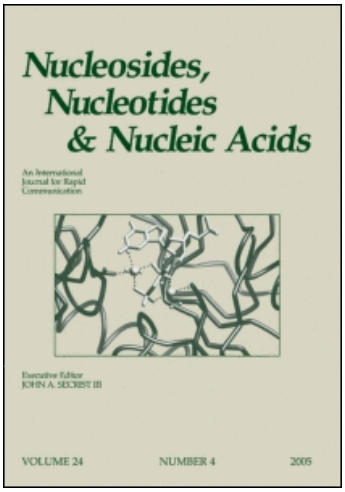

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PREPARATION AND CHARACTERIZATION OF OLIGONUCLEOTIDES OF D- and L-2' DEOXYURIDINE

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Abstract. The synthesis of oligonucleotides of 2'deoxyuridine containing both the natural D-2'deoxyribose and the unnatural L-2'deoxyribose is described. Units up to the 18-mer have been made via a modified triester procedure and characterized by HPLC.

INTRODUCTION

We had previously shown[1] that cytosine L-arabinoside lacked the bioactivity of the D-enantiomer. However, the work of Ts'o demonstrated[2] that dinucleotides of adenosine (ApA) containing both D- and L-ribose possessed similar binding properties with poly U. These observations stimulated our thoughts and ideas about the potential properties and utilities of oligonucleotides containing the unnatural L-sugars rather than sugars of the natural D-configuration. It was expected that such molecules would be devoid of template activity and be resistant to the usual degradative enzymes. Potentially, they may possess therapeutic value in selected disease states, especially of genetic origin. For example, such an oligonucleotide "anti-gene" could sequester the expression of abnormal β-chains of hemoglobin in human sickle cell disease. Similarly, such molecules have great promise as specific anti-viral agents.

Although there existed[3] chemical preparations of the usual nucleosides containing L-ribose and L-deoxyribose, generally these procedures were not very adaptable to larger scale synthesis in order that reasonable quantities be available for solution oligonucleotide synthesis.

A reasonable goal of an 18-mer of an L-deoxynucleoside was set since this length would approach almost two turns in a double helix of DNA (and may possess substantial biological specificity) and on paper could be prepared from trinucleotide blocks.

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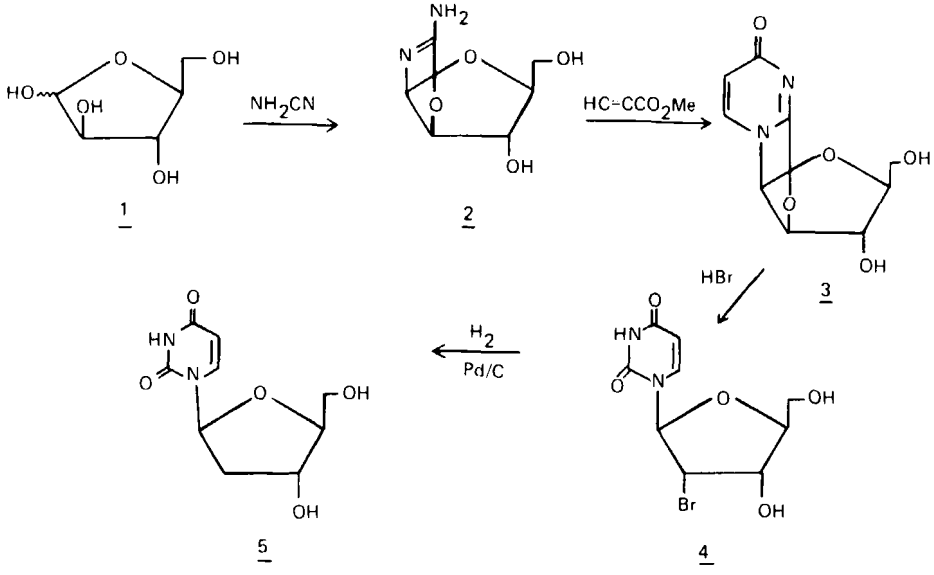
These laboratories had at hand expertise in cytosine arabinoside chemistry[1] and with developments at the Salk Institute[4] it was reasoned that large quantities of L-2'deoxyuridine (L-2'dU) could be prepared conveniently and inexpensively and serve as a building block for oligonucleotides.

METHODOLOGY

By applying the chemistry developed by Orgel[4] with D-arabinose, a route was developed for the conversion of the readily available L-arabinose to L-2' deoxyuridine. L-arabinose could be converted to the aminooxazoline 2 with cyanamide, and 2 could be elaborated to the anhydronucleoside 3 with methyl propiolate. It was subsequently found that the anhydro derivative 3 could be opened with HBr to give the 2'-bromo nucleoside 4, which upon catalytic hydrogenation afforded L-2' deoxyuridine 5. Although reactions of these types have been described in the literature,[3b,5,6] the authors have generally had to resort to protection of sugar -OH functions and chromatography, whereas our series of reactions shown in Scheme 1 can be accomplished without either protection or chromatography.



Scheme 1

Oligonucleotide Synthesis Strategy. Utilizing modifications and improvements of the triester approach,[7,8] we successfully synthesized protected 18-mers of D-thymidine[9] and L-2'dU. Since we anticipated that enzymatic characterizaton

of the oligonucleotides of L-2'dU would be impossible, we conducted parallel oligonucleotide experiments with D-2'dU for eventual enzymatic characterization.

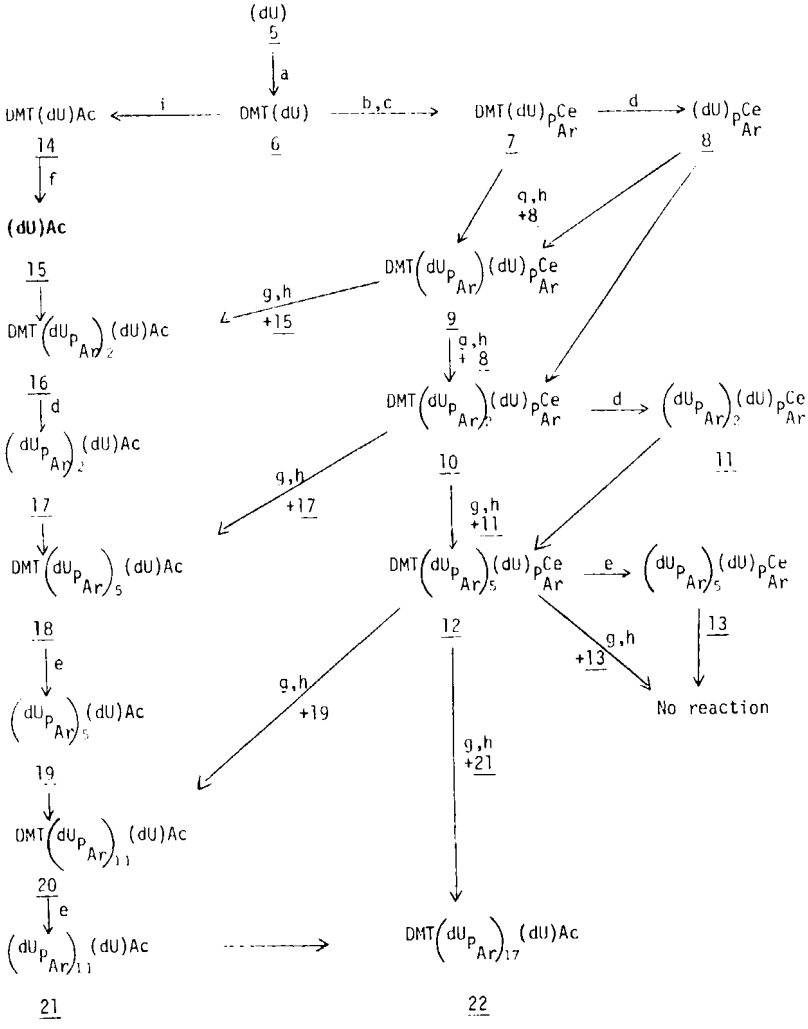
The synthetic strategy is shown in Scheme 2 and applies to both D-and L-2'dU. The 5'-DMT (dU) 6 was phosphorylated on the 3'-position[8] to give the phosphotriester 7, containing the labile cyanoethyl (Ce) and the more stable p-chlorophenyl (Ar) protecting groups. Detritylation of 7 was accomplished with benzenesulfonic acid to afford 8. (We found it far more convenient to use trifluoroacetic acid for subsequent detritylations.) The cyanoethyl group of 7 was removed with triethylamine[8] and subsequently coupled with 8 to give the dinucleotide 9. The coupling reaction was facilitated by the use of mesitylenesulfonyl tetrazole[8]. Normally this catalyst is isolated and used in the solid form. However, since it is rather unstable and aged samples give irreproducible results, we found it more convenient to use it in situ, and generate it in solution. In this manner a fresh volumetric amount could be used as required. This gave consistent results and most couplings were complete within 30 min. at 25°.

Using similar techniques as described above, the trimer 10 was produced which could be detritylated to 11. Subsequent coupling of 10 and 11 afforded the hexanucleotide 12. The hexamer 19 (bearing a terminal 3'-acetate) was prepared analogously and coupled to the hexanucleotide 12 to afford the dodecamer 20. Up until this stage silica gel chromatography had been adequate in isolating pure materials, however it was impossible to obtain a separation of unreacted detritylated hexanucleotide 19 and the fully protected product, dodecanucleotide 20. Examination of the problem on reverse phase TLC plates demonstrated that DMT containing oligomers were well retained, whereas the detritylated species had much greater mobilities. The solution to the separation was solved using RP-2 derivatized silica gel as a gravity column adsorbent. Increasing amounts of acetone (up to 70%) in water eventually eluted pure 12-mer 20. Due to the limited solubility of 20 in the eluting solvent, the fractions containing 20 were generally cloudy and easily visualized. The 12-mer 20 was detritylated to afford 21, which was coupled to the hexaphosphate 12 to give the 18-mer 22. The latter was similarly purified by RP-2 chromatography, which we thoroughly recommend as a separation medium. (Several attempts to couple the detritylated hexamer 13 with the hexanucleotide 12 inexplicably failed with D- and L-2'dU.)

During the course of this work it was reported[10] that 2'deoxyoligonucleotides could be prepared in good yields with little or no protection of the sugar hydroxyls. The method relies mainly on the greater reactivity of the primary 5'-OH over secondary 3'-OH to couple with an appropriate 3'-phosphate. We investigated this technique with L-2'dU but were only able to get as far as the hexanucleotides 25 and 26 before the coupling yields became unacceptable (20% for the formation of 25 from tetramer and dimer).

L-DMT$\left(dUp_{Ar}\right)_5$ (dU) L-$\left(dUp_{Ar}\right)_5$ (dU)

25 26



Reagents, (a) 4,4'-dimethoxytrityl chloride-pyridine (b) p-chlorophenyl phosphodichloridate-1H-1,2,4-triazole (c) cyanoethanol (d) benzenesulfonic acid (e) trifluoroacetic acid (f) acetic acid (g) triethylamine-water-pyridine (h) mesitylenesulfonyl tetrazole (i) acetic anhydride-pyridine.

Key DMT = terminal 5'-dimethoxytrityl

Ac = terminal 3'-acetyl

P^{Ce}_{Ar} = terminal 3'-phosphotriester

Ce = cyanoethyl

Ar = p-chlorophenyl

P_{Ar} = internal 3'-5'phosphotriester.

Scheme 2
The Preparation of the 18 mers of D- and L-2'dU

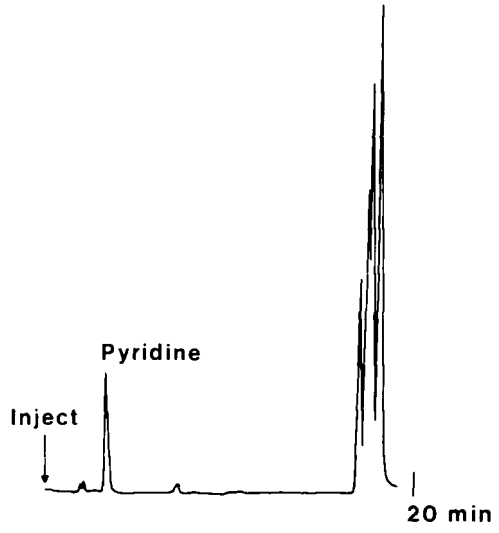


Figure 1: Trinucleotide L-DMT(dUp_{Ar})$_2$(dU)Ac, 16 (μBondapak C18; 25→100% MeCN in 0.1M NH_4OAc over 30 min; 2ml/min)

HPLC. Since the majority of the oligomers that we prepared were foams, gums, or glasses, it was impossible to obtain satisfactory elemental analyses on these materials. Instead we relied heavily on tlc and HPLC to assess their purity utilizing a reverse phase μBondapak C18 column and a single gradient elution system (25 → 100% acetonitrile in 0.1M ammonium acetate over 30 min). Retention times for oligomers increased with chain length and thus the elution times of new oligomers became quite predictable. The same oligomers of L-2'dU and D-2'dU had identical retention times. Crude coupling reaction mixtures (diluted with chloroform/methanol) could be injected directly on to the column. Certain trends in a similar series of oligomers existed and will be reported in full elsewhere[11]. As a representative example, the profile of the trimer 16 is shown in Figure 1. The multiple peaks are due to phosphorus diastereomers.

Deprotection. Initial studies with the 18-mer of thymidine indicated that conc. ammonia/50°/6hr removed the terminal 3'-acetate and internucleotidic p-chlorophenyl groups. Subsequent treatment with 80% acetic acid/50°/2hr removed the 5'-trityl group. However, HPLC showed (Figure 2) that there was significant internucleotide cleavage and 17 distinct small fragments could be observed. The major peak was isolated by preparative HPLC (Figure 3). The multiplicity of the smaller oligomer peaks (Figure 2) represent products of random internucleotide cleavage during the deprotection sequence. The multiplets may represent oligomers bearing respectively 5'- or 3'-terminal phosphates, or both.

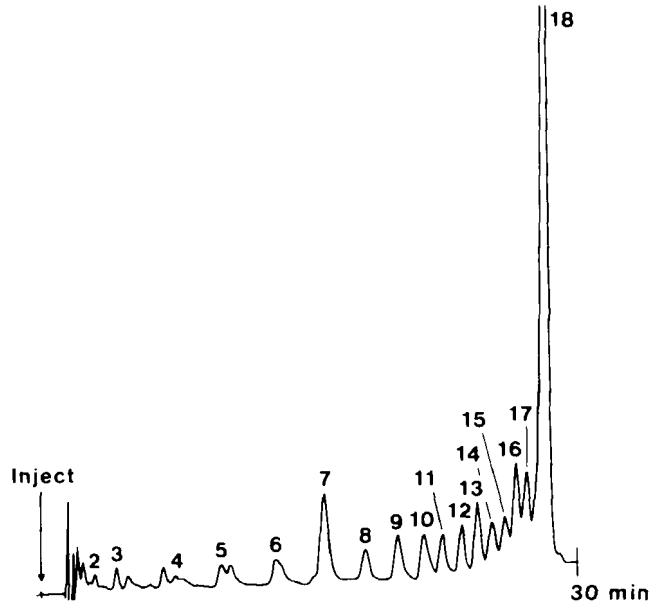


Figure 2: Crude from deprotection of D-Tr(Tp_{Ar})$_{17}$(T)Ac (μBondapak C18; 10-20% MeCN in 0.1M Et_3NHOAc over 60 min; 2ml/min)

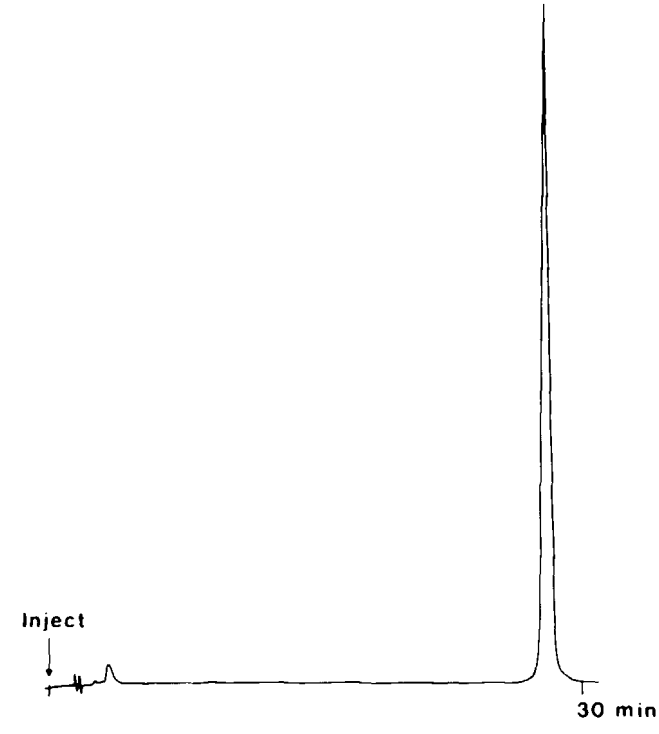


Figure 3: Pure (Tp)$_{17}$T (Conditions as for Figure 2)

When the conc. ammonia treatment was applied to the 18-mers from D- and L-2'dU, HPLC indicated an unacceptable amount of internucleotide cleavage. When p-nitrobenzaldoximate[12] was used to remove the internucleotidic p-chlorophenyl groups, the overall deprotection of the 18-mers was much cleaner (Figure 4). The major peak was isolated by preparative HPLC (Figure 5).

Subsequent studies with smaller oligomers demonstrated that the oxime also removed the terminal 3'-acetate.

The deprotected oligonucleotides derived from D- and L-2'dU had identical retention times on HPLC.

Enzymatic degradation of D-2'dU oligomers. As confirmatory evidence of the chain length of the 2'dU oligomers, each deprotected species (in the D-series) was subjected to digestion with snake venom phosphodiesterase (PDE). Only two products were formed, dU and its 5'-phosphate (pdU). These two materials were conveniently separated by HPLC, and the ratio of pdU:dU easily computed.

$$dU(pdU)_n \xrightarrow[PDE]{\text{snake venom}} dU + npdU$$

In a control experiment it was found that 0.8% of pdU was dephosphorylated to dU under the digest conditions. This correction factor was added and allowances were made for the different extinction coefficients of pdU and dU at the monitoring wavelength (260 nm) (ε_{260}dU = 9.85 x 10^3; ε_{260}pdU = 9.4 x 10^3). The corrected ratios are compiled in Table 1. In addition to the absolute

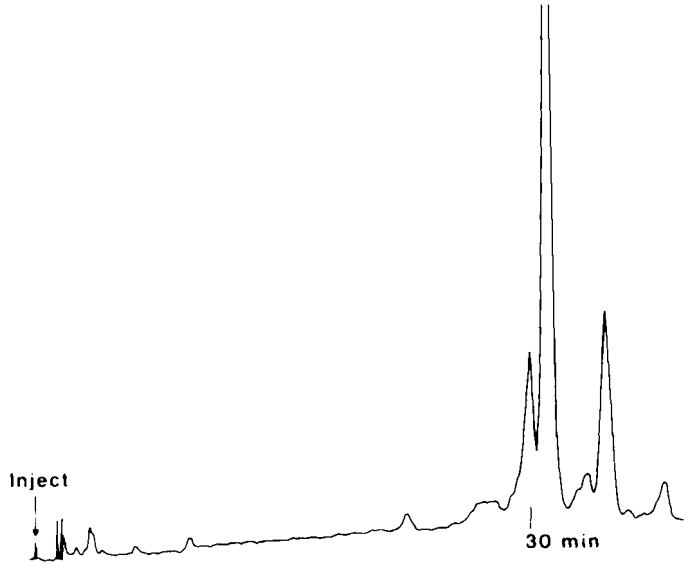


Figure 4: Crude from deprotection of L-DMT(dUp_{Ar})$_{17}$(dU)Ac, 22
(Conditions as for Figure 2)

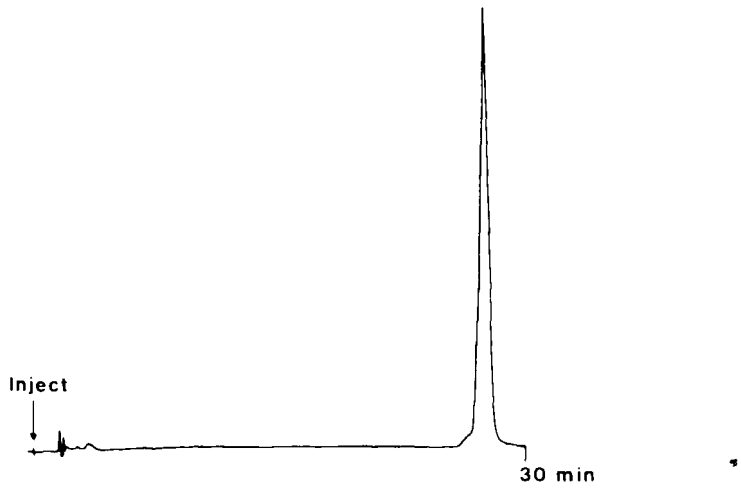


Figure 5: Pure L-$(dU_p)_{17}dU$ (Conditions as for Figure 2)

ratios obtained above, if the enzymatic digests were run with less enzyme and at lower temperature, then it was possible to observe (by HPLC) the stepwise removal of individual units of pdU from the 3'-end of the chain. Indeed the overall chain length could be additionally confirmed by simply counting the fragments. Although all the individual peaks from the 18-mer digestion were not present on a single chromatogram, they were observed on about four different traces obtained over about 24 hours. A representative trace is shown in Figure 6. Thus this technique can be applied to rapidly determine the length of a homooligonucleotide.

Table 1.

Snake venom PDE digest ratios of oligonucleotides

Oligonucleotide	Ratio
$dU(pdU)_n$	dU:pdU
n = 1	1:1.02
2	1:1.87
5	1:4.7
8	1:8.3
11	1:11.2
17	1:15.7

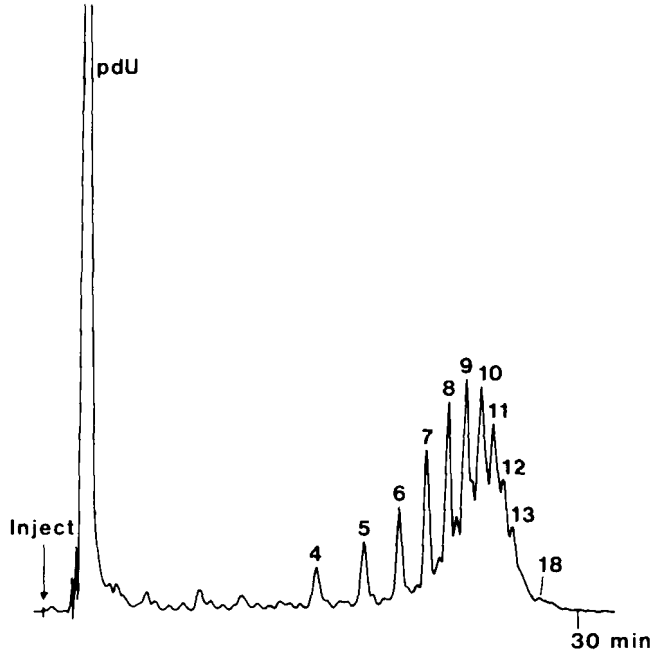


Figure 6: Partial PDE digest of D-$(dUp)_{17}$dU (Conditions as for Figure 2)

We examined the digestion of the tetramer of L-2'dU with snake venom PDE and found that after 20 hr/37° about 2% of L-pdU and the trimer were formed, which increased to about 7% after four days. Under the same conditions the tetramer of D-2'dU was completely digested within 4 hr/37°. In the absence of PDE the tetramer of L-2'dU was completely stable.

Binding Studies. The literature revealed[14] that mixtures of poly dU and poly dA exhibited a temperature dependent absorbance profile characteristic of binding, with T_m values of 43-78° depending upon sodium ion strength (0.038 to 2.04M). With commercially available materials (P-L Biochemicals) we observed a T_m of 40° for 1:1 poly dU:poly dA at 0.5M sodium ion concentration. However, with the synthetic oligonucleotides (up to 18-mers) of D- and L-dU, we were unable to observe any temperature dependent interactions with poly dA in the range 5 to 99°, at various sodium ion concentrations, and in the presence of 0.01M $MgCl_2$. This was particularly disappointing, but may be compared to the results with oligomers of thymidine where it is necessary to have at least hexamers before binding to poly dA can be observed[15]. Similarly, it has recently been shown[16] that the inclusion of uracil into deoxyribonucleotide polymers reduces their template-primer activity.

The biological evaluation of the L-oligonucleotides as potential antiviral agents has been inconclusive. We are not confident of the techniques that are available to deliver them into cells. As more reliable methods are developed, we plan to reevaluate them.

EXPERIMENTAL

Pyridine and triethylamine were distilled from CaH_2 and stored over 3A molecular sieves. Tetrahydrofuran was distilled from LAH and used immediately.

The water-acetone mixtures used in the RP-2 chromatography were degassed by filtering twice through a Millipore filter. RP-2 grade silica gel was prepared by Mallinckrodt, St. Louis, Missouri.

The HPLC equipment consisted of a basic Waters unit (ALC202) comprising Models 6000 and 6000A pumps linked to a Model 660 solvent programmer. Injection on to the Waters reverse phase μBondapak C18 column was via a septumless Valco 7000 loop-injector. Detection at 260nm was via a Tracor variable wavelength spectrometer on to a Texas Instrument flat bed x-y recorder. Protected oligonucleotides were generally dissolved in a chloroform-methanol mixture just prior to injection. The solvent for the column consisted of linear gradients of increasing amounts of acetonitrile in aqueous 0.1M ammonium acetate (about pH 6.8). The aqueous solutions were made up with Baker HPLC grade water and were degassed twice by filtering through a Millipore filter. For deprotected oligonucleotides the aqueous buffer was replaced[13] with 0.1M triethylammonium acetate (TEAA) and samples were dissolved in water prior to injection.

Snake venom phosphodiesterase was obtained from Sigma (catalog #P6877), and made up as a stock solution (1 mg/ml) in 0.1M TRIS buffer. It was stored in the freezer.

The 0.1M TRIS buffer [tris (hydroxymethyl) aminomethane] containing 0.01M $MgCl_2$ was made up from standard reagents in Baker HPLC grade water, then adjusted to pH 8.9 by the addition of 1M HCl.

Thermal melting (T_m) profiles were obtained on a Gilford 2400-2 spectrometer.

L-2'-Bromo-2'deoxyuridine 4. Anhydrous hydrogen bromide was bubbled into a stirred suspension of the L-anhydronucleoside 3[4] (1063g, 4.7 mole) in anhydrous ether (13.6 l) for 12 hours. After which time the ether was removed *in vacuo* and to the resultant solid was added dry dioxane (16 l.). The suspension was refluxed for 8 hours then allowed to cool overnight. The mixture was further cooled in an ice bath and then filtered to afford the L-2'-bromo compound 4 as a white solid mp 198°d (916g, 63%). $C_9H_{11}BrN_2O_5$ requires: Br, 26.02%; fd: 25.94%.

L-2'Deoxyuridine 5. To a solution of L-2'-bromo-2'deoxyuridine 4 (610g, 1.987 mole) in dimethylformamide (4 l) was added magnesium oxide (870g, 21.6 mole) and 10% palladium on charcoal (60g). The hydrogenation of the mixture was run in two 1-gallon autoclaves overnight, when the theoretical amount of hydrogen had been taken up. The mixture was filtered and the filtrate concentrated *in vacuo* to afford an oil. The oil was azeotroped 3 times with absolute ethanol (2l. each) and the residual oil was dissolved in warm 95% ethanol (1 l.). Cooling to ice temperatures afforded a solid (283g) which was recrystallized by dissolving it in absolute ethanol (6 l) and concentrating to

ca. 1.5 l. L-2'Deoxyuridine 5 was thus obtained as a white solid mp 160-3° (268g, 59%); $[\alpha]_D^{22}$ = -49°; CD spectrum 268 (-6000), 252 (0): (lit[3b]: mp 158°; CD spectrum[3b]: 268 (-6720), 252(0); $[\alpha]_D^{22}$ for D-2'deoxyuridine[17] = +50°).

D-and L-DMT(dU)p^{Ar}_{Ce} 7. 2'Deoxyuridine (34.2g, 0.15 mole) was dissolved in dry pyridine (280 ml), then 50 ml of solvent was azeotroped off. 4,4'-Bis dimethoxytrityl chloride (Aldrich) (53.4g, 0.1575 mole) was added over about 5 min, and the reaction stirred for 2 hr. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2 (800 ml) and washed with water (2 x 500 ml). After drying (Na_2SO_4) and filtering, tlc (chloroform) showed about 90 to 95% 5'-DMT and 5 to 10% 3',5'-diDMT compounds. Removal of the solvent gave a golden yellow foam (105g), containing a little pyridine. The foam was azeotroped with reagent pyridine (2 x 250 ml) but the weight remained the same.

1,2,4-Triazole (Aldrich) (27.6g, 0.4 mole) and dry triethylamine (40.4 g, 56 ml, 0.4 mol) were stirred in dry THF (600 ml) and cooled in an ice bath. p-Chlorophenylphosphodichloridate[7] (49.1g, 0.2 mol) was added over 15 min. and the temperature rose to about 45°, and triethylamine HCl precipitated. The suspension was stirred for 30 min. during which time it cooled to room temperature. The crude 5'-dimethoxytrityl-2'-deoxyuridine (approx. 0.15 mol) was dissolved in dry pyridine (300 ml) and added to the phosphorylating mixture. After 30 min. the solution was deep maroon and tlc (20:1, $CHCl_3$:MeOH) indicated the reaction was complete. Cyanoethanol (Aldrich) (42.6g, 41 ml, 0.6 mol) was added and stirred for an additional 1 hr after which time tlc indicated the reaction was complete. The solvent was removed in vacuo at 40° and the deep purple residue treated with CH_2Cl_2 (1000 ml) and water (500 ml). The organic layer was washed with extra water (500 ml) and dried overnight (Na_2SO_4). Filtration and removal of the solvent gave a purple gum which was dissolved in CH_2Cl_2 (100 ml) and added to a column (1000g) of SiO_2 made up in 1% py-CH_2Cl_2. The column was eluted with 2 l. of the same solvent, 5 l. 1% MeOH, 2 l. 2% MeOH and finally 5% MeOH. The mononucleotide 7 was eluted pure (73g) in the later fractions. Impure product (34g) was rechromatographed over 500g, SiO_2 as before to give 7 as a foam (30g). Total yield of 7, based on 2'dU (103g, 89%). tlc: 2 spots Rf 0.4 (1 x ether, 1 x 5% MeOH-$CHCl_3$).

D-and L-(dU)p^{Ar}_{Ce} 8. The mononucleotide 7 (5.5g 0.007 mole) was dissolved in a mixture of $CHCl_3$-MeOH [(7:3), 50 ml] and a 2% solution of benzenesulfonic acid (50 ml) in $CHCl_3$/MeOH (7:3) added. The reddish-orange mixture was stirred at room temperature for 45 min. after which tlc indicated the reaction was complete. The reaction was quenched with pyridine (1 ml) giving a clear solution. The solvent was removed in vacuo and the residue dissolved in $CHCl_3$ (15 ml) and added to a Merck (size C) column of SiO_2 made in 5% MeOH-$CHCl_3$. The column was eluted with 3 l. of the same solvent. The mononucleotide 8 was eluted in the later fractions (2.8g, 84%) giving a hard white foam. tlc: Rf 0.34 (7.5% MeOH-$CHCl_3$).

Dinucleotides D-and L-DMT $\left(dUp_{Ar}\right)$ $(dU)p_{Ar}Ce$ 9. The mononucleotide 7 (1.0g, 1.29 mmole) was dissolved in pyridine (10 ml) and triethylamine (10 ml) added. Water (5 ml) was added to the reaction mixture and the amber solution stirred at room temperature for 15 min. TLC indicated the reaction was complete and the solvent was removed in vacuo. The residue was azeotroped with stock pyridine (3 x 25 ml) and then with dry pyridine (1 x 25 ml). The detritylated mononucleotide 8 (0.85g, 1.8 mmole) was azeotroped with dry pyridine (2 x 25 ml) and redissolved in dry pyridine (25 ml) and added to the above residue. Meanwhile mesitylene sulfonyl chloride (0.85g, 3.9 mmoles) was added to a solution of 1,2,3,4-tetrazole (0.275g, 3.9 mmole) and triethylamine (0.39g, 3.9 mmole) in dry THF (20 ml). The mixture was stirred at room temperature for 30 min. and then the white precipitate ($Et_3N{\cdot}HCl$) removed by filtration and washed with dry THF (5 ml). The THF filtrate was immediately added to the above pyridine solution. The THF was removed in vacuo at 35° leaving a reddish-brown gum. The residue was dissolved in $CHCl_3$ and added to a column of SiO_2 (150g) made up in 1% pyridine-$CHCl_3$. The column was eluted with MeOH-$CHCl_3$-py (3:96:1) yielding the dinucleotide 9 (0.56g, 37%) tlc: Rf 0.4 (7.5% MeOH-$CHCl_3$).

Trinucleotides D-and L-DMT $\left(dUp_{Ar}\right)_2$ $(dU)p_{Ar}Ce$ 10. These were prepared in a similar manner to the dinucleotide 9: from 9 (1.0 mmole), mononucleotide 8 (0.85 mmole), and mesitylene sulfonyl tetrazole (MSTet) (3.0 mmole). Isolated from SiO_2 column with MeOH-$CHCl_3$-pyridine (5:94:1) as a pale yellow hard glass (64%) tlc: Rf 0.5 (7.5% MeOH-$CHCl_3$).

Trinucleotides D-and L-$\left(dUp_{Ar}\right)_2$ $(dU)p_{Ar}Ce$ 11. These were prepared by detritylation of 10 and isolated from SiO_2 chromatography with MeOH-$CHCl_3$ (5:95) as a hard white glass (97%) tlc: Rf 0.7 (10% MeOH-$CHCl_3$).

Hexanucleotides D-and L- DMT $\left(dUp_{Ar}\right)_5$ $(dU)p_{Ar}Ce$ 12. Prepared from trinucleotide 10 (3.0 mmole), trinucleotide 11 (2.7 mmole) and MSTet (10 mmole), and isolated with MeOH-$CHCl_3$-pyridine (6:93:1) from a SiO_2 column as a hard white glass (50%). tlc: Rf 0.4 (15% MeOH-$CHCl_3$).

Trinucleotides D-and L- DMT $\left(dUp_{Ar}\right)_2$ (dU) Ac 16. Prepared from dinucleotide 9 (2.13 mmole), 3'-acetyl-2'deoxyuridine[18] 15 (2.04 mmole), and MSTet (6.4 mmole). Isolated as a white foam (70%) from SiO_2 with $CHCl_3$-MeOH-pyridine (93:6:1). tlc: Rf 0.32 (10% MeOH-$CHCl_3$).

Trinucleotides D-and L-$\left(dUp_{Ar}\right)_2$ (dU) Ac 17. Prepared from trinucleotide 16 as a clear glassy gum (97%) after SiO_2 chromatography with 8% MeOH-$CHCl_3$. tlc: Rf 0.21 (10% MeOH-$CHCl_3$).

Hexanucleotides D-and L-DMT $\left(dUp_{Ar}\right)_5$ (dU) Ac 18. From trinucleotide 10 (0.953 mmole), trinucleotide 17 (0.934 mmole), and MSTet (3 mmol) as a foamy glass (86%) from SiO_2 with $CHCl_3$-MeOH-pyridine (93:6:1). tlc: Rf 0.42, (1 x ether; 1 x 10% MeOH-$CHCl_3$).

Hexanucleotides D-and L-$\left(dUp_{Ar}\right)_5$ (dU) Ac 19. From detritylation of 18 with CF_3CO_2H. Isolated as a white solid (81%) from SiO_2 with 15% MeOH-$CHCl_3$. tlc: Rf 0.24 (10% MeOH-$CHCl_3$), 0.51 (15% MeOH-$CHCl_3$).

Dodecanucleotides D-and L-DMT $\left(dUp_{Ar}\right)_{11}$ (dU) Ac 20. The crude material from the reaction of hexanucleotide 12 (0.43 mmole), hexanucleotide 19 (0.35 mmole), and MSTet (3 mmole) was rapidly chromatographed over SiO_2 with $CHCl_3$-MeOH-pyridine (89:10:1); and the crude 12-mer thus obtained was dissolved in 15% MeOH-$CHCl_3$ and adsorbed on to 10g of RP-2 support. This was added to a column of the same support (100g) made up in 50% acetone-water. Gradient elution up to 70% acetone gave the dodecamer 20 as a white glass (30%). tlc: Rf 0.6 (15% MeOH-$CHCl_3$).

Dodecanucleotides D-and L- $\left(dUp_{Ar}\right)_{11}$ (dU) Ac 21. From detritylation of 20 with trifluoroacetic acid. Isolated from SiO_2:15% MeOH-$CHCl_3$ as a clear glassy foam (97%). tlc: Rf 0.53 (15% MeOH-$CHCl_3$).

Octadecanucleotides D-and L-DMT $\left(dUp_{Ar}\right)_{17}$ (dU) Ac 22. From the hexanucleotide 12 (0.1142 mmole), dodecamer 21 (0.0535 mmole) and MSTet (0.614 mmole) via RP-2 chromatography (75% acetone-water) as a cream colored hard glass (51%) tlc: Rf 0.53 (15% MeOH-$CHCl_3$).

Deprotection of the 18-mers of D- and L-2'dU, 22. p-Nitro-benzaldoxime (166 mg 1 mmole) and tetramethylguanidine (115 mg, 1 mmole) were dissolved in 3 ml of water-dioxane (1:1) giving an orange-red solution. 300 µl of this solution was added to the octadecanucleotide 22 (20 mg) and the reaction mixture stirred at room temperature for 18 hr. The solvent was removed in vacuo, the residue dissolved in 80% aqueous acetic acid (1 ml) and stirred at room temperature for 1.5 hr, and then the solvent removed and the residue treated with conc. NH_3 (1 ml) for 18 hr at room temperature. Removal of the solvent left an oil that was suspended in water (2 ml) and extracted with CH_2Cl_2 (2 x 2 ml). The aqueous layer was filtered and lyophilized giving a cream solid. The product was purified by preparative HPLC. The product was lyophilized several times giving a white fluffy solid. HPLC: 21.85 min. (10 to 15% MeCN in 0.1M TEAA, 30 min.) (Figures 4 and 5).

The other oligonucleotides were deprotected and purified in a similar manner.

General procedure for complete enzymic digestion. The fully deprotected oligonucleotide (about 0.5 mg) was dissolved in the TRIS buffer (40 µl) and the solution of the enzyme (40 µl) added. The solution was then incubated at 37°. The reaction was monitored by HPLC and was almost complete by 4 hr. The reaction was allowed to stay at 37° for 20 hr (overnight) by which time it was complete. It was immediately analyzed by HPLC or frozen. Samples of 2 µl were injected directly on to the µBondapak C18 column under the following conditions and the ratios of pdU:dU computed.

Flow Rate:	2 ml/min.
Solvent Gradient:	0-20% MeCN in 0.1M NH_4OAc (30 min.)
Retention Times:	pdU 1.8 min.
	dU 4.65 min.

General procedure for slow enzymic digestion. The oligonucleotide (about 0.5 mg) was dissolved in the TRIS buffer (100 µl) and a solution of the enzyme (10 µl) added. The hydrolysis was allowed to proceed at room temperature (25°). Samples (5 µl) were withdrawn at 1 hr intervals and assayed by HPLC using the appropriate gradient for the particular oligonucleotide. A representative chromatogram is shown in Figure 6.

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