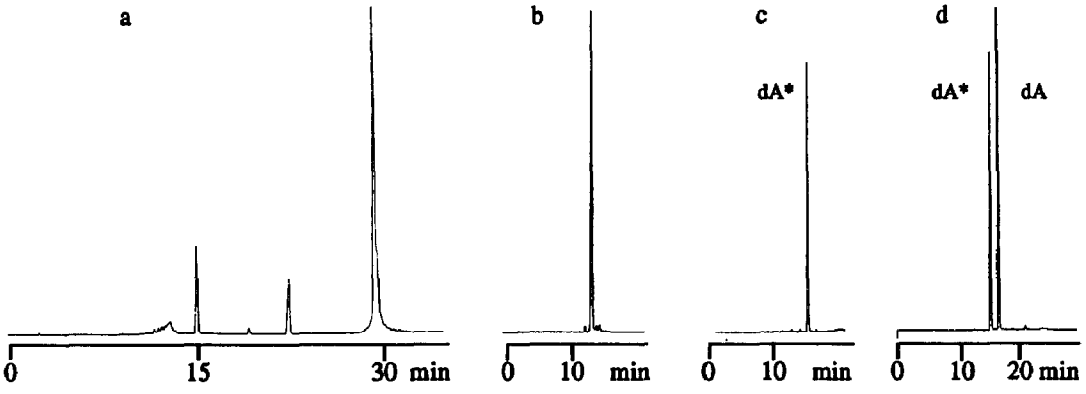


Fig. 1. a) Analytical RP HPLC of crude DMTr-$d(A^*_{10})p$. b) Analytical IE HPLC of RP HPLC purified $d(A^*_{10})p$. c) Analytical RP HPLC of dA* from enzymatic digest of $d(A^*_{10})p$. d) Analytical RP HPLC of coinjected digests of $d(A^*_{10})p$ and of $d(A_{10})p$. For HPLC conditions, see reference 11.

The synthesized oligomers were characterized by thermal dissociation experiments, with their melting temperatures (T_ms) summarized in Tables 1 and 2.

TABLE 1

Melting temperature of duplexes,[a] Buffer A.[12]

Exp.	Oligonucleotide	Target	X=A	X=A*	ΔT_m
1	d(XXX XXX XXX X)p[b]	DNA	40.5	41.0	0.5
2	d(XXX XXX XXX X)p[c]	RNA	34.0	58.0	24.0
3	d(XXX XGX XXX GGG GGG X)p	DNA	56.5	63.5	7.0
4	d(XXX XGX XXX GGG GGG X)p	RNA	45.5	58.0	12.5
5	d(GGG GGX GGX GX)p	DNA	50.0	55.5	5.5
6	d(GGG GGX GGX GX)p	RNA	49.0	54.5	5.5
7	d(CTT CCT TXX XGG GCT T)	DNA	56.5	60.0	3.5
8	d(CTT CCT TXX XGG GCT T)	RNA	54.0	59.5	5.5
9	d(XXG GGC TTC TTC CTT X)p	DNA	58.5	60.5	2.0
10	d(XXG GGC TTC TTC CTT X)p	RNA	62.5	62.5	0.0

[a]To nearest 0.5 °C. Standard deviations ±0.3 to ±0.7 °C. [b]Hybridized to poly-dT. [c]Hybridized to poly-U.

TABLE 2

Melting temperature of duplexes,[a] Buffer B.[13]

Exp.	Oligonucleotide	Target	X=A	X=A*	ΔT_m
1	d(XXX XXX XXX X)p[b]	DNA	59.0	54.5	-4.5
2	d(XXX XXX XXX X)p[c]	RNA	59.0	78.0	19.0
3	d(XXX XGX XXX GGG GGG X)p	DNA	61.0	66.5	5.5
4	d(XXX XGX XXX GGG GGG X)p	RNA	51.0	67.5	16.5
5	d(GGG GGX GGX GX)p	DNA	54.5	60.0	5.5
6	d(GGG GGX GGX GX)p	RNA	58.0	64.5	6.5
7	d(CTT CCT TXX XGG GCT T)	DNA	61.5	63.0	1.5
8	d(CTT CCT TXX XGG GCT T)	RNA	62.0	68.0	6.0
9	d(XXG GGC TTC TTC CTT X)p	DNA	63.5	65.0	1.5
10	d(XXG GGC TTC TTC CTT X)p	RNA	66.5	69.0	2.5

[a]To nearest 0.5 °C. Standard deviations ±0.3 to ±0.6 °C. [b]Hybridized to poly-dT. [c]Hybridized to poly-U.

T_M data for the homooligomers suggested that conversion of dA to dA* does not improve binding to DNA, although it significantly enhances binding to RNA (exps. 1,2). Experiments with mixed-base oligomers did show stabilization of duplexes formed with DNA (exps. 3,5,7,9), although on average, RNA binding was affected more (exps. 4,6,8,10).

It has been reported that dA tracts bind very poorly to RNA, presumably because dA disfavors the A-form helical structure favored by RNA duplexes.[14] In 150 mM NaCl (Buffer A), there is an apparent destabilization of RNA duplexes by dA residues; for the mixed-base sequences, RNA preference increases with decreasing dA content and dispersion of dA tracts (exps. 3-10, Table 1). Substitution of dA with dA* countered this destabilization, generally improving binding to both DNA and RNA, but affecting DNA:RNA hybrids with greater variation. Substitution with dA* enhanced binding to RNA the most where the original destabilization by dA residues was the greatest.

Addition of 10 mM Mg^{2+} (Buffer B, Table 2) improved the stability of all duplexes studied, but especially of DNA:RNA duplexes. Mg^{2+} also enhanced the RNA preference of dA*-containing oligomers.

In conclusion, the substitution of dA* for dA generally improves the stability of hybrids formed with both DNA and RNA. The greatest potential appears to lie in applications such as antisense therapeutics and diagnostics which may require binding of DNA to RNA targets where significant dA tracts might otherwise preclude strong binding.

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8. ^{1}H NMR ($CDCl_3$): δ 2.6 (m, 1H), 2.8 (m, 1H), 3.4 (m, 2H), 3.8 (s, 6H), 4.2 (m, 1H), 4.7 (br, 1H), 4.8 (br m, 1H), 4.9 (br, 1H), 6.6 (dd, 1H), 6.8 (dd, 4H), 7.0-7.1 (m, 6H), 7.2-7.4 (m, 14H), 8.1 (s, 1H)
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11. DNA was purified DMT-on by RP HPLC, eluted with a gradient of 1%/min acetonitrile versus 0.05 M triethylammonium acetate, flow 1 mL/min, Hypersil ODS 4 X 250 mm column. The DNA was detritylated with 80% acetic acid 5 min., evaporated to dryness, and ethanol precipitated. RNA was purified by IE HPLC, with a gradient of 1%/min 1.5 M NaCl in 10 mM NaOH on a Dionex NucleoPac™ column. Collected fractions were immediately neutralized with dilute acetic acid, evaporated to 0.5 mL, and desalted on Pharmacia NAP-5™ columns. Purified oligomers were analyzed by IE HPLC using the same elution conditions as described for purification of RNA.
12. Buffer A: 150 mM NaCl, 10 mM Tris·HCl; pH 7.0, 3 μM oligomer concentration
13. Buffer B: 10 mM $MgCl_2$, 150 mM NaCl, 10 mM Tris·HCl; pH 7.0, 3 μM oligomer concentration
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