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Synthesis and properties of 2′-*O*-[*R*- and *S*-(2-amino-3-methoxy) propyl] (*R*-AMP and *S*-AMP) nucleic acids

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ABSTRACT

Substitution at 2′-position by either amino- or methoxy-pendant groups of the antisense oligonucleotides (AONs) is known to enhance their therapeutic value. A simple modification is described here in which we introduce both these groups in the form of enantiospecific tethers at 2′-position. Practical synthesis of modified nucleosides using natural L-serine, en route to *R*-AMP- and *S*-AMP-AONs is presented. Such tethered ONs formed stable DNA:RNA duplexes and the stability was found to be marginally better than the methoxyethyl/methoxypropyl-substituted MOE/MOP-AONs. The stereochemistry of the tether effectively differentiated the hydrolytic cleavage of AONs and the *R*-AMP-AON was three times more stable than the *S*-AMP-AONs after 4 h. In comparison, the MOE- or MOP-AONs were almost completely digested by SVPD after 1 h.

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1. Introduction

Synthetic antisense or siRNA oligonucleotides (ONs) as gene silencing agents inhibit viral replication and expression of disease-causing genes based on the simple concept of nucleic acid sequence recognition via Watson–Crick hydrogen bonding by a complementary base sequence.[1] Amongst the large number of 2′-*O*-alkyl derivatives studies so far,[2] the 2′-*O*-methoxyethyl substituted antisense oligonucleotides (MOE-AONs) are being studied[3] in several ongoing in vivo studies and have shown excellent results.

The presence of amino functionality in the AONs could reduce electrostatic charge-repulsions during duplex formation in some backbone/nucleobase modifications of DNA that could reduce the overall negative charges of the ONs.[4] Conjugation or complexation with positively charged peptides with oligonucleotides improves cellular uptake of oligonucleotides and analogues.[5] Introduction of positively charged substitution as in 2′-*O*-aminoethyl/2′-*O*-aminopropyl derived DNA oligomers shows increased stability towards hydrolytic enzymes.[6] The protonated amino group in the 2′-*O*-substituent of these modifications was found to displace a catalytically important metal cation of the hydrolytic enzyme.[7] The methoxy substitution as in OMe or MOE-AONs improves duplex stability by improving hydration in the minor groove and by increasing conformational pre-organization through a stable water structure.[8] The MOP-AONs marginally destabilize the duplexes with complementary RNA compared to MOE-AONs.[2] The thermal stability of the duplexes containing the 2′-*O*-aminoethyl/2′-*O*-aminopropyl is also relatively less compared to the OMe or MOE-AONs.[2,6] We planned the synthesis of a novel modification by combining the amino- and OMe-substitution patterns. The presence of a positively charged amino-functionality in conjunction with the methoxy substituent on the propyl chain would ensure that the resulting oligomers have advantages of both methoxy and amino groups (Fig. 1). The aminomethoxypropyloxy (AMP) substitution at 2′-position, thus derived, might improve nuclease stability of the AONs while maintaining duplex stability, comparable to MOE-AONs. Although a large number of 2′-*O*-chemical

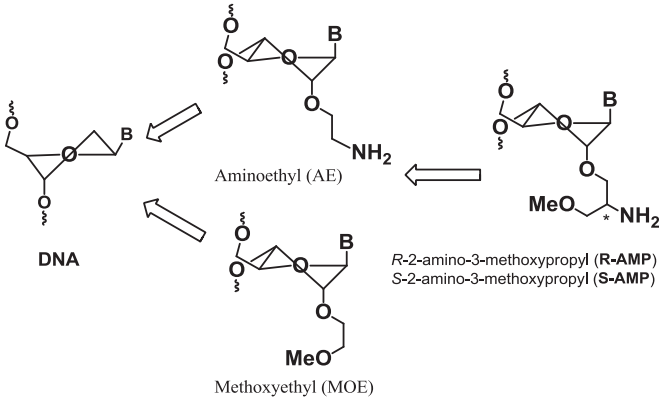

Fig. 1. Design of *R*-AMP and *S*-AMP.

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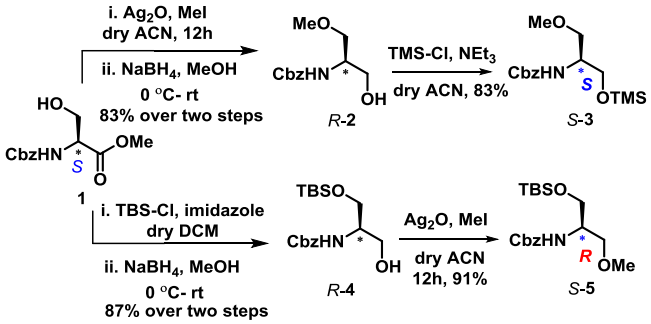
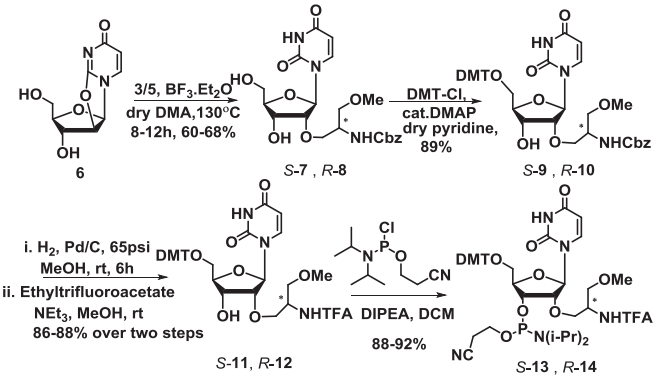
modifications are reported in the literature, this is perhaps the first report where branched chain is tethered stereospecifically, to the 2′-*O*-position of AONs. In addition to the presence of charged amino group, the hydrolytic susceptibility of the functionalized AONs is also known to be reduced with increasing size of the 2′-O-substitution.[2b] For the present enantiomeric *R*/*S*-AMP tethers, due to the conformational freedom, the steric bulk and electronic factors may be expected to be similar for each of the isomers when incorporated in the in the ssDNA.[9] We report here the synthesis of two diastereomeric nucleoside analogues differing in chirality at the 2′-*O*-sustituent groups with a synergistic combination of methoxy and amino pendant groups. Stereospecific synthesis of protected [*R*/*S*-(2-amino-3-methoxy)propanol tethers (*R*-AMP and *S*-AMP) from L-serine, synthesis of appropriately protected *R*-AMP and *S*-AMP phosphoramidite derivatives of uridine starting from 2,2′-anhydrouridine are presented. The stereochemistry of the tether effectively differentiated the hydrolytic cleavage of AONs and the *R*-AMP-AON was three times more stable than the *S*-AMP-AONs after 4 h. We also show that the thermal stability of *S*-AMP- and *R*-AMP-derivatized AONs is marginally better than the MOE/MOP-AONs. These oligomers exhibit stereochemistry-dependent protection against hydrolysis by snake venom phosphodiesterase enzyme (SVPD) when the MOE/MOP ONs are completely digested.

2. Results and discussion

There are previous literature reports[2] aimed to achieve selective alkylation of uridine at 2′-position, such as treatment of uridine with alkyl halides using DBTO[10] or by employing 3′,5′-*O*- and *N*-protected uridine in reactions with alkyl halides[2a] or cyanoethylation via a Michael addition of 3′,5′-*O*-protected uridine to the acrylonitrile.[11] Alternatively, regiospecific 2,2′-anhydrouridine ring opening is effected by reaction with borate esters generated from BH_3[12] and large excess of appropriate alcohols to get the desired products at high temperature and pressure or by treatment with metal alkoxides.[13] The yields were variable in each case and in the case of *N*-2-hydroxyethylphthalimide, the reported yield was 21%.[14] In the present design, we need to synthesize both the enantiomeric amino alcohol tethers separately. In the above mentioned protocols, the alcohol components are used as reaction medium or the alkylation is low yielding and we could not adopt them for the present studies. Alternatively, we came across $BF_3{\cdot}OEt_2$-promoted 2,2′-anhydro ring opening strategy used by Saneyoshi,[15] using excess trimethylsilylated alcohol as a nucleophile. We decided to employ this methodology by some variance in the strategy. The synthesis of protected [*S*/*R*-(2-amino-3-methoxy)propanol *S*-**3** and *R*-**5** was thus undertaken. We achieved this starting from the same chiral source, i.e., L-serine. Just by altering the sequence of reactions we arrive at the enantiomeric tethers *S*-**3** and *R*-**5** having suitable activation/protection, as shown in Scheme 1. Thus, *N*-Cbz-protected-L-serine methyl ester **1** was *O*-methylated using silver oxide and methyl iodide. After reduction, hydroxy compound *R*-**2** was obtained (83% overall yield) and was further activated as a silyl ether *S*-**3** (83%) using trimethylsilylchloride (TMS-Cl). Alternatively, ester **1** was first protected as *tert*-butyldimethylsilyl ether (TBS) and then the ester was reduced to get alcohol *R*-**4** (87%). Methylation of free hydroxyl group in *R*-**4** gave TBS protected ether *R*-**5** in 91% yield. We slightly improved Saneyoshi[15] strategy to get the desired 2,2′-anhydro ring-opened products *S*-**7** and *R*-**8** by employing 2 equiv of *S*-**3** and *R*-**5**, Scheme 2. We found that both the TMS- and TBS-silyl ethers were equally reactive as nucleophiles in 2,2′-anhydrouridine ring-opening and the yields were comparable (60, 68%, respectively). Compounds *S*-**7** and *R*-**8** were then protected as 5′-*O*-DMT to get *S*-**9** and *R*-**10**, respectively, (Scheme 2). The *N*-Cbz protecting group of the 2′-alkoxy derivatives was removed under hydrogenation conditions using Pd/C under pressure. The primary amino group was then protected to get the trifluoroacetyl derivatives *S*-**11** and *R*-**12**, Scheme 2[16] suitable for the solid-phase DNA synthesis protocols.



Scheme 1. Synthesis of protected/activated [*R*/*S*-(2-amino-3-methoxy)propanol.



Scheme 2. Synthesis of protected 2′-*O*-[*R*/*S*(2-amino-3-methoxy)propyl uridine phosphoramidite.

The phosphoramidite derivatives *S*-**13** and *R*-**14** were then prepared by phosphitylation using known method[17] starting from *S*-**11** and *R*-**12**, to be used in automated DNA synthesis. We chose a DNA sequence of biological relevance that was used in the splice-correction assay developed by Kang et al.[18] The unmodified oligomers were synthesized using Bioautomation MM4 DNA synthesizer and commercially available phosphoramidite building blocks. The phosphoramidites *S*-**13** and *R*-**14** were incorporated into sequences using increased coupling time (6 min) to yield the modified sequences. The site of the modified units in the sequences was decided so that these units were in the middle of the sequence (**16**–**19**, Table 1), were separated by 4–5 nucleosides (**20**–**23**, Table 1) or were at consecutive 3′-end positions (**24**–**28**, Fig. 2). The AONs were purified by HPLC subsequent to ammonia treatment and characterized by MALDI-TOF mass spectrometric analysis. Their purity was also re-checked by analytical HPLC and found to be >95% prior to their use in experiments. The thermal stability of DNA:RNA duplexes was evaluated using temperature dependent UV measurements and the results were comparable to U_{MOE} containing oligomers (Table 1). The vicinal electronegative substituents, namely, O4′, -2′-*O*- and 2-amino- and 3-methoxy- on the propyl chain at 2′-*O*-position of uridine would be expected to maintain the *gauche* orientations with respect to the O4′-oxygen to induce conformational change and hydration in the minor groove as is known for 2′-MOE derivatives.[7,8] We examined the *S*-type to *N*-type conformational equilibria for 2′-MOE-uridine with *S*-**7** and *R*-**8** and found that they are comparable (please see SI). This might indeed be the case even in the oligomers as the stability of the duplex structures was marginally higher with 2′-*O*-*R*-AMP (**19** and

Table 1
Modified DNA sequences, their MALDI-TOF mass analysis and biophysical evaluation by UV-T_m (°C) measurements[a]

Sequences no.[b]	Mass		T_m °C
	Calcd	Obsd	RNA[c]
cctcttacctcagttaca **15**			56.6
cctcttacctcagtU_{MOE}aca **16**	5429.9	5430.0	55.8(−0.8)[d]
cctcttacctcagtU_{MOP}aca **17**	5440.9	5474.1	55.3(−1.3)
cctcttacctcagt$U_{S\text{-}AMP}$aca **18**	5459.6	5460.2	56.2(−0.4)
cctcttacctcagt$U_{R\text{-}AMP}$aca **19**	5459.6	5460.6	56.2(−0.4)
cctcttaccU_{MOE}cagtU_{MOE}aca **20**	5490.9	5492.7	57.4(+0.8)
cctcttaccU_{MOP}cagtU_{MOP}aca **21**	5515.0	5541.6	56.5(−0.1)
cctcttacc$U_{S\text{-}AMP}$cagt$U_{S\text{-}AMP}$aca **22**	5549.0	5549.8	57.3(+0.7)
cctcttacc$U_{R\text{-}AMP}$cagt$U_{R\text{-}AMP}$aca **23**	5549.0	5551.4	57.5(+0.9)

[a] UV-T_m values were measured by annealing 1 μM sequences with 1 μM cDNA/RNA in sodium phosphate buffer (0.01 M, pH 7.2) containing 100 mM NaCl and is an average of three independent experiments. (Accuracy is ±0.5 °C).
[b] The lower case letters indicate unmodified DNA, U_{MOE} denotes 2′-*O*-methoxyethyl uridine, U_{MOP} denotes 2′-*O*-methoxypropyl uridine, $U_{S\text{-}AMP}$ and $U_{R\text{-}AMP}$ denote 2′-*O*-[*S*-(2-amino-3-methoxy)propyl and 2′-*O*-[*R*-(2-amino-3-methoxy)propyl uridine derivatives, respectively.
[c] 5′-uguaacugagguaagagg was the complementary RNA sequence.
[d] Values in parenthesis denote ΔT_m °C compared to the unmodified duplex.

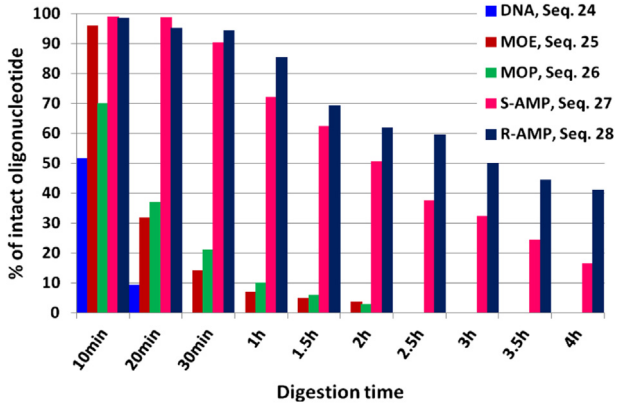


Fig. 2. Preferential protection of AONs containing *S*-AMP and *R*-AMP substituents at 3′-**27**–**28** (ttttttttttttt$U_{S\text{-}AMP}U_{S\text{-}AMP}$ **27**, ttttttttttttt$U_{R\text{-}AMP}U_{R\text{-}AMP}$ **28**) compared to native ttttttttttttttt **24**, MOE-modified DNA ttttttttttttt$U_{MOE}U_{MOE}$ **25** and MOP-modified DNA ttttttttttttt$U_{MOP}U_{MOP}$ **26**.

23) and 2′-*O*-*S*-AMP AONs (**18** and **22**) when compared with 2′-*O*-MOE/MOP (**16**,**17**/**20**,**21**) AONs.

We further explored the in vitro studies regarding the protection of these newly synthesized oligomers tethered with $U_{S\text{-}AMP}$ and $U_{R\text{-}AMP}$ units against hydrolytic cleavage and compared the results with the U_{MOE} tether.[1,2] MOE is being developed as antisense therapeutic and is also finding newer applications in siRNA approach where only two terminal units are modified with MOE.[3] We therefore followed literature precedence and synthesized a homothyminyl sequence[19,20] **24** and modified it at the 3′-terminus with two consecutive units of U_{MOE}, U_{MOP}, $U_{S\text{-}AMP}$ or $U_{R\text{-}AMP}$ to get sequences **25**, **26**, **27** and **28**, respectively, for comparison (Fig. 2). We digested these sequences with SVPD under conditions reported earlier.[19] The products of the digestion were analyzed by RP-HPLC and percent intact AON was plotted against time to understand the degradation pattern for all the oligomers (Fig. 2). The results clearly indicate the dependence of enzyme digestion on steric bulk and/or the presence of protonable amino group as charged species, as both the oligomers containing $U_{S\text{-}AMP}$ **27** and $U_{R\text{-}AMP}$ **28** are much more stable than the unmodified **24**, MOE-modified **25** and MOP-modified **26** sequences. The stereochemical influence of the AMP tethers in $U_{S\text{-}AMP}$ (**27**) and $U_{R\text{-}AMP}$ (**28**) AONs was clearly reflected in SVPD digestion experiment, (Fig. 2). The MOE- and MOP-AONs were almost completely digested by SVPD at the end of 1 h. At the same time, almost 90% of *R*-AMP-AONs and 80% *S*-AMP-AONs were intact and were effectively able to resist the hydrolytic cleavage. Furthermore, ~42% of *R*-tethered AON stereoisomer was still intact after 4 h and was almost thrice more stable than the *S*-stereoisomer. Introduction of chirality in the minor groove as in the case of *R*-AMP and *S*-AMP, did indeed allow differential protection of one of the isomers while interacting with the active site of the enzyme[6] and thus this interaction may be energetically different for two isomers, compared to the achiral, acyclic, alkyl substitution, such as MOE or AE/AP.[7,8] It could be presumed that the projection of the amino substituent in the minor groove while interacting with chiral environment of the enzyme[7] would play a crucial role for this result. The chirality of our new compounds containing stereospecific 2′-tethers thus allowed preferential protection against nucleases synergistically while retaining the duplex stability. The constrained chiral analogue of MOE synthesized earlier also could effectively protect the AONs from enzymatic degradation but without chiral discrimination.[21] The chirality effect on protection of AONs against digestive enzymes, such as SVPD was also observed when currently therapeutically used, fully modified, stereorandom phosphorothioate oligonucleotides were synthesized in chirally pure form.[22]

3. Conclusions

In this paper, efficient synthesis of novel, chiral *R*/*S*-AMP-AONs is presented. The synergy of chirally placed methoxy and amino groups substituents marginally improved the DNA:RNA duplex stability. The oligomers were highly stable to nuclease digestion as compared to MOE or POM where amino groups are absent. The *R*-AMP-AONs were thrice as stable to enzymatic degradation compared to the *S*-AMP-AONs.

4. Experimental section

4.1. (*R*)-Benzyl (1-hydroxy-3-methoxypropan-2-yl)carbamate, *R*-2

N-Cbz protected-L-serine-methyl ester **1** (39.5 mmol, 10 g) was dissolved in dry acetonitrile (250 mL), followed by the addition of Ag_2O (98.8 mmol, 22.8 g) and MeI (197.5 mmol, 12.7 mL) and the reaction mixture was vigorously stirred at room temperature for 12 h. Reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give the *O*-methylated derivative of **1**. The crude colourless residue was dissolved in MeOH (500 mL) and $NaBH_4$ (150 mmol, 5.6 g) was added fraction wise at 0 °C for a period of 1 h and the mixture was kept for stirring at room temperature for another 6–8 h. Excess $NaBH_4$ was quenched with saturated NH_4Cl solution, followed by the removal of MeOH under reduced pressure. The crude reaction mixture was extracted with ethyl acetate. The organic extract was washed with brine solution and dried over anhydrous sodium sulfate. Ethyl acetate was removed under reduced pressure to give the crude product and was purified through column chromatography (eluted in 20% ethyl acetate in petroleum ether) to yield *R*-**2** as a colourless liquid in 83% (7.8 g) over two steps. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.53 (br s, 1H), 3.35 (s, 3H), 3.55–3.86 (m, 5H), 5.11 (s, 2H), 5.43 (br s, 1H), 7.36–7.37 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.8, 58.7, 62.1, 66.4, 71.7, 127.7, 128.1, 136.0, 156.2. HRMS(EI) mass calcd for $C_{12}H_{18}O_4N$ (M+H) 240.1230, found 240.1229.

4.2. (*S*)-Benzyl (1-methoxy-3-((trimethylsilyl)oxy)propan-2-yl)carbamate, *S*-3

To a stirred solution of *R*-**2** (16.7 mmol, 4 g) and NEt_3 (83.5 mmol, 11.7 mL) in dry acetonitrile (70 mL), TMS-Cl

(25.1 mmol, 3.1 mL) was added and stirring was continued for another 1 h. The reaction mixture was diluted with ethyl acetate. Water wash and brine wash were given to the organic layer. The organic layer was dried over anhydrous sodium sulfate, solvents removed in vacuo and the crude compound was purified through column chromatography (eluted in 5% ethyl acetate in petroleum ether) to give *S*-**3** as a colourless liquid in 83% yield (4.3 g). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.10 (s, 9H), 3.32 (s, 3H), 3.35–3.39 (m, 1H), 3.47–3.50 (m, 1H), 3.56–3.60 (m, 1H), 3.68–3.73 (m, 1H), 3.84 (m, 1H), 5.09 (s, 2H), 5.27 (br s, 1H), 7.29–7.34 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 0.8, 51.3, 58.6, 60.6, 66.4, 70.3, 127.8, 128.2, 136.3, 155.7. HRMS(EI) mass calcd for $C_{15}H_{26}O_4NSi$ (M+H) 312.1626, found 312.1622.

4.3. (*R*)-Benzyl (1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypropan-2-yl)carbamate, *R*-4

N-Cbz protected-L-serine-methyl ester 1 (39.5 mmol, 10 g) was dissolved in dry DCM (200 mL), followed by the addition of imidazole (98.8 mmol, 6.7 g) and TBS-Cl (47.4 mmol, 7.1 g). The reaction mixture was diluted with DCM and the DCM layer was washed with water and brine solution. Organic layer was dried over anhydrous sodium sulfate and solvent removed in vacuo to result the crude TBS protected ester, which was directly subjected to $NaBH_4$ reduction. The colourless residue was dissolved in methanol (500 mL) and $NaBH_4$ (150 mmol, 5.6 g) was added fraction wise at 0 °C for a period of 1 h and then continued stirring at room temperature for another 6 h. Excess $NaBH_4$ was quenched with saturated NH_4Cl solution, followed by the removal of MeOH under reduced pressure and the crude compound was extracted with ethyl acetate. The organic extract was washed with brine and dried over anhydrous sodium sulfate. Ethyl acetate was removed under reduced pressure to give the crude product and was purified through column chromatography (eluted in 15% ethyl acetate in petroleum ether) to yield *R*-**4** as a colourless liquid in 87% (11.6 g) over two steps. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.05 (s, 6H), 0.88 (s, 9H), 2.64 (br s, 1H), 3.66–3.84 (m, 5H), 5.11 (s, 2H), 5.38–5.41 (m, 1H), 7.35–7.38 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ −5.6, 18.1, 25.7, 53.0, 63.0, 63.4, 66.7, 128.0, 128.4, 136.2, 158.2. HRMS(EI) mass calcd for $C_{17}H_{30}O_4NSi$ (M+H) 340.1939, found 340.1945.

4.4. (*R*)-Benzyl (1-((*tert*-butyldimethylsilyl)oxy)-3-methoxypropan-2-yl)carbamate, *R*-5

To a stirred solution of *R*-**4** (29.4 mmol, 10 g) and MeI (147.4 mmol, 9.5 mL), Ag_2O (73.5 mmol, 16.9 g) was added and the reaction mixture was vigorously stirred at room temperature for 12 h. Reaction mixture was filtered and the filtrate was concentrated under reduced pressure. Crude compound was purified through column chromatography (eluted in 5% EtOAc in petroleum ether) to result *R*-**5** as a colourless liquid in 91% (9.4 g) yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.05 (s, 6H), 0.88 (s, 9H), 3.33 (s, 3H), 3.37–3.77 (m, 4H), 3.78–3.86 (m, 1H), 5.10 (s, 2H), 7.35–7.38 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −5.6, 18.1, 25.7, 51.5, 58.8, 61.3, 66.6, 70.4, 128.0, 128.4, 136.4, 155.9. HRMS(EI) mass calcd for $C_{18}H_{32}O_4NSi$ (M+H) 354.2095, found 354.2089.

4.5. A typical procedure for 2′-O-functionalization of 2,2′-anhydrouridine

Desiccated 2,2′-anhydrouridine **6** (6.6 mmol, 1.5 g) was dissolved in dry DMA (10 mL) followed by the addition of $BF_3\cdot OEt_2$ (10 mmol, 1.2 mL) under argon atmosphere. After 2 min, the activated/protected silyl ethers 3/5 (13.2 mmol) were added and stirred at 130 °C for 8–12 h. DMA was removed partially in vacuo, followed by the dilution with MeOH and the reaction mixture was column purified (eluted in 5% MeOH in DCM) to result *S*-**7**/*R*-**8** as a white solid in 60–68% yield. *S*-**7** ^{1}H NMR (DMSO-d_6, 200 MHz): δ 3.21 (s, 3H), 3.49–3.63 (m, 5H), 3.82–3.9 (m, 3H), 4.03–4.10 (m, 1H), 5.01–5.05 (m, 3H), 5.14 (t, 1H, *J*=4.69, 9.75 Hz), 5.60–5.65 (m, 1H), 5.79 (d, 1H, *J*=4.15 Hz), 7.35 (m, 5H), 7.92 (d, 1H, *J*=7.76 Hz). ^{13}C NMR (DMSO-d_6, 200 MHz): δ 50.0, 58.1, 59.9, 65.3, 68.1, 69.1, 70.9, 81.5, 84.4, 86.1, 101.6, 127.7, 128.3, 136.9, 140.3, 150.3, 155.8, 163.1. HRMS(EI) mass calcd for $C_{21}H_{27}O_9N_3Na$ (M+Na) 488.1640, found 488.1630. *R*-**8** ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.22 (s, 3H), 3.51–3.68 (m, 5H), 3.78–3.93 (m, 3H), 4.09–4.13 (m, 1H), 4.97–5.051 (m, 3H), 5.16 (t, 1H, *J*=4.77, 9.54 Hz), 5.64–5.66 (m, 1H), 5.84 (d, 1H, *J*=4.77 Hz), 7.36 (m, 5H), 7.92 (d, 1H, *J*=8.03 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 50.3, 58.4, 60.6, 65.5, 68.6, 69.3, 71.4, 81.8, 85.0, 86.3, 102.0, 128.0, 128.5, 137.2, 140.6, 150.7, 156.0, 163.4 HRMS(EI) mass calcd for $C_{21}H_{28}O_9N_3$ (M+H) 466.1820, found 466.1822.

4.6. 5′-*O*-DMT-2′-*O*-(*N*-benzyloxycarbonyl-2′-*O*-*S*-AMP) and 2′-*O*-(*N*-benzyloxycarbonyl-2′-*O*-*R*-AMP) uridine: *S*-9/*R*-10

(3.44 mmol, 1.6 g) was dissolved in dry pyridine (10 mL) and DMT-Cl (3.61 mmol, 1.22 g) and catalytic amount of DMAP (~20 mg) were added. Reaction mixture was kept for stirring at room temperature for 5–6 h. Pyridine was removed under reduced pressure and the residue was diluted with ethyl acetate. 10% aq $NaHCO_3$, water and brine solution wash were given to the organic layer. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness. Crude compound was column purified (eluted in 70% ethyl acetate in petroleum ether) to result *S*-**9**/*R*-**10** as a white foam in 89% yield. *S*-**9** ^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.33 (s, 3H), 3.42–3.54 (m, 5H), 3.8 (s, 6H), 3.91–4.04 (m, 4H), 4.44 (br s, 1H), 5.09 (s, 2H), 5.23 (d, 1H, *J*=8.21 Hz), 5.32 (d, 1H, *J*=7.96 Hz), 5.89 (s, 1H), 6.83–6.87 (m, 4H), 7.31–7.40 (m, 14H), 8.02 (d, 1H, *J*=8.08 Hz), 8.26 (br s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 50.1, 55.0, 58.9, 61.0, 66.6, 68.2, 70.9, 71.4, 82.8, 82.9, 86.8, 87.5, 101.8, 113.1, 126.9, 127.8, 128.0, 128.3, 129.9, 130.0, 134.9, 135.2, 136.2, 139.9, 144.2, 150.2, 156.6, 158.4, 158.5, 163.8. HRMS(EI) mass calcd for $C_{42}H_{45}O_{11}N_3Na$ (M+Na) 790.2946, found 790.2931. *R*-**10** ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.31 (s, 3H), 3.50–3.52 (m, 5H), 3.76 (m, 6H), 3.90–3.91 (m, 1H), 4.02–4.04 (m, 3H), 4.39–4.42 (m, 1H), 5.07–5.08 (m, 2H), 5.27 (d, 1H, *J*=8.24 Hz), 5.64 (d, 1H, *J*=8.24 Hz), 5.92 (d, 1H, *J*=0.92 Hz), 6.82–6.84 (m, 4H), 7.27–7.39 (m, 14H), 7.96 (d, 1H, *J*=8.24 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 50.2, 55.0, 58.9, 61.2, 66.7, 68.3, 70.9, 71.5, 83.1, 83.2, 86.8, 87.2, 101.9, 113.1, 127.0, 127.8, 128.0, 128.3, 129.9, 130.0, 134.9, 135.1, 136.1, 139.8, 144.2, 150.3, 156.2, 158.50, 158.54, 163.7. HRMS(EI) mass calcd for $C_{42}H_{45}O_{11}N_3Na$ (M+Na) 790.2946, found 790.2946.

4.7. 5′-*O*-DMT-2′-*O*-(*N*-trifluoroacetyl-2′-*O*-*S*-AMP) and 2′-*O*-(*N*-trifluoroacetyl-2′-*O*-*R*-AMP) uridine: *S*-11/*R*-12

The 5′-DMT protected 2′-*O*-functionalized uridine derivative *S*-**7**/*R*-**8** (2.3 mmol, 1.8 g) was dissolved in MeOH (10 mL) followed by the addition of 10% Pd–C (10% w/w, 0.18 g). Then reaction mixture was subjected to catalytic hydrogenation at 65 psi of hydrogen pressure for 6 h. After the TLC analysis, reaction mixture was filtered over Celite and the removal of methanol in vacuo gave the free amine. Without further purification, the amine was subjected to trifluoroacetyl protection. To the crude amine (2.2 mmol, 1.4 g) dissolved in MeOH (15 mL), NEt_3 (3.3 mmol, 0.46 mL) was added. Ethyltrifluoroacetate was added to reaction mixture and the mixture was kept for stirring at room temperature for 8–10 h. MeOH was removed on rota evaporator and the reaction mixture was diluted with ethyl acetate. The organic layer was washed with water and 5% aq $NaHCO_3$ and the organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo. Crude compound was column purified to furnish *S*-**11**/*R*-**12** as a white foam in 86–88%

yield (eluted in 55% ethyl acetate in pet-ether). *S*-**11** ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.40 (s, 3H), 3.52–3.63 (m, 5H), 3.81 (s, 6H), 3.82–3.85 (m, 1H), 3.93 (dd, 1H, J=1.8 and 4.9 Hz), 4.03–4.06 (m, 1H), 4.09 (dd, 1H, J=4.8 and 10.0 Hz), 4.33–4.37 (m, 1H), 4.42–4.46 (m, 1H), 5.30 (dd, 1H, $J_{5,6}$=2.1 and 8.24 Hz), 5.92 (d, 1H, J=1.5 Hz), 6.85–6.86 (m, 4H), 7.25–7.39 (m, 9H), 8.00 (d, 1H, $J_{5,6}$=8.24 Hz). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 49.43, 55.2, 59.3, 61.2, 68.5, 70.1, 70.6, 83.4, 83.6, 87.1, 87.5, 102.3, 113.2, 113.3, 127.2, 128.0, 128.1, 130.0, 130.1, 135.0, 135.2, 139.7, 144.2, 150.2, 158.7, 162.7. HRMS(EI) mass calcd for $C_{36}H_{38}O_{10}N_3F_3Na$ (M+Na) 752.2402, found 752.2391. *R*-**12** ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.24 (s, 3H), 3.43–3.45 (m, 3H), 3.64–3.68 (m, 2H), 3.74 (s, 6H), 3.83 (m, 1H), 3.94–3.99 (m, 3H), 4.16–4.19 (m, 3H), 5.17 (d, 1H, J=7.50 Hz), 5.27 (dd, 1H, $J_{5,6}$=2.0 and 8.17 Hz), 5.77 (d, 1H, J=2.75 Hz), 6.89–6.91 (m, 4H), 7.24–7.39 (m, 9H), 7.70 (d, 1H, $J_{5,6}$=8.19 Hz). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 49.44, 55.2, 59.2, 60.9, 68.4, 69.3, 70.4, 83.1, 83.4, 87.1, 87.8, 102.2, 113.1, 113.3, 127.0, 128.0, 128.1, 130.0, 131.0, 135.0, 135.2, 139.7, 144.3, 150.3, 158.6, 158.7. HRMS(EI) mass calcd for $C_{36}H_{38}O_{10}N_3F_3Na$ (M+Na) 752.2402, found 752.2405.

4.8. General procedure was followed for the synthesis of phosphoramidite derivatives *S*-13 and *R*-14

To the compound *S*-**11**/*R*-**12** (0.68 mmol, 500 mg) dissolved in dry DCM (10 mL), DIPEA (1.7 mmol, 0.29 mL) was added. 2-Cyanoethyl-*N*,*N*-diisopropyl-chloro phosphine (0.81 mmol, 0.18 mL) was added to the reaction mixture at 0 °C and continued stirring at room temperature for 3 h. The contents were diluted with DCM and washed with 5% $NaHCO_3$ solution. The organic phase was dried over anhydrous sodium sulfate and concentrated to white foam. The residue was re-dissolved in DCM and the compound was precipitated with *n*-hexane to obtain corresponding phosphoramidite derivatives in 88–92% yield.

Phosphoramidite *S*-**13**: ^{31}P NMR (Acetonitrile, D_2O as external standard, 400 MHz): δ 149.22, 149.68. HRMS(EI) mass calcd for $C_{45}H_{55}O_{11}N_5F_3PNa$ (M+Na) 952.3480, found 952.3516.

Phosphoramidite *R*-**14**: ^{31}P NMR (Acetonitrile, D_2O as external standard, 400 MHz): δ 149.52, 150.62. HRMS(EI) mass calcd for $C_{45}H_{56}O_{11}N_5F_3P$ (M+H) 930.3661, found 930.3674.

4.9. SVPD digestion: stability of the oligonucleotides Seq

22–**25** towards *exo*-nucleases SVPD (snake venom phosphodiesterase) were analyzed by RP-HPLC. 7.5 μM of oligonucleotide in 300 μL of Tris–HCl buffer (pH=7.5, 10 mM Tris–HCl, 8 mM $MgCl_2$) were incubated at 37 °C for 15 min. SVPD 5 ng/μL was added to the oligonucleotide incubated at 37 °C and aliquots of 20 μL were removed at time intervals of 0, 2, 5, 10, 20, 30, 60, 90, 120, 150, 180, 210 and 240 min. Aliquots were kept at 90 °C for 2 min prior to their analysis on RP-HPLC with an increasing gradient (A: 5% acetonitrile and B: 30% acetonitrile in 0.1 N triethylammonium acetate, pH 7.0). And the % of the intact oligonucleotides (based on the %area of the peaks) was plotted against the time intervals.

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Supplementary data

^{1}H, ^{13}C and HRMS mass spectra of selected compounds in Scheme 1 and HPLC and MALDI-TOF-TOF mass spectra of oligomer **15**–**28**, temperature dependent UV-melting profiles of oligomers with complementary DNA/RNA sequences and overlay of HPLC chromatograms of SVPD digestion experiments of oligomers **24**–**28**. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.05.104.

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