Preparation and hybridization properties of oligonucleotides containing 1- α -D-arabinofuranosylthymine

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ABSTRACT

A pentadecanucleotide was prepared from 1- α -arabinofuranosylthymine. This novel oligonucleotide was found to hybridize to oligodeoxyadenylate, although not as strongly as pentadecathymidylate. It formed duplex hybrids with both DNA and RNA complements, and triplex structures with a duplex containing a (dT)₁₅-(dA)₁₅ tract within a more complex strand. The T_m of the duplex with polyadenylate was almost the same as that of (dT)₁₅ and polyadenylate, while its T_m with (dA)₁₅ was substantially lower than that of the natural counterpart. A selective benzylation of the 2'-O of a 5'-blocked α -ara-thymine was developed to greatly simplify the preparation of suitable blocked material for use in preparation of oligonucleotides on the automated DNA synthesizer.

INTRODUCTION

Efforts to enhance the biological activity of oligonucleotides as inhibitors of gene expression ('antisense' oligonucleotides) have focused on the improvement of their stability to nuclease digestion, among other aspects. This problem has been approached in several ways,¹ including alteration of the phosphate and the sugar moieties in the oligonucleotides. The most thoroughly investigated of the latter modifications has been the use of α -2'-deoxy nucleosides in construction of the oligonucleotides.²

Recent investigations into the properties of oligomers composed of the α -anomers of the natural 2'-deoxyribofuranosyl nucleosides have revealed interesting properties for this unusual class of compounds. The α -oligomers are highly nuclease resistant.^{3,4} Deoxy- α -(G₂T₁₂G₂) binds to (dA)₁₂ with a T_m of 53°, whereas the corresponding oligo- β -anomer binds with a T_m of 27°C.⁵ Oligo-(α -thymidylate) binds to polyadenylate more strongly than oligomers composed of the natural β -anomer.⁴ Addition of a tethered intercalator to the oligomer's terminus enhances the binding of these compounds, just as it does with the β -oligomers.⁶ In addition, as might be expected (because of the 'inverted' sugar), the oligodeoxy- α -nucleotide usually binds parallel to its β -oligomer complement.⁷ Interestingly, Hélène and coworkers have also shown that an intercalator-linked (α -dT)₈ forms triple helices with an oligo-dT- α -oligo-dA double

helix.^{8,9} The α -oligomers can bind to DNA and RNA (in a parallel manner) and sequence-specifically inhibit RNase H degradation of RNA and the priming capacity of β -oligomers for reverse transcriptase.¹⁰ The hybrids with RNA are not RNase H substrates.⁵

From a practical point of view, however, α - and β -anomeric mixtures are usually encountered in the preparation of the α -nucleosides. In order to make use of the good hybridization properties and excellent stability properties of α -oligomers in antisense therapeutics, it would be desirable to have similar nucleoside units that are stable and easy to prepare. Nucleosides of α -arabinofuranose should be easy to prepare because of the *trans*-relationship of the glycosidic linkage and the 2'-OH. Oligomers of these nucleotides should be straightforward to prepare and should have the hybridization properties of the α -2'-deoxy oligomers. We report here a method for the preparation of such oligomers, and the hybridization properties of the first member of this class, a pentadecamer [(α -ara-T)₁₅] derived from 1- α -arabinofuranosylthymine.

MATERIALS AND METHODS

General

All reagents used in the syntheses were purchased from Aldrich. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained at 200 MHz on a Varian Gemini 200 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Infrared spectra (IR) were recorded on a Perkin-Elmer Perkin-Elmer 783 spectrophotometer and are expressed in reciprocal centimeters. Routine ultraviolet spectra (UV) were recorded on a Beckman DU-40 spectrophotometer. Elemental analyses were performed by Robertson Laboratories. EM Reagents silica gel (230-400 mesh) was used for flash column chromatography.¹¹ All columns were 5×20 cm. Thin-layer chromatography was run on silica gel 60 F-254 (EM Reagents) aluminum backed plates. TLC solvents used were: A= 50% acetone/methylene chloride, B= 50% acetone/hexanes, C= acetone/hexanes/triethylamine (4/6/1), D= 5% acetone/methylene chloride. Components were detected on TLC by UV light and with 5% H₂SO₄ in MeOH spray followed by heating.

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Evaporations were carried out under reduced pressure with a bath temperature below 30°C. HPLC was performed using Rainin HPX pumps and a Gilson 116 UV-Vis detector.

1-(2,3,5-Tri-*O*-benzoyl- α -D-arabinofuranosyl)thymine

A mixture of dry thymine (0.5 g, 3.96 mmol), hexamethyldisilazane (30 mL), ammonium sulfate (50 mg), and dry pyridine (1.0 mL) was refluxed for 2 h. The solvents were removed under vacuum. The residue was added to a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose (2.0 g, 3.96 mmol) in dry MeCN (50 mL). Trimethylsilyl trifluoromethanesulfonate (0.765 mL, 3.96 mmol) was added and the mixture was stirred 18 h. Solvents were evaporated and the residue was dissolved in EtOAc. This solution was washed with saturated NaHCO₃, then saturated NaCl, and dried over Na₂SO₄. Flash chromatography on silica gel using a step gradient of 10% acetone/hexane to 50% acetone/hexanes gave the product, which was dried to give 1.0 g (44.3%); mp >90°C.

HNMR (CDCl₃): 8.83 (1H, s, NH), 8.2–7.4 (15H, m, ArH), 7.30 (1H, s, H-6), 6.29 (1H, d, H-1', J=3.29 Hz), 5.95 (1H, t, J=2.85), 5.77 (1H, t, J=3.0), 4.98 (1H, m, H-4'), 4.73 (2H, m, H-5'), 1.95 (3H, s, CH₃).

Anal. Calc. for C₃₁H₂₆N₂O₉: C, 65.26; H, 4.59; N, 4.91; Found: C, 64.87; H, 4.75; N, 4.51

1- α -D-Arabinofuranosylthymine (1)

1-(2,3,5-Tri-*O*-benzoyl- α -D-arabinofuranosyl)thymine (18.6 g, 32.6 mmol) was dissolved in dry MeOH, adjusted to 'pH 11' (moist pH test paper) with freshly prepared methanolic NaOMe. After stirring over night the solution was neutralized with Dowex-50 H⁺ resin, filtered, and evaporated to dryness. The residue was washed with EtOAc and dried, giving a crude yield of 8.0 g (69%). This was used without further purification. UV: $\lambda_{\max}$ (pH7) 267 nm. TLC (solvent A) R_f = 0.25.

HNMR (d₆-DMSO): 11.27 (1H, s, NH), 7.60 (1H, s, H-6), 5.72 (1H, d, H-1', J=4.80), 5.64, 5.44, 4.89 (3H, br s, 2',3', 5'-OH), 1.90 (3H, s, CH₃), and other sugar protons.

1-[3,5-*O*-(1,1,3,3-tetraisopropylidisiloxan-1,3-diyl)- α -D-arabinofuranosyl]thymine (2)

To a solution of 1- α -D-arabinofuranosylthymine (1, 300 mg, 0.85 mmol) in dry pyridine (15 mL) was added 1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane (0.38 mL, 0.85 mmol). The solution was stirred 18 h. Pyridine was evaporated and the residue was partitioned between EtOAc and water. The organic phase was washed with cold 1N HCl, H₂O, saturated NaHCO₃, and saturated NaCl, then dried over Na₂SO₄. Flash chromatography using 5% acetone/CH₂Cl₂ gave 230 mg (54%), mp 80°–96°C. TLC (solvent D) R_f = 0.27.

HNMR (CDCl₃): 9.1 (1H, br s, NH), 7.35 (1H, s, H-6), 5.55 (1H, d, H-1', J=4.8), 4.5–3.8 (6H, m, other sugar protons), 1.95 (3H, s, CH₃), 1.2–0.9 (24H, m, *i*-Pr).

Anal. Calc. for C₂₂H₄₀N₂O₇Si₂: C, 52.77; H, 8.05; N, 5.59; Found: C, 52.50; H, 7.93; N, 5.37

1-(2-*O*-Benzoyl- α -D-arabinofuranosyl)thymine (4)

To a cold solution of 1-[3,5-*O*-(1,1,3,3-tetraisopropylidisiloxan-1,3-diyl)- α -D-arabinofuranosyl]thymine (2, 0.9 g, 1.8 mmol) in dry pyridine (10 mL) was added benzoyl chloride (0.2 mL, 1.8 mmol). After stirring at ambient temperature 4 h, the mixture was poured into ice/water, extracted with EtOAc. The organic phase was dried (Na₂SO₄) and evaporated to give 600mg (55%)

of the 3',5'-disiloxanyl-2'-*O*-benzoyl derivative (3, R_f = 0.40, solvent D) was used without further purification.

To this product (3, 600 mg, 1.2 mmol) in THF (10 mL) was added 10 mL of 1 *N* tetrabutylammonium fluoride in THF, and stirring was continued 2 h. The solvent was evaporated, and the residue was flash chromatographed on silica gel, using 10% acetone/hexanes. Fractions were monitored by TLC (solvent B, R_f = 0.19). The fractions containing product were collected and evaporated to give 100 mg (12%) of 4.

HNMR (d₆-DMSO): 11.34 (1H, s, NH), 8.1–7.5 (5H, m, ArH), 7.73 (1H, s, H-6), 5.97 (1H, d, H-1', J=4.80), 5.89 (1H, d, 3'-OH, J=4.80), 5.51 (1H, t, H-2', J=4.95), 4.99 (1H, t, 5'-OH, J=5.70), 4.45–4.25 (2H, m, H-3' and H-4'), 3.7–3.5 (2H, m, H-5'), 1.82 (3H, s, CH₃).

1-[5-*O*-(4,4'-Dimethoxytrityl)- α -D-arabinofuranosyl]thymine (6)

To 1- α -D-arabinofuranosylthymine (1, 0.5 g, 1.4 mmol) in dry pyridine (20 mL) was added 4, 4'-dimethoxytrityl chloride (0.656 g, 1.9 mmol). The mixture was stirred for 20 h at ambient temperature. The mixture was evaporated to dryness, and the residue was dissolved in EtOAc (100 mL) and poured into a saturated solution of sodium bicarbonate. The organic layer was washed with H₂O and dried over anhydrous sodium sulfate, then evaporated to dryness. The residue was flash chromatographed on silica gel using a step gradient of EtOAc/hexanes (1:1 to 2:1). Fractions were monitored by TLC (solvent A, R_f = 0.63). The major product was collected and dried to give 0.4 g (49%), mp 77–80°C.

Anal. Calc. for C₃₁H₃₂N₂O₈ · 0.75 H₂O: C, 64.85; H, 5.88; N, 4.88;

Found: C, 65.23; H, 6.04; N, 4.45.

1-(2-*O*-Benzoyl-5-*O*-dimethoxytrityl- α -D-arabinofuranosyl)thymine (5)

Method A. To a cold solution of 1-(5-*O*-dimethoxytrityl- α -D-arabinofuranosyl)-thymine (6, 330 mg, 0.57 mmol) in dry pyridine (15 mL) was added benzoyl chloride (0.068 mL, 0.58 mmol). The mixture was stirred at ambient temperature for 4 h, then poured into a saturated solution of sodium bicarbonate and extracted with EtOAc. The organic phase was washed with saturated sodium chloride solution and dried over sodium sulfate, then evaporated. The residue was flash chromatographed on silica gel using a step gradient of 20% EtOAc/hexanes to 60% EtOAc/hexanes. The product was monitored by TLC (solvent C, R_f = 0.50). The product was rechromatographed on preparative HPLC (80% MeCN/H₂O) using a C₁₈ column (Rainin Dynamax, 22.4 mm × 50 cm) to give 185 mg (51%) of 5, homogeneous on TLC.

HNMR (CDCl₃): 8.1–6.7 (19H, m, ArH), 6.02 (1H, d, H-1' J=4.5 Hz), 5.39 (1H, m, H-2'), 4.55 (2H, m, H-3' and H-4'), 3.78 (6H, s, OCH₃), 3.67 (2H, m, H-5'), 2.00 (3H, s, CH₃).

Anal. Calc. for C₃₈H₃₆N₂O₉ · 0.25 H₂O: C, 68.20; H, 5.50; N, 4.19;

Found: C, 68.25; H, 5.52; N, 4.01.

Method B. To 1-(2-*O*-benzoyl- α -D-arabinofuranosyl)thymine (4, 60 mg, 0.13 mmol) in pyridine (10 mL) was added 4,4'-dimethoxytrityl chloride (80 mg, 0.23 mmol), and the mixture was stirred 18 h at ambient temperature. Solvents were removed in vacuo and the product was isolated on preparative

TLC (silica gel, 1:1 hexane/EtOAc). The HNMR was identical with the compound as prepared above.

1-(2-*O*-Benzoyl-5-*O*-dimethoxytrityl- α -D-arabinofuranosyl)thymine 3'-*O*-(2-Cyanoethoxy-*N,N*-diisopropylphosphoramidite) (7)

To a solution of 1-(2-*O*-benzoyl-5-*O*-dimethoxytrityl- α -D-arabinofuranosyl)thymine (**5**, 460 mg, 0.72 mmol), diisopropylethylamine (0.5 mL), and dichloromethane (40 mL) was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (448 mL, 2 mmol) in two portions. The solution was stirred 2 h. The reaction was followed by TLC (solvent C, R_f = 0.60). Methanol (0.1 mL) was added, and the solution was poured into a solution of EtOAc (100 mL) and triethylamine (10 mL). This solution was washed with 10% aqueous Na_2CO_3 , then with saturated aqueous NaCl. The organic layer was dried over sodium sulfate, filtered, and evaporated. The residue was coevaporated with MeCN, dissolved in xylenes (10 mL), and poured into hexanes (300 mL). The precipitated solid was filtered and dried to give 260 mg (43%), which was used for oligonucleotide synthesis without further purification. TLC of the phosphoramidite in solvent C gave the characteristic two spots observed for commercial amidites.

Oligonucleotide Synthesis and Purification

Oligonucleotides were synthesized on a Milligen 7500 DNA synthesizer using standard cyanoethyl phosphoramidite chemistry. Synthesis of the (α -ara-T)₁₅ was accomplished using an RNA protocol (Milligen) with a 3' amino-modifier CPG solid support (Glen Research Corporation). Average trityl yields for the (α -ara-T)₁₅ and for (dT)₁₅ were estimated at 97% and 99%, respectively, as judged by the spectrophotometric detection of trityl cation by the Milligen 7500. Deblocking was performed with concentrated aqueous ammonia at 40°C for 4 h. Oligodeoxynucleotides and (α -ara-T)₁₅ were purified by the same method, as follows. 5'-Dimethoxytrityl oligonucleotides were purified on HPLC by direct injection of the deprotected solution (aqueous ammonia) onto a Hamilton PRP-1 column (305 $\times$ 7.0 mm). The products were eluted using a linear gradient from 20% MeCN/triethylammonium acetate (TEAAc, 0.1 M, pH 7.5) to 45% MeCN/TEAAc over 20 min (UV detection at 254 nm). Appropriate fractions were combined and concentrated to dryness by lyophilization on a Savant Speed-Vac. The residue was detritylated in 80% acetic acid (300 μ L, 30°C, 90 min), precipitated with butanol (1 mL), washed with 95% ethanol (1 mL), and reconstituted in sterile distilled water (500 μ L). Oligonucleotide purity was confirmed by denaturing PAGE analysis; each oligomer afforded a single band with a mobility consistent with the specified length when stained with methylene blue (0.2% in water). The overall yield, after purification, of (α -ara-T)₁₅ was 17%, calculated using a value of 33 μ g/OD, the same value as thymidylate-containing nucleotides. The reported ϵ of **1** is 10,000,¹² within 4% of the ϵ of thymidine (9600) which was used in the calculations. Oligonucleotide ϵ values were estimated by summing the ϵ values of the individual nucleotides.

Thermal Denaturation

Thermal transitions were recorded at 260 nm using a Gilford System 2600 UV-VIS spectrophotometer equipped with a Gilford 2527 Thermo-programmer. Oligonucleotides (2 μ M/strand) were dissolved in 10 mM cacodylate, pH 7.2, containing EDTA,¹³ with various concentrations of NaCl and MgCl_2 . The

oligonucleotide mixtures were heated at 65°C, then cooled to 10–15°C to allow hybridization. The samples were then heated to 85°C with a temperature increase of 0.5°/min. Absorbance versus time was recorded, and the first derivative was computed automatically. The T_m values were determined using the first derivative maxima. Duplicate runs produced nearly identical values ($\pm 1^\circ\text{C}$). Data from one representative run is given.

RESULTS AND DISCUSSION

Synthesis

The strategy adopted for the synthesis of the α -arabinosyl oligonucleotides was protection of the 2'-*O* position of the sugar with groups removable by ammonia; no silyl groups were necessary because no neighboring group attack at phosphate by the 2',3'-*trans*-hydroxyl system in arabinofuranose was possible. In the synthesis of oligonucleotides containing β -arabinofuranosides, this approach was adopted for the preparation of a self-complementary 12-mer in which one dCyd had been replaced by 1- β -D-arabinofuranosylcytosine.¹⁴

The route shown in Scheme 1 depicts the preparation of a blocked phosphoramidite, derived from 1- α -D-arabinofuranosylthymine (**1**), which is suitable for direct use on the automated DNA synthesizer for the preparation of α -arabino-oligomers. We prepared 1- α -D-arabinofuranosylthymine (**1**) by a variation on the published route¹². We used the same tri-*O*-benzoyl-D-arabinofuranosyl acetate as the original workers, but used the more convenient trimethylsilyl triflate-catalyzed condensation of the bis-trimethylsilylated thymine¹⁵ for the synthesis of the nucleoside.

In initial studies, the 2'-*O*-benzoyl group was introduced in an unambiguous manner. Treatment of **1** with

(a) 1,3-Dichloro-1,1,3,3-tetraisopropylidisiloxane, pyridine. (b) BzCl, pyridine. (c) $\text{Bu}_4\text{N}^+\text{F}^-$. (d) DMTrCl, pyridine. (f) $[\text{NC}(\text{CH}_2)_2\text{O}]_2\text{P}(\text{Cl})\text{N}(\text{iPr})_2$.

1,3-dichloro-1,1,3,3-tetraisopropylidisiloxane gave the 3',5'-*O*-(1,1,3,3-tetraisopropylidisiloxan-1,3-diyl) derivative **2**. This has previously been shown to give a 3',5'-cyclic disiloxanyl derivative with arabinofuranosyl nucleosides.¹⁶ This protected arabinoside was benzoylated with benzoyl chloride, and the crude product (**3**) was desilylated with fluoride ion to give the 1-(2-*O*-benzoyl- α -D-arabinofuranosyl)thymine (**4**).

This route allowed the assignment of the site of benzoylation, but it was subsequently found that the 2'-*O*-protecting group could be introduced regioselectively onto nucleoside **1** once the 5'-position was blocked. Selective dimethoxytritylation of **1** gave the 5'-DMT derivative (**6**). As described in *Method A* in the Materials and Methods section, careful treatment with benzoyl chloride/pyridine (1 equiv) gave the 2'-*O*-benzoate (**5**) in 58% yield, purified by preparative HPLC. This nucleoside was identical by chromatography and NMR spectrum to the product prepared by dimethoxytritylation of 2'-*O*-benzoate **4** (*Method B*). This nucleoside was converted to the synthesizer-ready reagent **7** by treatment with cyanoethyl *N,N*-diisopropylchlorophosphoramidite using standard procedures.^{17,18}

For synthesis of the new oligonucleotide derivatives on an automated synthesizer, we used the 3'-amino modifier column available from Glen Research, Herndon, VA, which places the 3-amino-2-hydroxyprop-1-yl ester of a 3'-phosphate. This allows preparation of oligomers without the normal deoxy- β -riboside that would be present at the 3'-terminus if the usual commercial CPG columns were used. Although we have found that this terminal modification gives degradation products in deblocking conditions more strenuous than those used here,¹⁹ these conditions gave a single product. The automated synthesizer was programmed using the cycle times for RNA preparation (particularly, 6 min for each phosphoramidite cycle) to allow for any reduction in coupling reaction rate that might occur due to the presence of a 2'-substituent. The yield after purification (17%), albeit somewhat low, was in the range often observed in the synthesis and purification of oligonucleotides.

Hybridization Properties

As noted in the introduction, the α -deoxyoligomers have been found, in some cases, to give hybrids with higher T_m values than their β -oligomer counterparts. These comparisons are made, of course, between α - and β -oligomers of opposite polarity, since the α -oligomers generally bind in a parallel manner to their complement. Studies of α -oligomers incorporating all the natural bases have been somewhat inconclusive regarding the effect of the modification: some cases give better hybrid stability, some lower.

The thermal melting data is shown in Table 1. The 15-mer we have prepared from α -arabinothymine was found to form a hybrid with a complementary DNA strand, although the duplexes were not as stable as those formed by pentadecathymidylate under identical conditions. The complement used for all of our studies was (dC)₃(dA)₁₅(dC)₃. In a solution which approximates physiological conditions (150mM NaCl and 5 mM Mg⁺⁺), the (α -ara-T)₁₅ hybrid has a T_m of 17°C, compared to a T_m of 42°C for the hybrid between (dT)₁₅ and the same complement. Increasing the salt concentration to 1 M increased the T_m to 31° with the α -ara-oligomer, and a somewhat larger increase in the T_m of the natural β -oligomer. It appears that the hybrid strength of the α -ara-oligomer is somewhat less than that of its β -counterpart in all conditions tested with DNA complements.

The new α -ara-oligomer was found to form a triple stranded

Table 1. T_m Values for Duplexes and Triplexes Formed Between Synthetic Oligonucleotides Under the Conditions Given.^a

Additions, mM				
NaCl	150	150	1000	0 ^b
MgCl ₂	0	5	0	50
Strands ^c		T_m , °C		
A,C	12	17	31	31.2
B,C	38	42	52	51
D,C	60	63	70	67
A,C,D	< 10,61	12,62	29,70	28, 67
B,C,D	39,61	44,64	54,67	23, 65
A,E		38		
B,E		42		

^aThermal melts were performed as described in Methods. Except where indicated, solutions have 10mM cacodylate buffer, pH 7.2, and 1mM EDTA. NaCl and MgCl₂ were added as noted. Strand concentrations are 2 μ M. Duplexes have a excess (10%) of strand C, (dC)₃(dA)₁₅(dC)₃. The triplexes have an excess (10%) of (α -Ara-T)₁₅ or (dT)₁₅.

^bValues in this column obtained in 50mM HEPES buffer, pH 7.5.

^cStructures of the oligonucleotide strands used:

- A = (α -AraT)₁₅
- B = (dT)₁₅
- C = (dC)₃(dA)₁₅(dC)₃
- D = (dG)₃(dT)₁₅(dG)₃
- E = poly (riboA)

complex with a β -DNA duplex. When (dC)₃(dA)₁₅(dC)₃, (dG)₃(dT)₁₅(dG)₃, and (α -ara-T)₁₅ were mixed in a 1:1:1 ratio, two breaks in the melting curve were obtained. This is in analogy with similar results found for third-strand formation of oligodeoxyribonucleotides by Shea et al.,¹³ who used a system similar to ours, but with longer duplex oligomers. They annealed (dT)₁₅ to a complementary pair of 34-mers containing (dA)₁₅-(dT)₁₅ in the middle, and observed a triplex T_m of 32° and 46° at 100 and 500 mM NaCl, respectively, pH 7.2, and strand concentrations of 2 μ M. We found that the (dT)₁₅ triplex melted at 44°C (150 mM NaCl, 5 mM Mg⁺⁺) and the triplex formed by the new (α -ara-T)₁₅ melted at 12°C. At higher Mg concentrations (50 mM), the α -ara-oligomer actually has a higher T_m than (dT)₁₅.

Perhaps the most interesting result in the hybridization studies was the observation that the duplexes formed between either (α -ara-T)₁₅ or (dT)₁₅ and polyadenylate were of similar strength. Furthermore, this (α -ara-T)₁₅ duplex was much stronger than its duplex with a DNA complement, while (dT)₁₅ gave hybrids of equal strength with either DNA or RNA complements. This remarkable preference for hybridization of the α -ara-(dT)₁₅ with complementary RNA rather than DNA is similar to results found when comparing the α -DNA oligomers' ability to hybridize to β -DNA or RNA complements.²⁰

CONCLUSIONS

A facile selective benzoylation of the 2'-*O* position of 5'-DMT- α -arabinofuranosylthymine provided a simple and generally applicable synthesis of fully protected phosphoramidite from nucleoside **1**, ready for oligonucleotide synthesis on an automated DNA synthesizer. Oligomers were prepared from this phosphoramidite in acceptable yield. It is interesting to note that the new α -ara-oligomer forms a much more stable duplex with an RNA complement than with a DNA complement. It forms

a weaker duplex with DNA than the natural compound, while the RNA hybrids are similar in T_m .

Groups studying the α -deoxy oligomers have shown that it is difficult to generalize about the anticipated hybrid stability of these compounds; it seems to be influenced by the sequence. It is possible that duplexes formed by oligomers of α -arabinosides of the 4 natural DNA nucleobases would give T_m values close to those of the natural β -deoxy oligomers. We have not examined the nuclease resistance of this new oligomer, but, based on results obtained with the β -deoxy oligomers discussed above, it is most reasonable to assume the α -ara-oligomers would be resistant. Although it is expected that the α -ara-oligomers' hybrids with RNA would not be RNase H substrates, in analogy to results with α -DNA-RNA duplexes⁵, there will likely be several applications in antisense therapy for this new, readily prepared backbone modification.

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