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Synthesis of 2′-*O*-Methyl/2′-*O*-MOE-L-Nucleoside Derivatives and Their Applications: Preparation of G-Quadruplexes, Their Characterization, and Stability Studies

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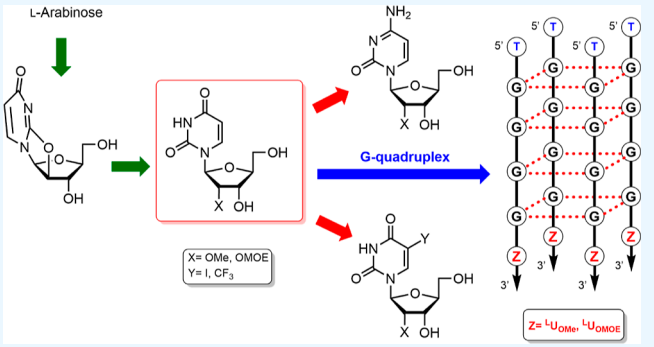
ABSTRACT: Nucleosides and their analogues constitute an important family of molecules with potential antiviral and antiproliferative activity. The enantiomers of natural nucleosides, L-nucleoside derivatives, which have comparable biological activity but more favorable toxicological properties and greater metabolic stability than D-nucleosides, have emerged as a new class of therapeutic agents. Furthermore, L-nucleosides can be used as a building block to prepare L-oligonucleotides, which have identical physical properties in terms of solubility, hybridization kinetics, and duplex thermal stability as D-oligonucleotides but completely orthogonal in nature. Consequently, they are resistant to nuclease degradation, nontoxic, and immunologically passive, which are desirable properties for biomedical applications. Herein, we describe the synthesis of several 2′-*O*-methyl/2′-*O*-MOE-L-nucleoside pyrimidine derivatives and their incorporation into G-rich oligonucleotides. Finally, we evaluated the stability and resistance against nucleases of these new G-quadruplexes, demonstrating the potential of the L-nucleosides described in this work in providing enhanced nuclease resistance with a minimal impact in the nucleic acid structural properties.



1. INTRODUCTION

Chemically modified nucleosides and their analogues are of enormous interest since they constitute an important family of molecules with well demonstrated antiviral and antitumor activity. For an extensive period of time, it was implicit that only natural nucleoside derivatives with a D configuration could display biological activity. However, in the early 1990s, this assumption was re-evaluated, and the L-nucleoside derivatives emerged as a new class of therapeutic agents.[1]

Although the first synthesis of a L-nucleoside dates back to 1964,[2] little attention was paid to this group of derivatives until 1992 with the discovery of lamivudine (**1**, 3TC, Figure 1),[3] used for the treatment of HIV types 1 and 2, and HBV. Since then, the discovery that several viral polymerases could accept phosphorylated L-nucleoside analogues as substrates for their own replication, causing the chain termination of DNA synthesis, has promoted the preparation and evaluation of a large number of L-nucleosides. Among the characteristics of this type of compounds, the following attributes stand out: their comparable biological activity, even greater in some cases, to the D enantiomers; more favorable toxicological profiles; and improved metabolic stability.[4]

A number of other L-nucleoside derivatives have been prepared as antiviral agents. These include the 5-fluoro analogue of lamivudine, emtricitabine (**2**, β-L-2′,3′-dideoxy-3′-thia-5-fluorocytidine, FTC) for the treatment of HIV infections, the β-L-thymidine (**3**, telbivudine), clevudine (**4**), and elvucitabine (**5**) analogues are approved antiviral drugs against HBV, as well as maribavir (**6**), another L-nucleoside against cytomegalovirus infection.[5]

In addition, L-nucleoside derivatives are promising anticancer agents. Troxacitabine (**7**, Figure 1) is the first compound with L isomerism that has shown activity against solid tumors and leukemia. Another example is its 5-fluoro analogue, 5-fluorotroxacitabine (**8**), which has shown in vitro activity against acute myeloid leukemia.[6]

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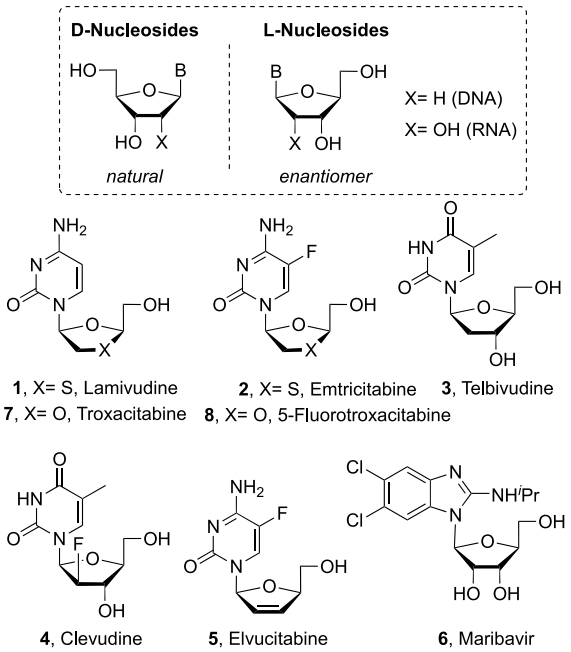


Figure 1. Structures of D- and L-nucleosides and some commercial antiviral (**1**–**6**) and antitumor (**7**–**8**) L-nucleosides.

On the other hand, L-nucleosides can be used as monomers to prepare L-oligonucleotides. Although L-oligonucleotides are not present in nature, they can be easily prepared synthetically in the laboratory.[7] There is a large interest in the synthesis of mirror-image DNA and RNA especially in the aptamer field. For example, Spiegelmers can have a strong binding affinity to target proteins but they are resistant to nuclease degradation while off−target interactions that plague traditional D-oligonucleotide-based technologies are circumvented.[8,9] It was though that L-oligonucleotides have only limited utility since D and L-oligonucleotides cannot form contiguous Watson−Crick base pairs with each other, but recently, L-RNA and L-DNA aptamers have been shown to interact with D-transactivation responsive RNA[10] and conserved structural elements from SARS-CoV-2 genome.[11]

Additionally, Yu and Sczepanski[12] revealed new therapeutic opportunities with L-oligonucleotides. They have described how L-oligonucleotides interact with biological systems at the cellular level, showing that G-rich L-oligonucleotides have potent cytotoxicity and a dramatic impact on gene expression via extensive interactions with endogenous proteins. Also, the anticoagulant/cytotoxic activities of the intramolecular G-quadruplex known as thrombin-binding aptamer have been shown to be modulated by the L-oligonucleotides.[13]

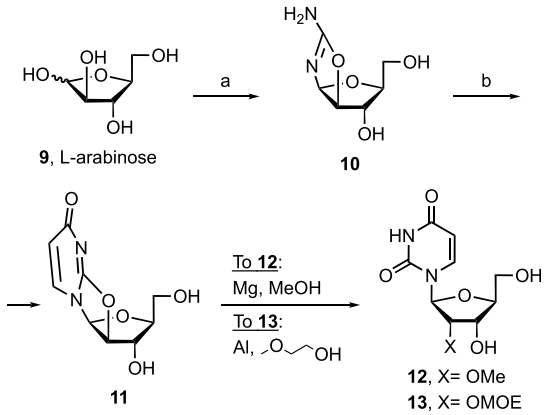
Although the preparation of L-nucleoside building blocks for oligonucleotide synthesis have been described, there is limited description of a 2′-modified L-nucleoside building block ready for incorporation.[14] 2′-Deoxy-2′-fluoro-L-uridine was found to have a superior stability to nuclease degradation and an increased double helix stability instilled by the fluoro modification in the sugar moiety.[14] Since 2′-*O*-methyl and 2′-*O*-methyloxyethyl (MOE) derivatives of the conventional D-nucleosides have proven to be ideal sugar modification for design of therapeutic oligonucleotides,[15] we were inspired to introduce these in L-nucleosides with the intent of increasing the arsenal of available chemical modifications.

Herein, we describe the synthesis of several 2′-*O*-methyl/2′-*O*-MOE-L-nucleoside derivatives and their application in oligonucleotide synthesis. The utility of L-nucleoside was demonstrated by incorporation into a G-quadruplex. Additionally, we characterized the structure of new quadruplexes and studied their stability against nucleases.

2. RESULTS AND DISCUSSION

2.1. Synthesis of 2′-*O*-Methyl/2′-*O*-MOE-L-Nucleoside Derivatives. The strategy for the synthesis of 2′-substituted L-nucleoside derivatives involved the displacement with several nucleophiles on $O^{2,2'}$-anhydro-L-uridine (**11**, Scheme 1),[16]

Scheme 1. Preparation of 2′-*O*-Methyl/2′-*O*-MOE-L-Uridine (12/13)[a]



[a]Reagents and conditions: (a) NC-NH_2, NH_4OH−MeOH, rt, 3 d, 70%; (b) methyl propiolate, EtOH−H_2O, reflux, 4 h, 65%; (to **12**) Mg, MeOH, reflux, 5 h, 85%; (to **13**) Al, 2-methoxyethanol, reflux, 48 h, 55%.

prepared from L-arabinose (**9**). First, the condensation between L-arabinose and cyanamide in a basic medium (MeOH−NH_4OH) for 3 days at rt afforded aminooxazoline **10** in 70% yield. The conformationally locked structure of **10** offered an efficient entry into desired L-nucleoside analogues. Subsequent treatment of **10** with methyl propiolate in an EtOH−H_2O mixture at reflux for 4 h furnished anhydro-L-uridine **11** in 65% isolated yield. To synthesize the 2′-*O*-Me derivative, we used the procedure described by Szlenkier et al.[17] for D-nucleosides due to the availability of reagents and the good yield of the reaction. Hence, compound **11** was added to a suspension of magnesium and iodine in methanol, and the mixture was stirred under reflux for 5 h. However, instead of the desired compound **12**, a derivative, resulting from the iodination of **11** at the nucleobase 5-position, was isolated (40%). To prevent the formation of this byproduct, iodine was not employed as a catalyst.[18] In order to ensure that $Mg(OMe)_2$ has been generated in situ, magnesium was added to methanol and stirred vigorously at 30 °C for 40−45 min, until no H_2 release was observed. Anhydrouridine **11** is then added, and the reaction stirred at reflux for 5 h. Under these conditions, 2′-*O*-methyl-L-uridine (**12**) was obtained in good yield (85%).

The use of 2-methoxyethanol instead of methanol during above procedure failed to generate 2′-*O*-MOE derivative **13**. The synthesis of compound **13** was carried out by adding the anhydrouridine **11** to a mixture of 2-methoxyethanol and aluminum, previously heated under reflux for 2 h, according to

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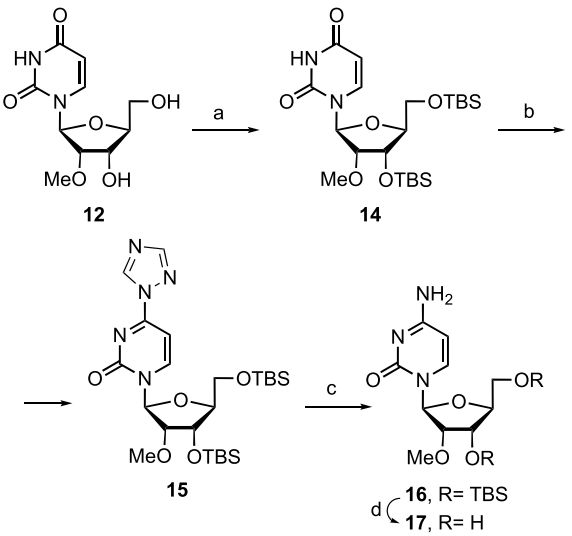
the conditions described by Legorburu et al.[19] After 48 h under reflux, 2′-*O*-MOE-L-uridine (**13**) was isolated in 55% yield.

For the preparation of cytidine derivatives, starting from the corresponding uridine compounds, the uracil base was transformed into cytosine following a conventional protocol.[20] First, 3′- and 5′-hydroxyl groups of **12** were protected as silyl ethers with *tert*-butyldimethylsilyl chloride (TBSCl), imidazole, and CH_2Cl_2 to give **14** (Scheme 2). Reaction of the latter with

Scheme 2. Preparation of 2′-*O*-Methyl-L-Cytidine (17) Using Silyl Protecting Groups[a]



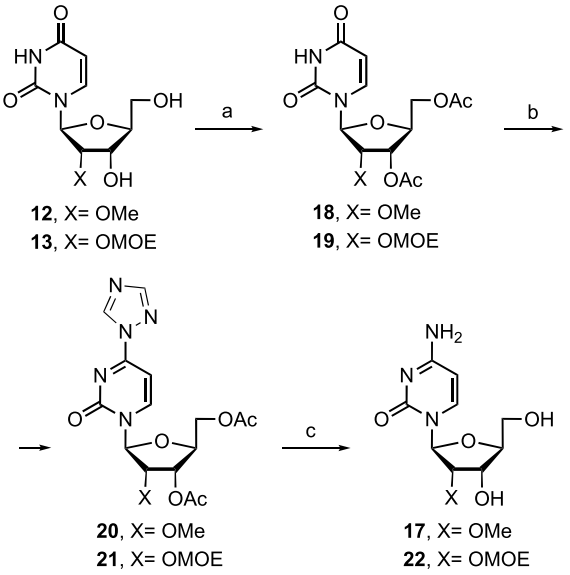
[a]Reagents and conditions: (a) TBSCl, imidazole, CH_2Cl_2, reflux, 5 h, 75%; (b) 1,2,4-triazole, $POCl_3$, Et_3N, MeCN, rt, 1.5 h, 90%; (c) NH_3 (aq), 1,4-dioxane, rt, 5 h, 90%; and (d) TBAF, THF, rt, 3 h, 95%.

tris(1*H*-1,2,4-triazole-1-yl)phosphine oxide, generated in situ with phosphorus oxychloride and 1,2,4-triazole in Et_3N, provided **15** in 90% yield. Subsequent treatment with aqueous ammonia (32%) in 1,4-dioxane afforded **16**. Finally, deprotection of the TBS groups with tetrabutylammonium fluoride (TBAF) in THF led to 2′-*O*-methyl-L-cytidine (**17**) in 58% overall yield and 4 steps from **12**.

In order to make the foregoing protocol more sustainable, the silyl ether protecting groups of the 3′ and 5′ positions were replaced by acetyl groups, avoiding the use of hazardous reagents such as TBSCl and TBAF. Thus, **12** was treated with Ac_2O in pyridine at rt to afford diacetyl compound **18** in 92% yield (Scheme 3). Following a sequence of reactions similar to those carried out above, **18** was easily transformed in **17** via triazole derivative **20**. Gratifyingly, treatment of **20** with aq. ammonia concomitantly deprotected the acetyl groups in situ furnishing **17** in excellent yield. Using a similar synthetic strategy, 2′-*O*-MOE-L-cytidine (**22**) was obtained from 2′-*O*-MOE-L-uridine (**13**; Scheme 3).

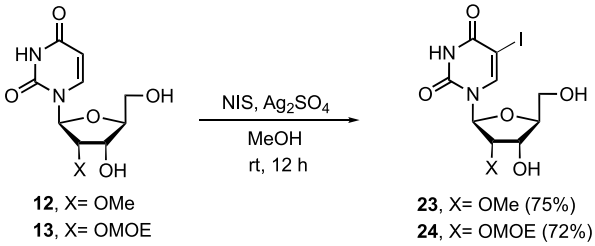
Base modifications of D-nucleoside have been yet another prolific area of research leading to discovery of antiviral drugs such as idoxuridine. Therefore, synthesis of C5-modified pyrimidine nucleosides is worthy of exploration in search for biologically active molecules.[21] In addition to its intrinsic biological value, its coupling with terminal alkenes or alkynes allowed the preparation of novel compounds with potential pharmacological activity.[22] In this vein, the synthesis of 2′-*O*-methyl-5-iodo-L-uridine (**23**, Scheme 4) was performed by reaction of **12** with iodine and $NaNO_2$ in MeCN or MeOH as

Scheme 3. Preparation of 2′-*O*-Methyl/2′-*O*-MOE-L-Cytidine (17/22) Using Acetyl-Protecting Groups[a]



[a]Reagents and conditions: (a) Ac_2O, Py, rt, 12 h, 92% (**18**) and 95% (**19**); (b) 1,2,4-triazole, $POCl_3$, Et_3N, MeCN, rt, 1.5 h, 80% (**20**) and 85% (**21**); and (c) (i) NH_3 (aq), 1,4-dioxane, rt, 5 h, (ii) NH_3 (aq)-MeOH, rt, 12 h, 95% (both, **17** and **22**).

Scheme 4. Preparation of 2′-*O*-Methyl/2′-*O*-MOE-5-Iodo-L-Uridine (23/24)



solvents at 30 °C. This protocol resulted in low yield (30%). In order to improve the yield, we used Ag_2SO_4 as an iodine-activating reagent.[23] The best results were obtained when compound **12** was reacted with 3 equiv of iodine and 3 equiv of Ag_2SO_4 under refluxing MeOH for 24 h. Under these conditions, iodinated compound **23** was isolated in 60% yield. We replaced iodine with *N*-iodosuccinimide (NIS) to further improve the yield. Treatment of **12** with NIS in the presence of Ag_2SO_4 and methanol as solvent worked favorably furnishing compound **23** with a 75% yield after 12 h of reaction at rt. Taking into account that the NIS−Ag_2SO_4 combination provided the best results, this protocol was implemented to carry out the iodination of the 2′-*O*-MOE derivative **13**, isolating the 2′-*O*-MOE-5-iodo-L-uridine compound (**24**) in 72% yield.

Bearing in mind the therapeutic activity of some nucleoside analogues such as 5-trifluorothymidine, an antiviral and anticancer drug,[24] it was worthy to introduce the trifluoromethyl group at position C5 of the base in L-nucleosides **12** and **13**. For this, the protocol described by Ji et al.,[25] which introduces the trifluoromethyl group into heterocycles through a radical mechanism, was selected. Thus, the addition of *tert*-butylhydroperoxide to a mixture of compound **12** and $NaSO_2CF_3$ in water provided 2′-*O*-methyl-5-trifluoromethyl-

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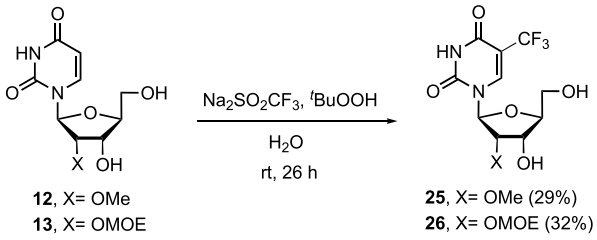
L-uridine (**25**, Scheme 5) in 29% yield after 26 h of reaction at rt. In a similar way, compound **13** was transformed into 2′-*O*-MOE-5-trifluoromethyl-L-uridine (**26**) in modest yield.

Scheme 5. Preparation of 2′-*O*-Methyl/2′-*O*-MOE-5-Trifluoromethyl-L-Uridine (25/26)



12, X= OMe
13, X= OMOE

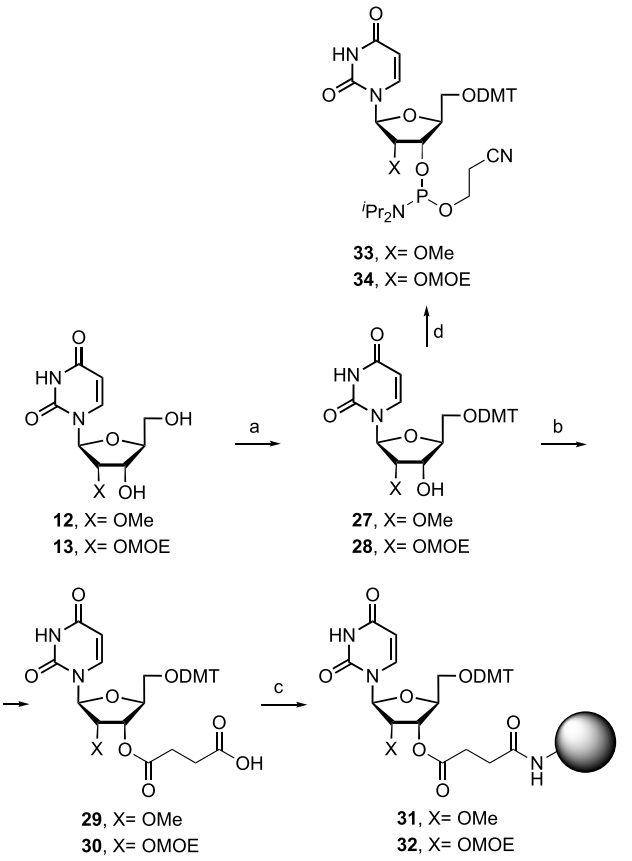
25, X= OMe (29%)
26, X= OMOE (32%)

2.2. Synthesis of Solid Supports Functionalized with 2′-*O*-Methyl/2′-*O*-MOE-L-Uridine Monomers 31 and 32. In order to incorporate the 2′-functionalized L-uridine monomers **12** and **13** in an oligonucleotide on its 3′-end using automated synthesis, the 5′-alcohol was protected with the dimethoxytrityl (DMT) group, and the 3′-hydroxyl group was succinylated (Scheme 6). Therefore, compounds **12** and **13** reacted with DMTCl in the presence of triethylamine using 1,4-dioxane as solvent. The mixture was stirred at 30 °C for 2 h providing compounds **27** and **28** with an 80 and 95% yield, respectively. Next, functionalization of a CPG solid support required a 3′-*O*-succinylated nucleoside. The 3′-secondary alcohol of each L-uridine derivatives **27** and **28** was reacted with succinic anhydride in the presence of 4-dimethylaminopyridine (DMAP) and anhydrous CH_2Cl_2 as the solvent at rt. The L-nucleoside succinate derivatives **29** and **30** were obtained with an 93 and 91% yield, respectively. These compounds were used for the functionalization of long-chain aminoalkyl controlled pore glass (LCAA-CPG) supports in a conventional manner to yield the CPG solid-supports **31** and **32** with 20 and 23 μmol/g loading, respectively.[26]

The synthesis of phosphoramidites derived from 2′-substituted L-uridine was carried out starting from the corresponding 5′-*O*-DMT-protected analogues (Scheme 6). Phosphitylation of **27** and **28**, with chloro-2-cyanoethoxy-*N*,*N*-diisopropylaminophosphine gave the expected phosphoramidites **33** and **34** in 67 and 65% yield, respectively.

2.3. Synthesis and Characterization of G-Quadruplexes with 2′-*O*-Methyl/2′-*O*-MOE-L-Uridine Functionalized at the 3′-End. To investigate the suitability of L-nucleosides in the structures and stability of G-quadruplex structures, we selected a short single-strand sequence TGGGGT (TG_4T), capable of self-assemble in a parallel G-quadruplex by a simple annealing process. The 2′-*O*-methyl-L-uridine (**12**) and 2′-*O*-MOE-L-uridine (**13**) were incorporated at the 3′-end replacing the 3′-T. In this way, we will be able to measure if the presence of L-nucleoside derivatives affects G-quadruplex stability. With this aim, we have synthesized two oligonucleotides and the corresponding controls sequences (Table 1). Four oligonucleotides were synthesized in a straightforward manner from the corresponding commercial or homemade functionalized solid-support of 5′-*O*-DMT-3′-*O*-succ-2′-*O*-methyl-L-uridine (**31**) and 5′-*O*-DMT-3′-*O*-succ-2′-*O*-MOE-L-uridine (**32**) on an automated DNA synthesizer using standard protocols (see the Supporting Information). Mass spectrometry data are shown in Table 1.

Scheme 6. Preparation of CPG Solid Supports Functionalized with 2′-*O*-Methyl/2′-*O*-MOE-L-Uridine and 3′-Phosphoramidites[a]



[a]Reagents and conditions: (a) DMTCl, triethylamine, 1,4-dioxane, 30 °C, 2 h, 80% (**27**) and 95% (**28**); (b) succinic anhydride, DMAP, CH_2Cl_2, rt, overnight, 93% (**29**) and 91% (**30**); (c) 2,2′-dithio-bis(5-nitropyridine), triphenylphosphine, DMAP, LCAA-CPG (69 μmol/g), CH_2Cl_2-acetonitrile, rt, 2 h, 20 μmol/g (**31**) and 23 μmol/g (**32**); and (d) chloro-2-cyanoethoxy-*N*,*N*-diisopropylaminophosphine, $^iPr_2NEt$, CH_2Cl_2, rt, 1 h, 67% (**33**) and 65% (**34**).

Table 1. Oligonucleotide Sequences and Characterization Data by Denaturation Curves and MALDI-TOF[a]

code	sequences (5′ → 3′)	T_m (°C)[b]	MW (calc.)	MW (found)
TG_4T	TGGGGT	67.1	1862	1861.2
TG_4U_{OMe}	TGGGGU$_{OMe}$	71.3	1878	1875.7
$TG_4{}^LU_{OMe}$	TGGGG^{L}U$_{OMe}$	65.8	1878	1878.4
$TG_4{}^LU_{OMOE}$	TGGGG^{L}U$_{OMOE}$	60.3	1921	1919.6

[a]The underlined nucleosides are L-RNA nucleosides. [b]Melting temperatures measured by CD in 10 mM sodium buffer (Figure S2).

The formation of the G-quadruplex structure containing L-uridine ribonucleoside was confirmed by circular dichroism (CD). CD spectra of $TG_4{}^LU_{OMe}$ and $TG_4{}^LU_{OMOE}$ are shown in Figure 2. Both L-uridine G-quadruplexes display the characteristic profile with a negative band at 238 nm and a positive maximum at 265, which are indicative of parallel-stranded tetramolecular G-quadruplex structure. CD techniques were employed to evaluated the thermal stability of $TG_4{}^LU_{OMe}$ and $TG_4{}^LU_{OMOE}$ (Figures S1 and S2, see Supporting Information). In the melting temperature experiments, we used 10 mM sodium chloride buffer to observe denaturation curves. CD

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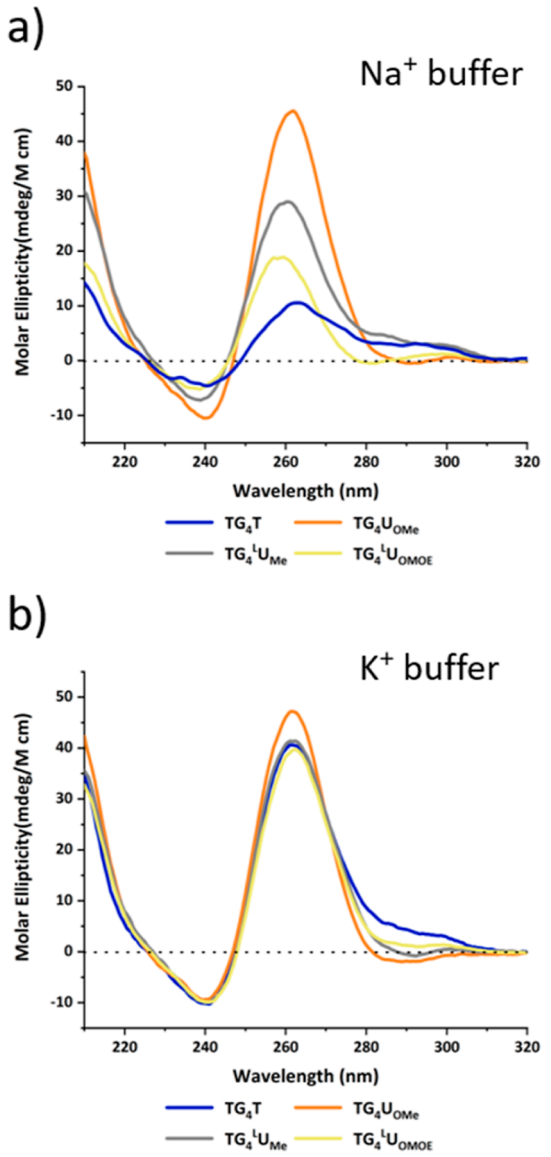

Figure 2. CD spectra of the G-quadruplex at 3 μM oligonucleotide concentration: (a) in 100 mM NaCl and (b) in 10 mM KCl.

spectra in 10 mM Na buffer and denaturation curves are shown in Figures S1 and S2. We used 10 mM Na buffer because at 100 mM sodium buffer, the denaturation curves were not complete at 90 °C. In potassium buffer, no denaturation of the tetrameric quadruplex was observed, as described previously for TG_4T.[27] The sigmoidal profiles of the change in ellipticity versus temperature, at 265 nm wavelength, allowed the estimation of their melting temperature which are between 65.8 °C ($TG_4{}^LU_{OMe}$) and 60.3 °C ($TG_4{}^LU_{OMOE}$) in 10 mM sodium buffer (Figure S2). These values were slightly lower than the corresponding G-quadruplex control sequences TG_4T (67.1 °C) and TG_4U_{OMe} (71.3 °C).

G-quadruplex formation of L-uridine derivatives was confirmed by 20% native polyacrylamide gel electrophoresis (PAGE) in 10 mM TBE buffer containing 100 mM KCl. The parental TG_4T and T_6 sequences as negative controls were used as references. Both L-uridine G-rich structures displayed a major band with reduced mobility. The mobility of the L-uridine G-rich structures was similar to the ones exhibited by G-quadruplex control sequences (TG_4T and TG_4U_{OMe}) in gel electrophoresis (Figure S5). In all cases, the mobility of G-rich sequences was different from the mobility of T_6 sequence, which runs faster. In some cases, small amounts of potentially higher-molecular-weight structures can be observed near the electrophoresis pockets. These data are consistent with the results obtained by the CD experiments confirming the formation of a parallel G-quadruplex structure, and it was not affected by the substitution of the terminal T for a 2′-modified L-uridine ribonucleoside. In addition, the presence of different substituents in the 2′- position of the ribose like the 2′-methoxy (OMe) or 2′-methoxyethyl (OMOE) allowed the formation of the tetrameric parallel structure.

2.4. Nuclease Stability Assay of G-Quadruplex with 2′-*O*-Methyl/2′-*O*-MOE-L-Uridine Functionalized at the 3′-End. The stability of G-quadruplex structures is one of the key elements for their potential use in therapeutic applications. Next, to test the resistance to nucleases of these two G-quadruplex, degradation assays with snake venom phosphodiesterase (SVP) were performed. Set point from 30 min, 1, 4, 8, and 24 h was analyzed by reversed phase high-performance liquid chromatography (HPLC) in comparison with the controls TG_4T and TG_4U_{OMe} analyzed at 5, 10, 15, and 20 min. Results are shown in Table 2 and Figure S3 (see Supporting Information). As anticipated, unmodified TG_4T and TG_4U_{OMe} were completely degraded in 20 min generating the monophosphates derivatives that eluted at the beginning of the chromatogram. Clearly, the two $TG_4{}^LU_{OMOE}$ and $TG_4{}^LU_{OMe}$ exhibited appreciable improvement in resistance to nucleases compared to the unmodified G-quadruplex.

3. CONCLUSIONS

In summary, we have described the synthesis of 2′-substituted L-nucleosides with OMe and OMOE groups derived from uridine, cytidine, 5-iodouridine, and 5-trifluoromethyluridine through a synthetic route that involves the formation of $O^{2,2'}$-anhydro-L-uridine, synthesized from L-arabinose, and its subsequent ring opening with different nucleophilic reagents.

The transformation of the uracil base into cytosine has been carried out using a *tert*-butyldimethylsilyl ether or acetyl-protecting groups for the sugar hydroxyls. The acetyl-protecting group has been more convenient as it involves one less reaction step and requires more economic and less toxic reagents for the protection/deprotection steps, important

Table 2. G-Quadruplex Degradation with Phosphodiesterase I

	degradation (%)								
code	5 min	10 min	15 min	20 min	30 min	1 h	4 h	8 h	24 h
TG_4T	45	52	72	100	100				
TG_4U_{OMe}	50	56	78	100	100				
$TG_4{}^LU_{OMe}$					0	0	57	85	90
$TG_4{}^LU_{OMOE}$					15	17	51	67	70

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aspects in the development of sustainable processes. However, the silyl ether group allows a selective deprotection of the amino function at the nucleobase that could be interesting for other applications.

A key step in the therapeutic application of oligonucleotides is to increase nuclease resistance. With this aim, we have successfully loaded the 2′-*O*-Me- and 2′-*O*-MOE-L-uridine monomers onto the solid support for automated synthesis. G-quadruplex oligonucleotides functionalized at the 3′-end with L-uridine ribonucleotides have been synthesized and characterized. Their structural features and resistant properties were analyzed in comparison with unmodified control oligonucleotide TG_4T. The CD and PAGE gel results suggested that both of them maintain the parallel G-quadruplex structure as TG_4T indicating that the substitution of the 3′ end T by different L-uridine ribonucleotides does not affect the topology of the G4-structure. The results concerning nuclease stability indicated that the incorporation of L-uridine ribonucleotides on the 3′-end increases significantly the parallel G-quadruplex stability in physiological media. Potential applications of these novel monomers include the incorporation of these compounds at the 3′-overhangs of siRNAs[28] and stabilization of aptamers or other therapeutic oligonucleotides.[8−13]

4. EXPERIMENTAL SECTION

4.1. General. *4.1.1. Reagents.* The standard phosphoramidites, reagents solutions, supports, and long-chain aminoalkyl (LCAA)-CPG (69 μmol amino/g) were purchased from Link Technologies Ltd. (Lanarkshire, Scotland, UK). Solvents and other chemicals were purchased from Merck chemicals (Merck-Sigma-Aldrich, Spain). Column chromatography was performed using Silica Gel (60 Å, 230 × 400 mesh). Thin-layer chromatography (TLC) was carried out on Silica-Gel 60 F_{254} plates (Merck-Sigma-Aldrich, Spain). MALDI-TOF experiments were made using a matrix composed of ammonium citrate dibasic and 2′,4′,6′-trihydroxyacetophenone monohydrate (Aldrich). The mobile phase used in HPLC analysis was prepared using triethylammonium acetate (TEAA) and acetonitrile. The HPLC buffer A was the same for analytical and for semipreparative experiments; Buffer A: 5% ACN in 0.1 M TEAA and Buffer B: 70 or 50% ACN in 0.1 M TEAA, respectively. Desalting was performed using molecular sieve columns filled with Sephadex G-25 (NAP-10 or NAP-5), and they were obtained from GE Healthcare (Little Chalfont, UK). Ultrapure water (Millipore) was used in the preparation of aqueous buffers.

4.1.2. Instrumentation. NMR spectra (^{1}H, ^{13}C, ^{19}F, and ^{31}P) were measured on a Varian Mercury-400 (^{1}H 400.13 MHz, ^{13}C 100.6 MHz, ^{19}F 376.5 MHz, and ^{31}P 162.0 MHz) spectrometer or on a Bruker DPX-300 (^{1}H 300.13 MHz, ^{13}C 75.5 MHz, and ^{31}P 121.5 MHz). Chemical shifts for ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR are given in parts per million (ppm) from the residual solvent signal. Coupling constant (J) values are given in Hertz (Hz). Oligonucleotides were synthesized on an Applied Biosystems 3400 DNA Synthesizer. Analytical and semipreparative reverse phase (RP) HPLC was performed on a Waters chromatography system with a 2695 Separations Module equipped with a Waters 2998 Photodiode Array Detector: (a) analytical column ACE 3 μm HILA-3-1546-A (4.6 × 150 mm) with automatic injection, flow of 1 mL/min, and two eluents; (b) semipreparative column Xbridge OST C_{18} 2.5 μm (10 × 50 mm) with manual injection, flow of 1 mL/min. High-resolution mass spectra (HRMS) were recorded on a mass spectrometer under electron spray ionization (ESI). Mass spectra of oligonucleotides were recorded on a MALDI Voyager DETM RP time-of-flight (TOF) spectrometer (Applied Biosystems). Molecular absorption spectra between 220 and 550 nm were recorded with a JASCO V650 spectrophotometer. Hellma quartz cuvettes were used. CD measurements were carried out with a J-810 spectropolarimeter. Enzymatic degradation was carried out in an Eppendorf Thermomixer at 37 °C. Degradation control was monitored by RP-HPLC (Chromatograph Waters) at 260 nm using a semipreparative column Xbridge OST C_{18} 2.5 μm (10 × 50 mm). The infrared spectra have been acquired with a Jasco FT/IR 4700 spectrometer.

4.2. Preparation of 2′-*O*-Methyl/2′-*O*-MOE-L-Uridine Derivatives (12/13). *4.2.1. Synthesis of Aminooxazoline Derivative (**10**).* An aqueous 6 M NH_4OH solution (2.8 mL) was added to a suspension of L-arabinose (1 g, 6.7 mmol) and cyanamide (677 mg, 16.1 mmol) in MeOH (17 mL). The mixture is stirred at rt for 3 days. Then, the temperature was lowered to −10 °C, and stirring is maintained for 12 h. The volume of the solvent is evaporated to half under reduced pressure, and the solid was filtered and washed with MeOH and Et_2O to afford **10** in 70% yield.

*4.2.1.1. 2-Amino-β-L-arabinofurano[1′,2′:4,5]oxazoline (**10**).* White solid. mp 179−181 °C; R_f: 0.40 (1% NH_3/MeOH); ^{1}H NMR (300.13 MHz, D_2O): δ 3.49 (dd, 1H, H_5, J = 12.1, 6.9 Hz), 3.57 (dd, 1H, H_5, J = 12.1, 5.4 Hz), 3.98 (m, 1H, H_4), 4.29 (dd, 1H, H_3, J = 3.1, 1.0 Hz), 4.89 (dd, 1H, H_2, J = 5.5, 1.0 Hz), 5.87 (d, 1H, H_1, J = 5.5 Hz) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ 61.2 (C_5), 75.2 (C_3), 84.3 (C_4), 88.7 (C_2), 98.6 (C_1), 164.8 (C_7) ppm; HRMS (ESI$^+$, m/z): calcd for $C_6H_{11}N_2O_4$ $[M + H]^+$, 175.0713; found, 175.0718, calcd for $C_6H_{10}N_2NaO_4$ $[M + Na]^+$, 197.0533; found, 197.0530, calcd for $C_6H_{10}KN_2O_4$ $[M + K]^+$, 213.0272; found, 213.0268.

*4.2.2. Synthesis of Anhydrouridine **11**.* Methyl propiolate (1.6 mL, 18.4 mmol) was added to a solution of **10** (1.6 g, 9.2 mmol) in 23 mL of EtOH/H_2O (1:1, v/v), and the mixture is stirred at reflux for 4 h. The volume of the solvent was evaporated to half under reduced pressure, and the solid was filtered and washed with EtOH and H_2O to afford **11** in 65% yield.

*4.2.2.1. $O^{2,2'}$-Anhydro-L-uridine (**11**).* White solid. mp 237−239 °C; R_f: 0.53 (40% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, D_2O): δ 3.56 (m, 2H, $H_{5'}$), 4.40 (m, 1H, $H_{4'}$), 4.67 (m, 1H, $H_{3'}$), 5.47 (d, 1H, $H_{2'}$, J = 5.9 Hz), 6.19 (d, 1H, H_5, J = 7.4 Hz), 6.54 (d, 1H, $H_{1'}$, J = 5.9 Hz), 7.92 (d, 1H, H_6, J = 7.4 Hz) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ 60.9 ($C_{5'}$), 75.5 ($C_{3'}$), 89.6 ($C_{2'}$), 89.8 ($C_{4'}$), 91.0 ($C_{1'}$), 108.7 (C_5), 138.4 (C_6), 161.2 (C_2), 175.7 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_9H_{11}N_2O_5$ $[M + H]^+$, 227.0662; found, 227.0658, calcd for $C_9H_{10}N_2NaO_5$ $[M + Na]^+$, 249.0482; found, 249.0477, calcd for $C_9H_{10}KN_2O_5$ $[M + K]^+$, 265.0221; found, 265.0216.

*4.2.3. Synthesis of 2′-O-Methyl-L-uridine (**12**).* A suspension of magnesium (430 mg, 17.6 mmol) in anhydrous MeOH (13 mL) was stirred vigorously at 30 °C for 45 min. Then, anhydrouridine **12** (200 mg, 0.88 mmol) was added and refluxed for 5 h. The mixture was cooled, first to rt and then to 0 °C, and glacial acetic acid was added until neutral pH. Subsequently, the solvents are evaporated until a foam was obtained which was redissolved in EtOH and refluxed for 2 h. After filtration over Celite, the crude was purified by column

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chromatography (5% MeOH/CH_2Cl_2), isolating **12** with 85% yield.

*4.2.3.1. 2′-O-Methyl-L-uridine (**12**).* White solid. mp 156−158 °C; R_f: 0.77 (30% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, D_2O): δ 3.50 (s, 3H, O−*Me*), 3.79 (dd, 1H, $H_{5'}$, J = 12.9, 4.3 Hz), 3.91 (dd, 1H, $H_{5'}$, J = 12.8, 2.8 Hz), 4.04 (dd, 1H, $H_{2'}$, J = 5.2, 4.0 Hz), 4.09 (ddd, 1H, $H_{4'}$, J = 6.3, 4.2, 3.0 Hz), 4.32 (t, 1H, $H_{3'}$, J = 5.7 Hz), 5.88 (d, 1H, H_5, J = 8.1 Hz), 5.96 (d, 1H, $H_{1'}$, J = 3.9 Hz), 7.89 (d, 1H, H_6, J = 8.1 Hz) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ 58.2 (CH_3), 60.4 ($C_{5'}$), 68.1 ($C_{3'}$), 82.6 ($C_{2'}$), 84.2 ($C_{4'}$), 87.6 ($C_{1'}$), 102.2 (C_5), 141.7 (C_6), 151.4 (C_2), 166.1 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{10}H_{15}N_2O_6$ [M + H]$^+$, 259.0925; found, 259.0928, calcd for $C_{10}H_{14}N_2NaO_6$ [M + Na]$^+$, 281.0744; found, 281.0747, calcd for $C_{10}H_{14}KN_2O_6$ [M + K]$^+$, 297.0483; found, 297.0486.

*4.2.4. Synthesis of 2′-O-(2-Methoxyethyl)-L-uridine (**13**).* A suspension of aluminum powder (36 mg, 1.3 mmol) in 2-methoxyethanol (1 mL) was stirred at reflux for 2 h. After the mixture has cooled to rt, **11** (50 mg, 0.22 mmol) was added and refluxed for 48 h. Next, the mixture has cooled to rt and crushed and coevaporated with MeOH (three times). The crude was purified by column chromatography (5% MeOH/CH_2Cl_2), obtaining **13** in 55% yield.

*4.2.4.1. 2′-O-(2-Methoxyethyl)-L-uridine (**13**).* White solid. mp 116−118 °C; R_f: 0.77 (30% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, DMSO-d_6): δ 3.22 (s, 3H, O−*Me*), 3.44 (t, 2H, $H_{2''}$, J = 4.8 Hz), 3.62 (m, 4H, $H_{5'}$ + $H_{1''}$), 3.85 (q, 1H, $H_{4'}$, J = 3.1 Hz), 3.94 (t, 1H, $H_{2'}$, J = 5.0 Hz), 4.09 (q, 1H, $H_{3'}$, J = 5.0 Hz), 5.06 (d, 1H, $OH_{3'}$, J = 5.7 Hz), 5.16 (t, 1H, $OH_{5'}$, J = 5.0 Hz), 5.65 (d, 1H, H_5, J = 8.1 Hz), 5.84 (d, 1H, $H_{1'}$, J = 5.1 Hz), 7.92 (d, 1H, H_6, J = 8.1 Hz), 11.35 (sa, 1H, NH) ppm; ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 58.1 (CH_3), 60.6 ($C_{5'}$), 68.4 ($C_{3'}$), 69.0 ($C_{1''}$), 71.2 ($C_{2''}$), 81.4 ($C_{2'}$), 85.1 ($C_{4'}$), 86.2 ($C_{1'}$), 101.8 (C_5), 140.5 (C_6), 150.6 (C_2), 163.2 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{12}H_{19}N_2O_7$ [M + H]$^+$, 303.1187; found, 303.1190, calcd for $C_{12}H_{18}N_2NaO_7$ [M + Na]$^+$, 325.1006; found, 325.0997, calcd for $C_{12}H_{18}KN_2O_7$ [M + K]$^+$, 341.0746; found, 341.0736.

4.3. Preparation of 2′-*O*-Methyl-L-cytidine (17) Using Silyl Protecting Groups. *4.3.1. Synthesis of TBS Protected **14**.* Imidazole (78 mg, 1.2 mmol) and TBSCl (173 mg, 1.2 mmol) were added to a solution of **12** (60 mg, 0.23 mmol) in anhydrous CH_2Cl_2 (0.8 mL). The reaction was stirred at reflux for 5 h. Then, water/ice is added and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude was purified by column chromatography (20% EtOAc/Hexane), isolating **14** in 75% yield.

*4.3.1.1. 3′,5′-Bis-O-(tert-Butyldimethylsilyl)-2′-O-Methyl-L-uridine (**14**).* White solid. mp 65−66 °C; R_f: 0.29 (20% EtOAc/Hexane); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.06 (s, 3H, Si−*Me*), 0.08 (s, 3H, Si−*Me*), 0.09 (s, 3H, Si−*Me*), 0.10 (s, 3H, Si−*Me*), 0.88 (s, 9H, Si-^{t}*Bu*), 0.91 (s, 9H, Si-^{t}*Bu*), 3.54 (s, 3H, O−*Me*), 3.58 (dd, 1H, $H_{2'}$, J = 4.8, 1.4 Hz), 3.75 (dd, 1H, $H_{5'}$, J = 10.6, 1.0 Hz), 4.03 (m, 2H, $H_{4'}$ + $H_{5'}$), 4.21 (dd, 1H, $H_{3'}$, J = 7.4, 4.9 Hz), 5.67 (d, 1H, H_5, J = 8.1 Hz), 5.92 (d, 1H, $H_{1'}$, J = 1.4 Hz), 8.05 (d, 1H, H_6, J = 8.1 Hz), 9.99 (sa, 1H, NH) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −5.5 (Si−CH_3), −5.4 (Si−CH_3), −4.8 (Si−CH_3), −4.5 (Si−CH_3), 18.2 (SiCMe_3), 18.5 (SiCMe_3), 25.8 (CH_3-^{t}Bu), 26.0 (CH_3-^{t}Bu), 58.3 (O−CH_3), 60.8 ($C_{5'}$), 68.5 ($C_{3'}$), 83.8 ($C_{4'}$), 84.4 ($C_{2'}$), 87.6 ($C_{1'}$), 102.0 (C_5), 140.2 (C_6), 150.4 (C_2), 164.0 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{22}H_{43}N_2O_6Si_2$ [M + H]$^+$, 487.2654; found, 487.2653, calcd for $C_{22}H_{42}N_2NaO_6Si_2$ [M + Na]$^+$, 509.2474; found, 509.2472, calcd for $C_{22}H_{42}KN_2O_6Si_2$ [M + K]$^+$, 525.2213; found, 525.2211.

*4.3.2. Synthesis of Triazole Derivative **15**.* $POCl_3$ (33 μL, 0.35 mmol) was added to a suspension of 1,2,4-triazole (193 mg, 2.79 mmol) in anhydrous MeCN (2.3 mL). Then, anhydrous Et_3N (429 μL, 3.08 mmol) and a solution of **14** (68 mg, 0.14 mmol) in anhydrous MeCN (0.5 mL) were added at 0 °C. The reaction was stirred at rt for 1.5 h. Subsequently, 120 μL of Et_3N and 30 μL of water are added, and the mixture was stirred for 10 min. Solvents were evaporated, the residue was dissolved in CH_2Cl_2, and washed with $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (40% EtOAc/Hexane), isolating **15** in 90% yield.

*4.3.2.1. 3′,5′-Bis-O-(tert-Butyldimethylsilyl)-2′-O-methyl-4-(1,2,4-triazol-1-yl)-L-cytidine (**15**).* Hygroscopic solid. R_f 0.31 (40% EtOAc/Hexane); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.03 (s, 3H, Si−Me), 0.06 (s, 3H, Si−Me), 0.14 (s, 3H, Si−Me), 0.16 (s, 3H, Si−Me), 0.87 (s, 9H, Si-^{t}Bu), 0.97 (s, 9H, Si-^{t}Bu), 3.68 (m, 1H, $H_{2'}$), 3.69 (s, 3H, O−*Me*), 3.81 (d, 1H, $H_{5'}$, J = 11.1 Hz), 4.17 (m, 3H, $H_{3'}$ + $H_{4'}$ + $H_{5'}$), 5.98 (s, 1H, $H_{1'}$), 6.96 (d, 1H, H_5, J = 7.2 Hz), 8.12 (s, 1H, $H_{triazol}$), 8.86 (d, 1H, H_6, J = 7.2 Hz), 9.25 (s, 1H, $H_{triazol}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −5.3 (Si−CH_3), −5.2 (Si−CH_3), −4.8 (Si−CH_3), −4.4 (Si−CH_3), 18.2 (SiCMe_3), 18.7 (SiCMe_3), 25.8 (CH_3-^{t}Bu), 26.2 (CH_3-^{t}Bu), 58.6 (O−CH_3), 60.2 ($C_{5'}$), 67.6 ($C_{3'}$), 83.6 ($C_{4'}$), 84.0 ($C_{2'}$), 89.4 ($C_{1'}$), 94.3 (C_5), 143.4 ($C_{triazol}$), 147.3 (C_6), 154.1 ($C_{triazol}$), 154.4 (C_2), 159.4 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{24}H_{44}N_5O_5Si_2$ [M + H]$^+$, 538.2875; found, 538.2872, calcd for $C_{24}H_{43}N_5NaO_5Si_2$ [M + Na]$^+$, 560.2695; found, 560.2691, calcd for $C_{24}H_{43}KN_5O_5Si_2$ [M + K]$^+$, 576.2434; found, 576.2432.

*4.3.3. Synthesis of Silylated Cytidine Derivative **16**.* To a solution of **15** (26 mg, 0.05 mmol) in 1,4-dioxane (1 mL) at 0 °C was added 285 μL of aqueous NH_3 (32%). The reaction was stirred at rt for 5 h. Solvents were evaporated under reduced pressure, and the crude was purified by column chromatography (5% MeOH/CH_2Cl_2), isolating **16** in 90% yield.

*4.3.3.1. 3′,5′-Bis-O-(tert-Butyldimethylsilyl)-2′-O-Methyl-L-cytidine (**16**).* White solid. mp 104−105 °C; R_f: 0.57 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 0.05 (s, 3H, Si−*Me*), 0.07 (s, 3H, Si−*Me*), 0.10 (s, 3H, Si−*Me*), 0.11 (s, 3H, Si−*Me*), 0.88 (s, 9H, Si-^{t}*Bu*), 0.93 (s, 9H, Si-^{t}*Bu*), 3.60 (m, 1H, $H_{2'}$), 3.62 (s, 3H, O−*Me*), 3.77 (d, 1H, $H_{5'}$, J = 10.6 Hz), 4.05 (m, 2H, $H_{4'}$ + $H_{5'}$), 4.18 (dd, 1H, $H_{3'}$, J = 8.5, 4.7 Hz), 5.65 (d, 1H, H_5, J = 7.4 Hz), 5.94 (s, 1H, $H_{1'}$), 8.15 (s, 2H, NH_2), 8.17 (d, 1H, H_6, J = 2.1 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ −5.4 (Si−CH_3), −5.3 (Si−CH_3), −4.8 (Si−CH_3), −4.4 (Si−CH_3), 18.2 (SiCMe_3), 18.6 (SiCMe_3), 25.8 (CH_3-^{t}Bu), 26.1 (CH_3-^{t}Bu), 58.3 (O−CH_3), 60.5 ($C_{5'}$), 68.1 ($C_{3'}$), 83.1 ($C_{4'}$), 84.3 ($C_{2'}$), 88.3 ($C_{1'}$), 94.2 (C_5), 141.5 (C_6), 156.0 (C_2), 166.0 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{22}H_{44}N_3O_5Si_2$ [M + H]$^+$, 486.2814; found, 486.2810, calcd for $C_{22}H_{43}N_3NaO_5Si_2$ [M + Na]$^+$, 508.2633; found, 508.2629, calcd for $C_{22}H_{43}KN_3O_5Si_2$ [M + K]$^+$, 524.2373; found, 524.2368.

*4.3.4. Synthesis of 2′-O-Methyl-L-cytidine (**17**). 4.3.4.1. Method A: from **16**.* TBAF (3.7 mL, 3.72 mmol, 1.0 M in THF) was added to a solution of **16** (275 mg, 0.62

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mmol) in anhydrous THF (6.2 mL) at 0 °C. After 5 min, the ice bath was removed, and the mixture was stirred at rt for 3 h. Next, solvents were evaporated, and the residue was purified by column chromatography (10% MeOH/CH_2Cl_2), isolating **17** in 95% yield.

*4.3.4.2. Method B: from **20**.* To a solution of **20** (54 mg, 0.13 mmol) in 1,4-dioxane (2.5 mL) at 0 °C was added 0.74 mL of aqueous NH_3 (32%). The reaction was stirred at rt for 5 h. Subsequently, 2.5 mL of NH_3 (ac)/MeOH (1:1, v/v) was added, and stirring was maintained for 12 h. Next, solvents were evaporated under reduced pressure, and the crude was purified by column chromatography (20% MeOH/CH_2Cl_2), isolating **17** in 95% yield.

*4.3.4.3. 2′-O-Methyl-L-cytidine (**17**).* White solid. mp 252−253 °C; R_f: 0.48 (30% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, D_2O): δ 3.51 (s, 3H, O−*Me*), 3.79 (dd, 1H, $H_{5''}$, J = 12.9, 4.3 Hz), 3.92 (dd, 1H, $H_{5''}$, J = 12.9, 2.8 Hz), 3.98 (dd, 1H, $H_{2'}$, J = 5.2, 3.5 Hz), 4.07 (m, 1H, $H_{4'}$), 4.27 (dd, 1H, $H_{3'}$, J = 6.4, 5.5 Hz), 5.94 (d, 1H, $H_{1'}$, J = 3.4 Hz), 6.03 (d, 1H, H_5, J = 7.6 Hz), 7.86 (d, 1H, H_6, J = 7.6 Hz) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ 58.2 (CH_3), 60.4 ($C_{5'}$), 68.1 ($C_{3'}$), 82.9 ($C_{2'}$), 83.8 ($C_{4'}$), 88.4 ($C_{1'}$), 96.1 (C_5), 141.6 (C_6), 156.9 (C_2), 165.9 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{10}H_{16}N_3O_5$ [M + H]$^+$, 258.1084; found, 258.1092, calcd for $C_{10}H_{15}N_3NaO_5$ [M + Na]$^+$, 280.0904; found, 280.0910.

4.4. Preparation of 2′-*O*-Methyl/2′-*O*-MOE-L-Cytidine (17/22) Using Acetyl-Protecting Groups. *4.4.1. Synthesis of Acetyl-Protected **18** and **19**.* Acetic anhydride (0.26 mL, 2.73 mmol) was added to a solution of **12** (100 mg, 0.39 mmol) in anhydrous pyridine (1.3 mL) at 0 °C, and the reaction was stirred at rt for 12 h. After the addition of CH_2Cl_2, the organic phase was washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude was purified by column chromatography (90% EtOAc/Hexane-EtOAc as gradient eluent), isolating **18** in 92% yield. A similar procedure as that described for **18** starting from **13** afford compound **19** in 95% yield.

*4.4.1.1. 3′,5′-di-O-Acetyl-2′-O-methyl-L-uridine (**18**).* White solid. mp 60−61 °C; R_f: 0.31 (80% EtOAc/Hexane); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 2.12 (s, 3H, OCO−*Me*), 2.14 (s, 3H, OCO−*Me*), 3.47 (s, 3H, O−*Me*), 4.04 (dd, 1H, $H_{2'}$, J = 5.3, 3.4 Hz), 4.36 (m, 3H, $H_{4'}$ + $H_{5'}$), 4.98 (t, 1H, $H_{3'}$, J = 5.8 Hz), 5.76 (d, 1H, H_5, J = 8.2 Hz), 5.91 (d, 1H, $H_{1'}$, J = 3.3 Hz), 7.53 (d, 1H, H_6, J = 8.1 Hz), 9.52 (sa, 1H, NH) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.8 (OCO−CH_3), 20.9 (OCO−CH_3), 59.2 (O−CH_3), 62.6 ($C_{5'}$), 70.0 ($C_{3'}$), 79.3 ($C_{4'}$), 81.7 ($C_{2'}$), 88.7 ($C_{1'}$), 102.8 (C_5), 139.3 (C_6), 150.2 (C_2), 163.2 (C_4), 170.3 (2 OC═O) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{14}H_{19}N_2O_8$ [M + H]$^+$, 343.1136; found, 343.1142, calcd for $C_{14}H_{18}N_2NaO_8$ [M + Na]$^+$, 365.0955; found, 365.0963, calcd for $C_{14}H_{18}KN_2O_8$ [M + K]$^+$, 381.0695; found, 381.0703.

*4.4.1.2. 3′,5′-di-O-Acetyl-2′-O-(2-methoxyethyl)-L-uridine (**19**).* Clear oil. R_f: 0.45 (EtOAc); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 2.12 (s, 3H, OCO−*Me*), 2.14 (s, 3H, OCO−*Me*), 3.30 (s, 3H, O−*Me*), 3.47 (m, 2H, $H_{2''}$), 3.68 (m, 1H, $H_{1''}$), 3.82 (m, 1H, $H_{1''}$), 4.30 (dd, 1H, $H_{2'}$, J = 5.5, 3.9 Hz), 4.34 (d, 2H, $H_{5'}$, J = 3.4 Hz), 4.40 (dt, 1H, $H_{4'}$, J = 6.3, 3.3 Hz), 4.98 (t, 1H, $H_{3'}$, J = 5.9 Hz), 5.74 (d, 1H, H_5, J = 8.1 Hz), 5.89 (d, 1H, $H_{1'}$, J = 3.8 Hz), 7.50 (d, 1H, H_6, J = 8.2 Hz), 9.35 (sa, 1H, NH) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.7 (OCO−CH_3), 20.9 (OCO−CH_3), 59.1 (O−CH_3), 62.8 ($C_{5'}$), 70.3 ($C_{3'}$), 70.7 ($C_{1''}$), 72.2 ($C_{2''}$), 79.4 ($C_{4'}$), 80.4 ($C_{2'}$), 89.3 ($C_{1'}$), 102.6 (C_5), 139.7 (C_6), 150.2 (C_2), 163.2 (C_4), 170.28 (OC═O), 170.34 (OC═O) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{16}H_{23}N_2O_9$ [M + H]$^+$, 387.1398; found, 387.1406, calcd for $C_{16}H_{22}N_2NaO_9$ [M + Na]$^+$, 409.1218; found, 409.1226, calcd for $C_{16}H_{22}KN_2O_9$ [M + K]$^+$, 425.0957; found, 425.0966.

*4.4.2. Synthesis of Triazol Derivatives **20** and **21**.* A similar procedure as that described for **15** starting from **18** or **19** afford **20** (column chromatography in 10% Hexane/EtOAc) or **21** (column chromatography in EtOAc) in 80% or 85% yield, respectively.

*4.4.2.1. 3′,5′-di-O-Acetyl-2′-O-methyl-4-(1,2,4-triazol-1-yl)-L-cytidine (**20**).* White solid. mp 160−161 °C; R_f: 0.23 (80% EtOAc/Hexane); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 2.14 (s, 3H, OCO−*Me*), 2.16 (s, 3H, OCO−*Me*), 3.64 (s, 3H, O−*Me*), 4.17 (dd, 1H, $H_{2'}$, J = 5.0, 1.5 Hz), 4.44 (t, 2H, $H_{5'}$, J = 3.1 Hz), 4.54 (dt, 1H, $H_{4'}$, J = 9.1, 2.7 Hz), 4.79 (dd, 1H, $H_{3'}$, J = 9.1, 5.0 Hz), 6.01 (d, 1H, $H_{1'}$, J = 1.4 Hz), 7.08 (d, 1H, H_5, J = 7.3 Hz), 8.13 (s, 1H, $H_{triazol}$), 8.41 (d, 1H, H_6, J = 7.3 Hz), 9.26 (s, 1H, $H_{triazol}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.7 (OCO−CH_3), 21.0 (OCO−CH_3), 59.3 (O−CH_3), 61.7 ($C_{5'}$), 69.1 ($C_{3'}$), 79.1 ($C_{4'}$), 81.8 ($C_{2'}$), 90.5 ($C_{1'}$), 94.7 (C_5), 143.5 ($C_{triazol}$),145.8 (C_6), 154.1 (C_2), 154.3 ($C_{triazol}$), 159.6 (C_4), 170.1 (OC═O), 170.2 (OC═O) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{16}H_{20}N_5O_7$ [M + H]$^+$, 394.1357; found, 394.1353, calcd for $C_{16}H_{19}N_5NaO_7$ [M + Na]$^+$, 416.1177; found, 416.1172, calcd for $C_{16}H_{19}KN_5O_7$ [M + K]$^+$, 432.0916; found, 432.0912.

*4.4.2.2. 3′,5′-di-O-Acetyl-2′-O-(2-methoxyethyl)-4-(1,2,4-triazol-1-yl)-L-cytidine (**21**).* White solid. mp 131−133 °C;. R_f: 0.29 (EtOAc); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 2.12 (s, 3H, OCO−*Me*), 2.14 (s, 3H, OCO−*Me*), 3.33 (s, 3H, O−*Me*), 3.52 (t, 2H, $H_{2''}$, J = 4.4 Hz), 3.85 (dt, 1H, $H_{1''}$, J = 11.7, 4.2 Hz), 4.08 (dt, 1H, $H_{1''}$, J = 11.6, 4.6 Hz), 4.36 (dd, 1H, $H_{2'}$, J = 5.0, 1.5 Hz), 4.43 (m, 2H, $H_{5'}$), 4.60 (dt, 1H, $H_{4'}$, J = 9.0, 2.6 Hz), 4.76 (dd, 1H, $H_{3'}$, J = 8.9, 5.1 Hz), 5.98 (d, 1H, $H_{1'}$, J = 1.4 Hz), 7.07 (d, 1H, H_5, J = 7.3 Hz), 8.13 (s, 1H, $H_{triazol}$), 8.39 (d, 1H, H_6, J = 7.3 Hz), 9.26 (s, 1H, $H_{triazol}$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 20.6 (OCO−CH_3), 21.0 (OCO−CH_3), 59.0 (O−CH_3), 61.8 ($C_{5'}$), 69.3 ($C_{3'}$), 70.6 ($C_{1''}$), 71.6 ($C_{2''}$), 79.3 ($C_{4'}$), 80.4 ($C_{2'}$), 91.4 ($C_{1'}$), 94.7 (C_5), 143.5 ($C_{triazol}$),145.9 (C_6), 154.19 (C_2), 154.23 ($C_{triazol}$), 159.6 (C_4), 170.1 (OC═O), 170.3 (OC═O) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{18}H_{24}N_5O_8$ [M + H]$^+$, 438.1619; found, 438.1614, calcd for $C_{18}H_{23}N_5NaO_8$ [M + Na]$^+$, 460.1439; found, 460.1435, calcd for $C_{18}H_{23}KN_5O_8$ [M + K]$^+$, 476.1178; found, 476.1173.

*4.4.3. Synthesis of 2′-O-(2-Methoxyethyl)-L-cytidine (**22**).* Similar procedure as that described for **17** (method B) starting from **21**. Compound **22** was obtained in 95% yield.

*4.4.3.1. 2′-O-(2-Methoxyethyl)-L-cytidine (**22**).* White solid. mp 153−155 °C;. R_f: 0.35 (20% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, D_2O): δ 3.32 (s, 3H, O−*Me*), 3.60 (t, 2H, $H_{2''}$, J = 4.4), 3.73−3.91 (various m, 4H, $H_{5'}$ + $H_{1''}$), 4.08 (m, 2H, $H_{2'}$ + $H_{4'}$), 4.23 (dd, 1H, $H_{3'}$, J = 6.3, 5.4 Hz), 5.91 (d, 1H, $H_{1'}$, J = 3.6 Hz), 6.00 (d, 1H, H_5, J = 7.6 Hz), 7.82 (d, 1H, H_6, J = 7.6 Hz) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ 58.0 (O−CH_3), 60.4 ($C_{5'}$), 68.3 ($C_{3'}$), 69.5 ($C_{1''}$), 71.2 ($C_{2''}$), 81.7 ($C_{2'}$), 83.7 ($C_{4'}$), 88.7 ($C_{1'}$), 96.1 (C_5), 141.6 (C_6), 157.2 (C_2), 166.0 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{12}H_{20}N_3O_6$ [M + H]$^+$, 302.1347; found, 302.1342.

*4.4.4. Synthesis of 2′-O-Methyl/2′-O-MOE-5-Iodo-L-uridine (**23**/**24**).* To a 0.1 M solution of **12** or **13** in anhydrous

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MeOH were added 1 equiv of Ag_2SO_4 and 1.5 equiv of NIS. The reaction was stirred at rt for 12 h. Then, the mixture was filtered over Celite and washed with MeOH. Solvents were evaporated under reduced pressure, and the crude was purified by column chromatography (5% MeOH/CH_2Cl_2), isolating **23** (75% yield) or **24** (72% yield), respectively.

*4.4.4.1. 2′-O-Methyl-L-5-iodoridine (**23**).* Pale yellow solid. mp 241−243 °C; R_f: 0.31 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, DMSO-d_6): δ 3.37 (s, 3H, O−*Me*), 3.57 (m, 1H, $H_{5'}$), 3.70 (m, 1H, $H_{5'}$), 3.78 (t, 1H, $H_{2'}$, J = 4.5 Hz), 3.85 (m, 1H, $H_{4'}$), 4.11 (q, 1H, $H_{3'}$, J = 5.2 Hz), 5.19 (d, 1H, $OH_{3'}$, J = 6.0 Hz), 5.35 (sa, 1H, $OH_{5'}$), 5.78 (d, 1H, $H_{1'}$, J = 3.8 Hz), 8.53 (s, 1H, H_6) ppm; ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 57.7 (CH_3), 59.7 ($C_{5'}$), 67.9 ($C_{3'}$), 69.5 (C_5), 83.2 ($C_{2'}$), 84.9 ($C_{4'}$), 86.6 ($C_{1'}$), 145.0 (C_6), 150.3 (C_2), 160.7 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{10}H_{14}IN_2O_6$ [M + H]$^+$, 384.9891; found, 384.9898, calcd for $C_{10}H_{13}IN_2NaO_6$ [M + Na]$^+$, 406.9711; found, 406.9720, calcd for $C_{10}H_{13}IKN_2O_6$ [M + K]$^+$, 422.9450; found, 422.9461.

*4.4.4.2. 2′-O-(2-Methoxyethyl)-L-5-iodoridine (**24**).* White solid. mp 62−63 °C; R_f: 0.47 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, D_2O): δ 3.33 (s, 3H, O−*Me*), 3.61 (t, 2H, $H_{2''}$, J = 4.4 Hz), 3.80 (m, 3H, $H_{5'}$ + $H_{1''}$), 3.93 (dd, 1H, $H_{5'}$, J = 13.0, 2.7 Hz), 4.09 (m, 2H, $H_{2'}$ + $H_{4'}$), 4.27 (dd, 1H, $H_{3'}$, J = 6.8, 5.2 Hz), 5.88 (d, 1H, $H_{1'}$, J = 3.1 Hz), 8.46 (s, 1H, H_6) ppm; ^{13}C NMR (75.5 MHz, D_2O): δ 58.1 (CH_3), 59.6 ($C_{5'}$), 67.6 (C_5), 67.8 ($C_{3'}$), 69.5 ($C_{1''}$), 71.2 ($C_{2''}$), 81.8 ($C_{2'}$), 83.9 ($C_{4'}$), 87.9 ($C_{1'}$), 146.1 (C_6), 151.1 (C_2), 163.0 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{12}H_{18}IN_2O_7$ [M + H]$^+$, 429.0153; found, 429.0142, calcd for $C_{12}H_{17}IN_2NaO_7$ [M + Na]$^+$, 450.9973; found, 450.9961, calcd for $C_{12}H_{17}IKN_2O_7$ [M + K]$^+$, 466.9712; found, 466.9699.

*4.4.5. Synthesis of 2′-O-Methyl/2′-O-MOE-5-Trifluoromethyl-L-uridine (**25/26**).* To an 2.5 M aqueous solution of **12** or **13** and 3 equiv of $NaSO_2CF_3$, 5 equiv of *tert*-butylhydroperoxide was added slowly at 0 °C. After stirring the mixture at rt for 24 h, a second addition of $NaSO_2CF_3$ (3 equiv) and *tert*-butylhydroperoxide (5 equiv) were added. Progress of the reaction was monitored by TLC (10% MeOH/CH_2Cl_2) until complete consumption of the starting material (total time: 26 h). The mixture was poured into EtOAc, and the organic phase was washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude was purified by column chromatography (3% MeOH/CH_2Cl_2) to afford **25** (29% yield) or **26** (32% yield), respectively.

*4.4.5.1. 2′-O-Methyl-L-5-trifluorouridine (**25**).* Clear oil. R_f: 0.30 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, MeOD-d_4): δ 3.58 (s, 3H, O−*Me*), 3.77 (dd, 1H, $H_{5'}$, J = 12.1, 1.6 Hz), 3.86 (dd, 1H, $H_{2'}$, J = 4.9, 2.0 Hz), 3.98 (m, 2H, $H_{4'}$ + $H_{5'}$), 4.26 (dd, 1H, $H_{3'}$, J = 7.4, 5.0 Hz), 5.92 (d, 1H, $H_{1'}$, J = 1.9 Hz), 9.00 (d, 1H, H_6, J = 1.0 Hz) ppm; ^{13}C NMR (75.5 MHz, MeOD-d_4): δ 58.9 (CH_3), 60.3 ($C_{5'}$), 68.9 ($C_{3'}$), 85.4 ($C_{2'}$), 85.7 ($C_{4'}$), 89.3 ($C_{1'}$), 105.2 (q, C_5, J = 33.0 Hz), 124.0 (q, CF_3, J = 269.1 Hz), 143.7 (q, C_6, J = 5.8 Hz), 151.3 (C_2), 161.3 (C_4) ppm; ^{19}F NMR (282 MHz, MeOD-d_4): δ −64.4 ppm; HRMS (ESI$^+$, m/z): calcd for $C_{11}H_{14}F_3N_2O_6$ [M + H]$^+$, 327.0798; found, 327.0800, calcd for $C_{11}H_{13}F_3N_2NaO_6$ [M + Na]$^+$, 349.0618; found, 349.0626, calcd for $C_{11}H_{13}F_3KN_2O_6$ [M + K]$^+$, 365.0357; found, 365.0358.

*4.4.5.2. 2′-O-(2-Methoxyethyl)-L-5-trifluorouridine (**26**).* Clear oil. R_f: 0.35 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, MeOD-d_4): δ 3.38 (s, 3H, O−*Me*), 3.60 (t, 2H, $H_{2''}$, J = 4.5 Hz), 3.77 (dd, 1H, $H_{5'}$ J = 12.2, 1.8 Hz), 3.83 (m, 1H, $H_{1''}$), 3.93 (m, 1H, $H_{5'}$), 3.96 (m, 1H, $H_{1''}$), 4.04 (m, 2H, $H_{2'}$ + $H_{4'}$), 4.24 (dd, 1H, $H_{3'}$, J = 7.0, 5.0 Hz), 5.93 (d, 1H, $H_{1'}$, J = 2.4 Hz), 8.97 (d, 1H, H_6, J = 1.0 Hz) ppm; ^{13}C NMR (75.5 MHz, MeOD-d_4): δ 59.2 (CH_3), 60.6 ($C_{5'}$), 69.4 ($C_{3'}$), 70.9 ($C_{1''}$), 73.0 ($C_{2''}$), 84.2 ($C_{2'}$), 86.0 ($C_{4'}$), 89.7 ($C_{1'}$), 105.3 (q, C_5, J = 32.7 Hz), 124.0 (q, CF_3, J = 268.7 Hz), 143.8 (q, C_6, J = 5.7 Hz), 151.4 (C_2), 161.3 (C_4) ppm; ^{19}F NMR (282 MHz, MeOD-d_4): δ −64.4 ppm; HRMS (ESI$^+$, m/z): calcd for $C_{13}H_{18}F_3N_2O_7$ [M + H]$^+$, 371.1061; found, 371.1063, calcd for $C_{13}H_{17}F_3N_2NaO_7$ [M + Na]$^+$, 393.0880; found, 393.0883, calcd for $C_{13}H_{17}F_3KN_2O_7$ [M + K]$^+$, 409.0619; found, 409.0623.

4.5. Preparation of Solid-Supports Functionalized with 2′-O-Methyl/2′-O-MOE-L-Uridine (31/32). *4.5.1. Synthesis of DMT-Protected L-uridine **27** and **28**.* To a solution of **12** or **13** in anhydrous 1,4-dioxane (0.1 M), anhydrous Et_3N (10 equiv) and 4,4′-DMT chloride (1.5 equiv) were added. After magnetic stirring at 30 °C for 2 h, a saturated aqueous sodium bicarbonate solution was added and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography (40% EtOAc/hexane as the eluent), previously packed with silica gel using a 10% Et_3N solution in EtOAc/hexane (4:6, v/v). Compounds **27** or **28** were obtained in 80 and 95% yield, respectively.

*4.5.1.1. 5′-O-(4,4′-Dimethoxytrityl)-2′-O-Methyl-L-uridine (**27**).* White solid. mp decomposes from 97 °C;. R_f: 0.44 (5% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 3.54 (m, 2H, $H_{5'}$), 3.64 (s, 3H, O−*Me*), 3.73 (m, 1H, $H_{2'}$), 3.76 (s, 6H, *Me*-DMT), 3.99 (d, 1H, $H_{4'}$, J = 7.9 Hz), 4.47 (dd, 1H, $H_{3'}$, J = 7.7, 5.5 Hz), 5.27 (d, 1H, H_5, J = 8.2 Hz), 5.96 (s, 1H, $H_{1'}$), 6.84 (d, 4H, H_g, J = 8.8 Hz), 7.28 (m, 7H, H_c + H_d + H_f), 7.38 (d, 2H, H_b, J = 7.5 Hz), 8.03 (d, 1H, H_6, J = 8.1 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 55.4 (2 O−CH_3, DMT), 58.9 (O−CH_3), 61.3 ($C_{5'}$), 68.5 ($C_{3'}$), 83.4 ($C_{4'}$), 84.2 ($C_{2'}$), 87.15 ($C_{1'}$), 87.24 (C_7), 102.3 (C_5), 113.4 (4C_g), 127.3, 128.2, 128.3, 130.25, 130.34 (2C_b + 2C_c + C_d + 4C_f), 135.2 (C_e), 135.4 (C_e), 140.2 (C_6), 144.5 (C_a), 150.2 (C_2), 158.8 (C_h), 158.9 (C_h), 163.3 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{31}H_{33}N_2O_8$ [M + H]$^+$, 561.2231; found, 561.2210, calcd for $C_{31}H_{32}N_2NaO_8$ [M + Na]$^+$, 583.2051; found, 583.2028, calcd for $C_{31}H_{32}KN_2O_8$ [M + K]$^+$, 599.1790; found, 599.1765.

*4.5.1.2. 5′-O-(4,4′-Dimethoxytrityl)-2′-O-(2-methoxyethyl)-L-uridine (**28**).* White foam. mp collapsed at 30 °C;. R_f: 0.67 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 3.38 (s, 3H, O−*Me*), 3.52 (m, 2H, $H_{5'}$), 3.59 (m, 2H, $H_{2''}$), 3.78 (m, 1H, $H_{1''}$), 3.80 (s, 6H, *Me*-DMT), 4.01 (dd, 1H, $H_{2'}$, J = 5.0, 2.5 Hz), 4.09 (m, 2H, $H_{1''}$ + $H_{4'}$), 4.44 (t, 1H, $H_{3'}$, J = 5.7 Hz), 5.28 (d, 1H, H_5, J = 8.2 Hz), 5.95 (d, 1H, $H_{1'}$, J = 2.4 Hz), 6.84 (m, 4H, H_g), 7.17 (m, 2H, H_c), 7.28 (m, 5H, H_d + H_f), 7.38 (m, 2H, H_b), 7.97 (d, 1H, H_6, J = 8.2 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 55.4 (2 O−CH_3, DMT), 59.0 (O−CH_3, MOE), 61.7 ($C_{5'}$), 68.9 ($C_{3'}$), 70.4 ($C_{1''}$), 71.8 ($C_{2''}$), 83.3 ($C_{2'}$), 83.5 ($C_{4'}$), 87.1 (C_7), 87.9 ($C_{1'}$), 102.2 (C_5), 113.2, 113.4 (4C_g), 127.2, 127.9, 128.1, 128.3, 129.3, 130.2, 130.3 (2C_b + 2C_c + C_d + 4C_f), 135.2 (C_e), 135.5 (C_e), 140.2 (C_6), 144.5 (C_a), 150.4 (C_2), 158.77 (C_h), 158.80 (C_h), 163.6 (C_4) ppm; HRMS (ESI$^+$, m/z): calcd for $C_{33}H_{37}N_2O_9$ [M + H]$^+$, 605.2494; found, 605.2489, calcd for $C_{33}H_{36}N_2NaO_9$ [M + Na]$^+$, 627.2313; found, 627.2310, calcd for $C_{33}H_{36}KN_2O_9$ [M + K]$^+$, 643.2052; found, 643.2049.

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*4.5.2. Synthesis of Hemisuccinates of L-Uridine **29** and **30**.* 5′-*O*-DMT-monomers **27** or **28** (1 equiv) were dried by evaporation with CH_2Cl_2 and redissolved in anhydrous CH_2Cl_2 (0.02 M). To the mixture, 1.5 equiv of DMAP and 1.5 equiv of succinic anhydride were added. The solution was stirred at rt overnight. Then, the reaction mixture was diluted with CH_2Cl_2, and the resulting organic phase was washed with an 0.1 M aqueous NaH_2PO_4 (pH 5) solution, dried over Na_2SO_4, and evaporated to dryness affording **29** (93% yield) or **30** (91% yield). These hemisuccinates were used directly for the functionalization of the appropriate solid supports.

*4.5.2.1. 5′-O-(4,4′-Dimethoxytrityl)-2′-O-Methyl-3′-O-succinyl-L-uridine (**29**).* White solid. ^{1}H NMR (300.13 MHz, MeOD-d_4): δ 2.62 (m, 4H, $H_{1''}$ + $H_{2''}$), 3.45 (s, 3H, O−*Me*), 3.51 (m, 2H, $H_{5'}$), 3.79 (s, 6H, *Me*-DMT), 4.14, 4.23 (2m, 2H, $H_{2'}$ + $H_{4'}$), 5.30 (d, 1H, H_5, J = 8.1 Hz), 5.32 (m, 1H, $H_{3'}$), 5.94 (m, 1H, $H_{1'}$), 6.89 (d, 4H, H_{arom}, J = 8.9 Hz), 7.32 (m, 7H, H_{arom}), 7,41 (m, 2H, H_{arom}), 7.93 (d, 1H, H_6, J = 8.1 Hz) ppm.

*4.5.2.2. 5′-O-(4,4′-Dimethoxytrityl)-2′-O-(2-methoxyethyl)-3′-O-succinyl-L-uridine (triethylammonium salt) (**30**).* White solid; R_f: 0.38 (10% MeOH/CH_2Cl_2); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ 1.18 (t, 9H, *Me*−Et_3N, J = 7.3 Hz), 2.55, 2.68 (2m, 4H, $H_{1'''}$ + $H_{2'''}$), 2.93 (q, 6H, CH_2−Et_3N, J = 7.3 Hz), 3.29 (s, 3H, O−*Me*), 3.47 (m, 4H, $2H_{5'}$ + $2H_{2''}$), 3.68 (m, 1H, $H_{1''}$), 3.78 (s, 6H, *Me*-DMT), 3.83 (m, 1H, $H_{1''}$), 4.27 (m, 1H, $H_{4'}$), 4.34 (t, 1H, $H_{2'}$, J = 4.6 Hz), 5.29 (d, 1H, H_5, J = 7.7 Hz), 5.30 (m, 1H, $H_{3'}$), 6.00 (d, 1H, $H_{1'}$, J = 4.1 Hz), 6.83 (d, 4H, H_{arom}, J = 7.7 Hz), 7.25, 7,34 (2m, 9H, H_{arom}), 7.86 (d, 1H, H_6, J = 8.2 Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 8.8 (3 CH_3, Et_3N), 30.4, 31.4 ($C_{1'''}$ + $C_{2'''}$),44.9 (3 N−CH_2, Et_3N), 55.4 (2 O−CH_3, DMT), 59.1 (O−CH_3, MOE), 62.1 ($C_{5'}$), 70.4 ($C_{3'}$), 70.6 ($C_{1''}$), 72.3 ($C_{2''}$), 81.3, 81.4 ($C_{2'}$ + $C_{4'}$), 87.4 (C_7), 87.6 ($C_{1'}$), 102.3 (C_5), 113.4, 127.3, 128.16, 128.22, 130.2, 130.3 ($2C_b$ + $2C_c$ + C_d + $4C_f$ + $4C_g$), 135.1 (C_e), 135.3 (C_e), 140.3 (C_6), 144.3 (C_a), 150.4 (C_2), 158.8 ($2C_h$), 163.4 (C_4), 172.9 (C═O), 177.3 (C═O) ppm; MS (ESI$^-$, m/z): calcd for $C_{35}H_{35}N_2O_{11}$ [M − H]$^-$: 659.2; found, 659.2.

*4.5.3. Incorporation of 3′-O-Hemisuccinates of L-Uridine **29** and **30** to an LCAA-CPG Solid Support (Synthesis of **31** and **32**).* Amino-LCAA-CPG (CPG New Jersey, 69 μmol amino/g) 643 mg (for **29**) and 800 mg (for **30**) were placed into a polypropylene syringe fitted with a polypropylene disc and washed sequentially with CH_2Cl_2 and MeCN under vacuo. In an Eppendorf tube, 15 mg (∼1 equiv) of DMAP was dissolved in 100 μL or 1 mL of MeCN, and the solution was added dropwise over 55 mg (0.08 mmol) of 5′-*O*-DMT-2′-OMe-L-uridine (**29**) or 80 mg (0.11 mmol) of 5′-*O*-DMT-2′-OMOE-L-uridine (**30**) hemisuccinates, allowing the complete dissolution of the sample. In another tube, 25 mg (∼1 equiv) of Ph_3P was dissolved in 100−200 μL of MeCN, and 30 mg or 34 mg of 2,2′-dithiobis(5-nitropyridine) was dissolved in a solution of CH_2Cl_2/MeCN (3:1) (∼100 μL). Both solutions were mixed and added over the hemisuccinate-nucleoside solutions. A red-orange solution was obtained, which was added over the native resin. Reaction was left for 2 h at rt. When reaction finished, the resin was washed abundantly with CH_2Cl_2/MeCN. Finally, the capping steps were carried out passing through the cartridge 4 mL of a solution of acetic anhydride, pyridine, and 1-methylimidazole (Ac_2O/Py/NMI) (1:1:1) in THF. Loading achieved for the resin was calculated by trityl assay. The calculated loading values were 20 and 23 μmol/g for **31** and **32**, respectively.

*4.5.4. Synthesis of Phosphoramidites of L-Uridine **33** and **34**.* Compound **27** or **28** was dried by coevaporation with anhydrous MeCN and left in a freeze-dryer overnight. The product was dissolved in anhydrous CH_2Cl_2 (0.1 M), and ^{i}Pr_2NEt (3 equiv) was added to the solution. Then, the mixture was cooled in an ice bath, and chloro-2-cyanoethoxy-N,*N*-diisopropylaminophosphine (1.5 equiv) was added dropwise with a syringe. After the addition, the reaction was allowed to warm to room temperature and stirred for an additional 1 h. At that time, the reaction was stopped by addition of brine and extracted with CH_2Cl_2. The organic layer was dried and concentrated to dryness. The residue was purified by silica gel column chromatography previously packed with a 10% Et_3N solution in 20% hexane/EtOAc and eluted with 20% hexane/EtOAc yielding **33** (67%) or **34** (65%).

*4.5.4.1. 3′-(2-Cyanoethyl-N,N-diisopropylphosphoramidityl)-5′-O-(4,4′-dimethoxytrityl)-2′-O-methyl-L-uridine (**33**).* White solid; R_f: 0.61 and 0.68 (20% Hexane/EtOAc); ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 150.39 and 150.90 ppm; HRMS (ESI$^+$, m/z): calcd for $C_{40}H_{50}N_4O_9P$ [M + H]$^+$, 761.3310; found, 761.3307, calcd for $C_{40}H_{49}N_4NaO_9P$ [M + Na]$^+$, 783.3129; found, 783.3122.

*4.5.4.2. 3′-(2-Cyanoethyl-N,N-diisopropylphosphoramidityl)-5′-O-(4,4′-dimethoxytrityl)-2′-O-(2-methoxyethyl)-L-uridine (**34**).* White solid; R_f: 0.44 and 0.53 (20% Hexane/EtOAc); ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 150.15 and 150.21 ppm; HRMS (ESI$^+$, m/z): calcd for $C_{42}H_{54}N_4O_{10}P$ [M + H]$^+$, 805.3572; found, 805.3568, calcd for $C_{42}H_{53}N_4NaO_{10}P$ [M + Na]$^+$, 827.3392; found, 827.3384; calcd for $C_{42}H_{53}KN_4O_{10}P$ [M + K]$^+$, 843.3131; found, 843.3131.

4.6. Synthesis of G-Rich Oligonucleotide Sequences. All the G-quadruplexes (G-rich) oligonucleotide sequences used in this study (see Table 1) were DMT-ON synthesized in a 1 μmol scale on an Applied Biosystems DNA synthesizer using solid-phase phosphonamidite chemistry. Before the cleavage step, oligonucleotides were detritylated using a solution of trichloroacetic acid (3%) in CH_2Cl_2. Then, the solid support was treated with 1 mL of concentrated aqueous ammonia solution (32%) overnight at rt and then 1 h at 55 °C. Next, the oligonucleotides were desalted using a Sephadex cartridge. The homogeneity of the oligonucleotides was checked by analytical HPLC presenting a single peak with the expected mass by MALDI-TOF analysis (Table 1). The oligonucleotide concentrations were determined by their extinction coefficient and the absorbance measurements (260 nm). The total amounts of each ones of the G-rich sequences were divided in several aliquots of 1 OD_{260} units. All the samples were dried under vacuo to dryness and were stored at −20 °C until further used.

4.7. Quadruplex Formation. The four oligonucleotides were dissolved in a 10 mM solution of NaCl in Milli-Q H_2O, and they were annealed by heating the samples to 95 °C for 5 min and slowly cooling to rt. The resulting G-quadruplex oligonucleotide conjugates were stored at 4 °C.

Then, another batch of oligonucleotides were dissolved in a different buffer solution (20 mM of KH_2PO_4 and 70 mM of KCl in Milli-Q H_2O) and annealed again in the same previous conditions. The resulting formed quadruplex was kept at 4 °C.

4.8. Thermal Denaturation Experiments and CD Spectra. CD measurements were registered between 200 and 320 nm in the same buffer as for the denaturing curves.

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CD thermal denaturation experiments were performed in the temperature range of 10−90 °C using a heating rate of 0.5 °C min^{-1} and monitoring the CD values at 260 and 295 nm with a scanning speed of 100 nm/min, a response time of 4 s, a 0.5 nm data pitch, and a 1 nm bandwidth.

4.9. Native Polyacrylamide Electrophoresis Mobility Shift Assay. A native PAGE was prepared using 20% (v/v) acrylamide under native conditions. Samples were dissolved in glycerol, and the gel was run in 1 TBE (1×) buffer (supplemented with 100 mM KCl) at 100 V for approximately 2−3 h under a fixed temperature of 4 °C. Stain-all was used to stain the oligonucleotide bands according to the manufacturer's instructions. The TG_4T and T_6 oligonucleotides were used as positive and negative control sequence, respectively. A solution containing bromophenol blue and xylene cyanole as ladder was used for visual tracking of oligonucleotide migration during the electrophoretic process.

4.10. Enzymatic Degradation Assay. Enzymatic degradation assays of G-rich oligonucleotides were carried out followed by HPLC analysis. An amount of 1 OD_{260} units of oligonucleotide was dissolved in 93 μL of H_2O, 5 μL of 1 M aqueous Tris−HCl solution (pH 8.0), and 1 μL of 1 M aqueous $MgCl_2$ solution. Then, 1 μL of phosphodiesterase I from *Crotalus adamanteus* venom (SVP) was added, and the samples were incubated at 37 °C. An aliquot of 20 μL was taken off at 0.5, 1, 4, 8, and 24 h. Immediately, they were heated to 85 °C for 5 min, in order to denature the enzyme and stop the digestion. Finally, each time point sample was analyzed by HPLC comparing the results with a control G-rich oligonucleotide sample, dissolved in the same buffer but without the phosphodiesterase I enzyme. The HPLC analysis conditions were 20 min linear gradient from 0 to 30% buffer B. Sample volume injection: 40 μL.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06231.

^1H, ^{13}C, and ^{19}F NMR spectral data; level of purity indicated by the inclusion of copies of ^1H, ^{13}C, and ^{19}F NMR spectra; some 2D NMR experiments used to assign the peaks; CD spectra (10 mM Na buffer), denaturation curves (10 mM Na buffer) followed by CD and MS spectra of purified oligonucleotides; and G-quadruplex degradation after incubation with snake venom phosphodiesterase (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CPG, controlled pore glass; DBU, 1,8-diazabicyclo[5,4,0]-undec-7-ene; DMAP, *N,N*-dimethylaminopyridine; DMF, *N,N*-dimethylformamide; DMT, 4,4′-dimethoxytrityl; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; LCAA-CPG, long-chain amino alkyl-controlled pore glass; MALDI, matrix-assisted laser desorption/ionization; OPC, oligonucleotide purification cartridge; Py, pyridine; RP-HPLC, reverse phase high-performance liquid chromatography; TCA, trichloroacetic acid; TOF, time of flight

■ REFERENCES

(1) Focher, F.; Spadari, S.; Maga, G. Antivirals at the mirror: the lack of stereospecificity of some viral and human enzymes offers novel opportunities in antiviral drug development. *Curr. Drug Targets: Infect. Disord.* **2003**, *3*, 41−53.

(2) (a) Smejkal, J.; Sorm, F. Nucleic acids components and their analogues. LIII. Preparation of 1−2'-deoxy-β-L-ribofuranosylthymine, L-thymidine. *Collect. Czech. Chem. Commun.* **1964**, *29*, 2809−2813. (b) Acton, E. M.; Ryan, K. J.; Goodman, L. Synthesis of L-

https://doi.org/10.1021/acsomega.3c06231
ACS Omega 2023, 8, 44893−44904

ribofuranose and L-adenosine. *J. Am. Chem. Soc.* **1964**, *86*, 5352−5354.
(3) (a) Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L. S.; Beach, J. W.; Choi, W. B.; Yeola, S.; et al. Activities of the four optical isomers of 2', 3′-dideoxy-3′-thiacytidine (BCH-189) against human immunodeficiency virus type 1 in human lymphocytes. *Antimicrob. Agents Chemother.* **1992**, *36*, 672−676. (b) Cameron, J. M.; Collis, P.; Daniel, M.; Storer, R.; Wilcox, P. Lamivudine. *Drugs Future* **1993**, *18*, 319−323.
(4) Mathe, C.; Gosselin, G. L-nucleoside enantiomers as antivirals drugs: a mini-review. *Antiviral Res.* **2006**, *71*, 276−281.
(5) (a) De Jonghe, S.; Herdewijn, P. An Overview of Marketed Nucleoside and Nucleotide Analogs. *Curr. Protoc.* **2022**, *2*, No. e376. (b) Khawaja, F.; Spallone, A.; Kotton, C. N.; Chemaly, R. F. Cytomegalovirus infection in transplant recipients: newly approved additions to our armamentarium. *Clin. Microbiol. Infect.* **2023**, *29*, 44−50.
(6) Bankar, A.; Siriwardena, T. P.; Rizoska, B.; Rydergard, C.; Kylefjord, H.; Rraklli, V.; Eneroth, A.; Pinho, P.; Norin, S.; Bylund, J.; et al. Novel L-nucleoside analog, 5-fluorotroxacitabine, displays potent efficacy against acute myeloid leukemia. *Haematologica* **2021**, *106* (2), 574−579.
(7) Young, B. E.; Kundu, N.; Sczepanski, J. T. Mirror image oligonucleotides: history and emerging applications. *Chem.—Eur. J.* **2019**, *25*, 7981−7990.
(8) Vater, A.; Klussmann, S. Turning mirror-image oligonucleotides into drugs: the evolution of Spiegelmer® therapeutics. *Drug Discovery Today* **2015**, *20*, 147−155.
(9) Klußmann, S.; Nolte, A.; Bald, R.; Erdmann, V. A.; Fürste, J. P. Mirror-image RNA that binds D-adenosine. *Nat. Biotechnol.* **1996**, *14* (9), 1112−1115.
(10) Sczepanski, J. T.; Joyce, G. F. Binding of a Structured D-RNA Molecule by an L-RNA Aptamer. *J. Am. Chem. Soc.* **2013**, *135*, 13290−13293.
(11) Li, J.; Sczepanski, J. T. Targeting a conserved structural element from the SARS-CoV-2 genome using L-DNA aptamers. *RSC Chem. Biol.* **2022**, *3*, 79−84.
(12) Yu, C.-H.; Sczepanski, J. T. The influence of chirality on the behavior of oligonucleotides inside cells: revealing the potent cytotoxicity of G-rich L-RNA. *Chem. Sci.* **2023**, *14*, 1145−1154.
(13) Virgilio, A.; Esposito, V.; Pecoraro, A.; Russo, A.; Vellecco, V.; Pepe, A.; Bucci, M.; Russo, G.; Galeone, A. Structural properties and anticoagulant/cytotoxic activities of heterochiral enantiomeric thrombin binding aptamer (TBA) derivatives. *Nucleic Acids Res.* **2020**, *48*, 12556−12565.
(14) Dantsu, Y.; Zhang, Y.; Zhang, W. Synthesis and structural characterization of 2′-Deoxy-2′-fluoro-L-uridine nucleic acids. *Org. Lett.* **2021**, *23*, 5007−5011.
(15) Sanghvi, Y. S. A status update of modified oligonucleotides for chemotherapeutics applications. *Curr. Protoc. Nucleic Acid Chem.* **2011**, *46*, 4.1.1−4.1.22.
(16) Holý, A. Nucleic acid components and their analogues. CLIII. Preparation of 2'-deoxy-L-ribonucleosides of the pyrimidine series. *Collect. Czech. Chem. Commun.* **1972**, *37*, 4072−4087.
(17) Szlenkier, M.; Kamel, K.; Boryski, J. Regioselective Mitsunobu reaction of partially protected uridine. *Nucleos Nucleot. Nucleic Acids* **2016**, *35*, 410−425.
(18) Roy, S. K.; Tang, J. Efficient large Scale synthesis of 2'-O-alkyl pyrimidine ribonucleosides. *Org. Process Res. Dev.* **2000**, *4*, 170−171.
(19) Legorburu, U.; Reese, C. B.; Song, Q. Conversion of uridine into 2′-O-(2-methoxyethyl) uridine and 2′-O-(2-methoxyethyl) cytidine. *Tetrahedron* **1999**, *55*, 5635−5640.
(20) (a) Lavandera, I.; Fernández, S.; Ferrero, M.; Gotor, V. Novel and efficient syntheses of 3′,5′-diamino derivatives of 2′,3′,5′-trideoxycytidine and 2′,3′,5′-trideoxyadenosine. Protonation behavior of 3′,5′-diaminonucleosides. *Tetrahedron* **2003**, *59*, 5449−5456. (b) Divakar, K. J.; Reese, C. B. 4-(1,2,4-Triazol-1-yl)- and 4-(3-nitro-1,2,4-triazol-1-yl)-1-(β-D-2,3,5-tri-*O*-acetylarabino-furanosyl)-pyrimidin-2(1H)-ones. Valuable intermediates in the synthesis of derivatives of 1-(β-D-arabinofuranosyl)cytosine (ara-C). *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171−1176.
(21) (a) Shanmugasundaram, M.; Senthilvelan, A.; Kore, A. R. C-5 substituted pyrimidine nucleotides/nucleosides: Recent progress in synthesis, functionalization, and applications. *Curr. Org. Chem.* **2019**, *23*, 1439−1468. (b) Herdewijn, P. A. M. M. 5-Substituted-2′-deoxyuridines as anti-HSV-1 agents: Synthesis and structure activity relationship. *Antiviral Chem. Chemother.* **1994**, *5*, 131−146. (c) Lin, T. S.; Chen, M. S.; McLaren, C.; Gao, Y.-S.; Ghazzouli, I.; Prusoff, W. H. Synthesis and antiviral activity of various 3′-azido, 3′-amino, 2', 3′-unsaturated, and 2', 3′-dideoxy analogs of pyrimidine deoxyribonucleosides against retroviruses. *J. Med. Chem.* **1987**, *30*, 440−444.
(22) (a) Gaware, S.; Kori, S.; Serrano, J. L.; Dandela, R.; Hilton, S.; Sanghvi, Y. S.; Kapdi, A. R. Rapid plugged flow synthesis of nucleoside analogues via Suzuki-Miyaura coupling and heck Alkenylation of 5-Iodo-2'-deoxyuridine (or cytidine). *J. Flow Chem.* **2023**, *13*, 293−310. (b) Robins, M. J.; Barr, P. J. Nucleic acid related compounds. 39. Efficient conversion of 5-iodo to 5-alkynyl and derived 5-substituted uracil bases and nucleosides. *J. Org. Chem.* **1983**, *48*, 1854−1862. (c) Ardhapure, A. V.; Sanghvi, Y. S.; Kapdi, A. R.; García, J.; Sanchez, G.; Lozano, P.; Serrano, J. L. Pd−imidate complexes as recyclable catalysts for the synthesis of C5-alkenylated pyrimidine nucleosides via Heck cross-coupling reaction. *RSC Adv.* **2015**, *5* (31), 24558−24563.
(23) Sy, W. W. Iodination with silver sulfate and iodine. II. Uridines. *Synth. Commun.* **1990**, *20*, 3391−3394.
(24) Shet, H.; Sahu, R.; Sanghvi, Y. S.; Kapdi, A. R. Strategies for the synthesis of fluorinated nucleosides, nucleotides and oligonucleotides. *Chem. Rec.* **2022**, *22*, No. e202200066.
(25) Ji, Y.; Brueckl, T.; Baxter, R. D.; Fujiwara, Y.; Seiple, I. B.; Su, S.; Blackmond, D. G.; Baran, P. S. Innate C-H trifluoromethylation of heterocycles. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 14411−14415.
(26) Guzaev, A. P.; Pon, R. T. Attachment of nucleosides and other linkers to solid-phase supports for oligonucleotide synthesis. *Curr. Protoc. Nucleic Acid Chem.* **2013**, *52*, 3.2.1−3.2.23.
(27) Ferreira, R.; Alvira, M.; Aviñó, A.; Gómez-Pinto, I.; González, C.; Gabelica, V.; Eritja, R. Synthesis and Structural Characterization of Stable Branched DNA G-Quadruplexes Using the Trebler Phosphoramidite. *ChemistryOpen* **2012**, *1*, 106−114.
(28) Alagia, A.; Jorge, A. F.; Aviñó, A.; Cova, T. F. G. G.; Crehuet, R.; Grijalvo, S.; Pais, A. A. C. C.; Eritja, R. Exploring PAZ/3′-overhang interaction to improve siRNA specificity. A combined experimental and modeling study. *Chem. Sci.* **2018**, *9*, 2074−2086.

https://doi.org/10.1021/acsomega.3c06231
ACS Omega 2023, 8, 44893−44904