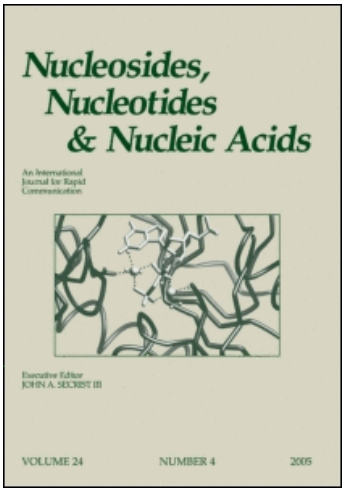

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An Unusual ''Senseless'' 2′,5′-Oligoribonucleotide with Potent Anti-HIV Activity†

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ABSTRACT

A new 32-mer 2′,5′-oligoribonucleotide of 1-methyl-6-thioinosinic acid (**10**) has been synthesized. The design of this unique oligoribonucleotide is based on the reported HIV inhibitory activities of both 2′,5′-oligonucleotides and the 3′,5′-oligoribonucleotides containing the 1-methyl-6-mercaptopurine base. Tm and CD studies of **10** revealed that it has no organized secondary structure, presumably due to the rigidity of the molecule. The synthesized oligomer, **10**, showed a potent inhibitory effect on HIV-1_{RF} and significantly inhibited HIV-1 reverse transcriptase.

Key Words: Oligoribonucleotide; HIV-1; Reverse transcriptase; ssRNA.

†In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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INTRODUCTION

The 2′,5′-oligonucleotide system represents a classical example of naturally occurring RNA isomers that are thought to be involved in the regulation of cell growth/differentiation and the antiviral effect of interferon.[1,2] These unique oligoribonucleotides are reported to inhibit the activities of HIV-1 reverse transcriptase[3,4] and DNA topoisomerase-I in HIV-1 infected cells.[5] Furthermore, their selective hybridization to single stranded RNA[6] (ssRNA) over ssDNA with a preexisting ability to activate RNase-L,[7,8] has led to the possibility of their use in antisense applications.[9] One serious limitation restricting the potential utility of these oligonucleotides, however, is in their rapid degradation by cellular nucleases.[10,11] There have been some recent efforts directed towards preparation of biologically stable analogs with 2′,5′-internucleotide connections.[7,11–14] Earlier, 2′,5′-linked 3′-deoxyoligonucleotides[13,15,16] were found to retain their selective affinity for ssRNA[17–19] and had a markedly prolonged biological half life compared to 3′,5′-linked DNA.[17]

Development of novel oligo-and polyribonucleotides as a new class of potent anti-HIV and HCMV drugs has been explored in this laboratory for several years. In contrast to the well-known antisense, sense and nonsense terminology used to describe nucleic acid interactions, these compounds are referred to as ‘‘senseless’’ because of

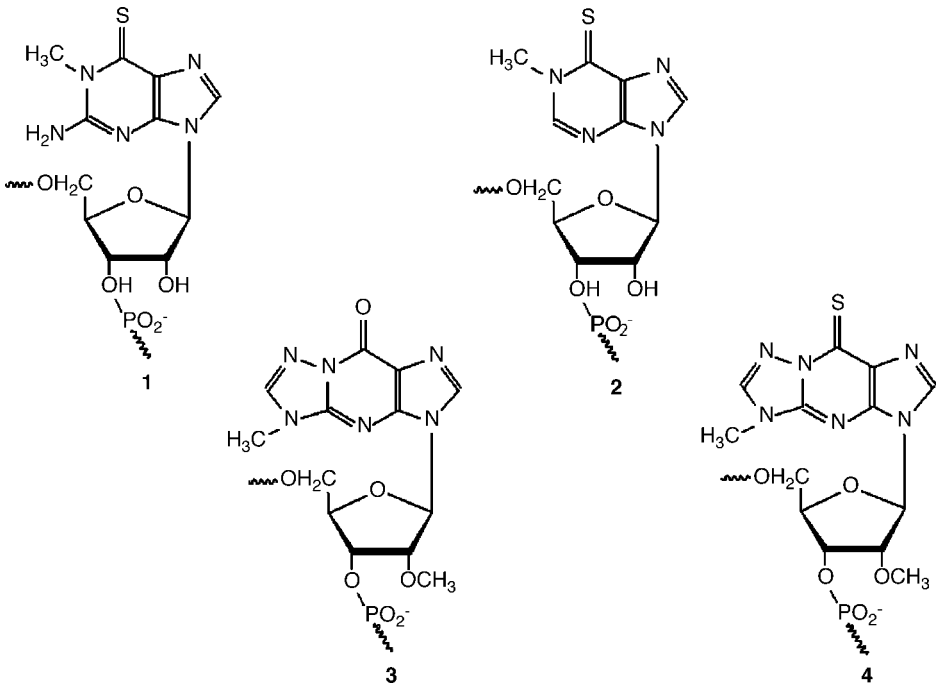

Chart 1. Anti-HIV active Oligo- and Polyribonucleotides. Abbreviations in the text are: 1, PMTG, poly(1-methyl-6-thioguanylic acid), 2, PMTI, poly(1-methyl-6-thoimosinic acid), 3, PTPR, poly[5-methyl-8-oxo-3-(β-D-ribofuramosyl)-1,2,4-triazolo[2,3-a]purinylic acid), 4, TTPR, a 32-mer oligonucleotide containing 5,2′-di-O-methyl-3-(β-D-ribofuranosy)-1.2.4-triazole[2.3-a]purine-9-thione.

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their complete lack of Watson-Crick hydrogen bonding sites. It was demonstrated earlier that PMTG (**1**), PMTI (**2**),[20,21] PTPR (**3**) and TTPR-32mer (**4**)[22,30] (Chart 1) all have effective anti-HIV activity with EC_{50} values ranging from 0.05 to 1.7 μM.

Preliminary SAR analysis reveals that the required structural elements for activity in this limited set of compounds are amphipathic character (hydrophobic base/hydrophilic backbone) and secondary structure (non–hydrogen bonded base-stacked array in solution).[20,23] The difference in activity of PMTI vs. PMI [poly(1-methylinosinic acid)] and TTPR-32mer vs. TPR-32mer also indicates a preference for sulfur over oxygen at the purine 6 position. Accordingly, this work involves synthesis and anti-HIV activity of a new type of 2′,5′-oligonucleotide containing the unusual nucleotide 1-methyl-6-thioinosinic acid.

CHEMISTRY

1,3′-O-Dimethyl-6-thioinosine (**6**) was synthesized starting with 3′-O-methyladenosine (**5**) according to the general procedures of Ueda as subsequently modified for large scale preparations.[24,25] Methylation of 3′-O-methyladenosine (**5**) by methyl iodide in dimethylacetamide (DMA) at room temperature afforded 1,3′-O-dimethyladenosine (**6**) in 87% yield. Application of the amino-thiono exchange reaction afforded 1,3′-O-dimethyl-6-thioinosine (**7**) in 60% yield. The synthon required for automated synthesis was prepared by protection of the 5′ position with DMTr and conversion of the 2′-OH to the phosphorodiamidite.[26] Compound **7** was dissolved in pyridine and treated with DMTrCl for overnight. The DMTr product (**8**) was purified by flash chromatography in the presence of triethylamine to avoid DMTr solvolysis. The phosphorodiamidite reaction was run in dry acetonitrile with diisopropylammonium tetrazolide salt and 2-cyanoethyl tetraisopropylphosphorodiamidite reagent. In order to prevent phosphoramidite oxidation during work-up, flash chromatography was carried out in a positive pressure argon atmosphere in the presence of triethylamine. Subsequently, oligomer synthesis was carried out (1 μmol) on an automated DNA synthesizer using **9** and a solid support resin conjugated to the 3′-posiiton of a thymidine. The coupling time was 25 minutes and tert-butyl peroxide was used for oxidation. At the end of synthesis oligomer (**10**) was removed from the solid support by treatment with NH_4OH at room temperature for 1 hour. Completely deblocked 32-mer (with the 3′-terminal T) was purified using a Poly-Pac II cartridge. The 5′-DMTr group of the last residue of the full-length oligomer was left in place in order to facilitate ''trityl-on'' purification. The oligomer was adsorbed onto the resin and an ammonium hydroxide wash was used to elute the failure sequences. The DMTr group was subsequently removed with TFA and then the oligomer was eluted with 50% aqueous acetonitrile. Completely deblocked 2′–5′-oligomer was further purified to homogeneity by reversed phase (C-8) HPLC according to the protocol described in the experimental section.

Verification of the structure of the oligomer involved both composition validation and mass spectral analysis. Digestion of **10** with alkaline phosphatase and venom phosphodiesterase yielded the starting nucleoside 1,3′-O-dimethyl-6-thioinosine (**6**) as the sole purine nucleoside component. The homogeneity of the oligonucleotide was confirmed by the appearance of a single band on size-exclusion HPLC. MALDI mass spectrometry revealed a mass of 11,815, quite consistent with the calculated mass of 11,807.

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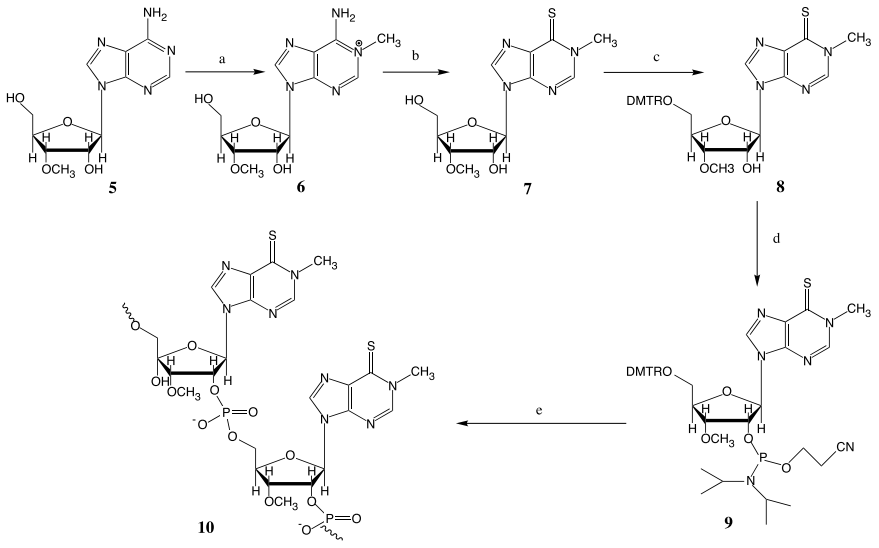
For biological studies, the oligomer was converted to its sodium salt using the same columns, followed by exhaustive dialysis against 0.1 M NaCl and then H_2O. Following this purification scheme, the oligomer was highly pure, eluting as a single peak on HPLC. The average yield was approximately 50% (Scheme 1).

Tm Investigation

UV absorbance versus temperature profiles of 2′–5′-linked oligomer, **10**, were essentially linear across a temperature range of 5 to 85°C (data not shown), showing no evidence of the sigmoidal transition typical of an ordered secondary structure. The total hyperchromicity observed over this temperature range was about 9%, consistent with some degree of base stacking. The relative rigidity of the 2′–5′ linkage compared to the 3′,5′-linked phosphodiesters probably limits secondary structure formation.[27–29]

CD Investigation

CD spectra of 10 run in ammonium acetate buffer (0.1 M) at room temperature and 0°C were similar in shape to those previously observed for the random coil form of PMTI.[30] A negative band at 309 nm and a positive band at 274 nm (data not shown) were observed and little temperature difference was seen in the spectra. Despite the identical 1-methyl-6-mercaptopurine chromophore in 10 and PMTI, however, the bands



Scheme 1. Synthesis of 2′-5′-Oligo(1-methyl-6-thioinosinic acid) 32-mer (**10**). Reagents and conditions. **a**. CH_3I/DMA, RT, 24 hours; **b**. H_2S/aq. pyridine, 80°C, steel bomb, 3 days; **c**. DMTRCl/pyridine; **d**. Diisopropylammonium tetrazolide, 2-cyanoethyl tetraisopropyl phosphorodiamidite; **e**. Automated RNA synthesis.

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Table 1. Anti-HIV activity of 2′-5′oligonucleotide **10**.

Compound	CEMSS/RF EC50 (μM/mL)	CEMSS TC50 (μM/mL)	Therapeutic index
10	0.228	> 4.238	> 18.8
AZT	0.001	> 1.0	> 1000

in 10 appeared at about 30 nm lower wavelength. Such differences in band positions have been previously noted in comparing 2′, 5′ with 3′, 5′-linked oligonucleotides, and have been attributed to differing interactions of the base transition moments in the two species.[27–29] The observed data are consistent with the reduced conformational flexibility and altered base stacking conformations previously reported for 2′, 5′-oligonucleotides.[27–29]

BIOLOGICAL RESULTS

The anti-HIV activity of **10** was evaluated against HIV-1_{RF} in comparison with AZT in the CEM–SS human cell line. The EC_{50} value of **10** was found to be 0.23 μM (Table 1) with a selectivity index > 18.8 based upon the highest dose tested. The IC_{50} value for inhibition of HIV-1 reverse transcriptase was 0.39 μM. Given that a higher concentration of oligonucleotide is required to inhibit RT than to reduce the viral cytopathic effect, it is unlikely that RT is the primary target for this molecule unless it can be concentrated in the cell against a gradient, which seems an improbable conjecture. Recent data with somewhat similar oligomers suggest that inhibition of virus entry is a more likely mechanism for these senseless oligonucleotides.[23,31] No information is currently available as to whether the compound is involved in any way with the interferon cascade, but given the structure of the base, this seems unlikely. Whatever the mechanism of action, it seems clear that senseless oligonucleotides, whether 3′, 5′-or 2′, 5′-linked, are capable of inhibition of viral replication at sub-micromolar levels without toxicity. Furthermore, the data support the fundamental hypothesis that ampathicity (hydrophilic backbone – hydrophobic base) is a requirement for potent antiviral activity.

CONCLUSIONS

The current investigation involved the utilization of a 6-mercaptopurine-containing nucleotide in the synthesis of a 2′,5′oligomer. Compounds of this type are characterized by a relatively rigid structure subject to relatively minor temperature-dependent conformational alteration, and the synthesized oligomer showed no tendency to form the kind of helical array previously seen with the 3′, 5′-linked oligomers containing the same purine base. Despite this inability, the compound showed sub-micromolar inhibition of HIV-1 cytopathicity, suggesting that further exploration of this system may be warranted.

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EXPERIMENTAL

ChemGenes Corporation, Waltham, MA was the source of 3′-O-methyladenosine. Other materials were purchased from Aldrich, Bio-Rad, Chem-Impex International, EM Science, Fisher, Selecto Scientific and Sigma and used without purification. Thin Layer Chromatography (TLC) was performed on silica gel coated Whatman aluminum backed flexible plates containing fluorescent indicator F_{254}. TLC spots were visualized with short wavelength UV. Column chromatography was performed with silica gel, particle size 63–200. Conversion to the NH_4^+ form of the mono-and diphosphates was carried out using BioRad 50W-X8 (200–400 mesh) cation exchange resin. Cation exchange chromatographic purification of the polymer was performed with AG® MP-50, 100–200 mesh, hydrogen form resin using a Wiz pump, ISCO type 6 optical unit (254 nm) and an ISCO UA-5 absorbance monitor. HPLC chromatography was performed with a Hitachi L6200 pump equipped with a L3000 photo diode array using a Bio Rad SEC-125 column. High resolution mass spectra were recorded with a MAT 95 spectrometer. Proton and phosphorus NMR spectra were recorded with an IBM AF200 MHz FT-NMR. UV spectra and T_m measurements were recorded with a Hewlett-Packard 8452A diode array spectrometer equipped with a Peltier variable temperature controller. Circular dichroism spectra were obtained using a Jeol J720 CD spectropolarimeter equipped with a variable temperature device.

HPLC Protocol. Size exclusion: 100%; phase a: 0.05 M phosphate buffer + 0.15 M NaCl + 0.01 M NaN_3. Reverse phase (C8): 0–60%, 45 min; phase a: acetonitrile; phase b: 0.1 M ammonium acetate.

5′-O-(4,4′-Dimethoxytrityl)-3′-O, 1-dimethyl-6-thioinosine (**8**). To a solution of dry 3′-O, 1-dimethyl-6-thioinosine (**7**) (5.61 g ,13.5 mmol) in pyridine (135 mL) was added DMTrCl (6.0 g ,17.8 mmol). The reaction mixture was vigorously stirred for overnight. One volume of MeOH was added and the reaction mixture was evaporated to a thick oil. The oil was dissolved in 100 mL $CHCl_3$, washed 3 times with 15 mL of both saturated $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The product was purified by flash chromatography under argon by gradient elution of ethyl acetate from 0 to 100% in $CHCl_3$ in the constant presence of 0.1% triethylamine. Product was recovered in the 100% $CHCl_3$. Evaporation of the solvent gave a white solid. Isolated yield was 7.24 g (87.5%).

^{1}H-NMR ($CDCl_3$): 8.11 (1H, s, H2); 8.04 (1H, s, H8); 7.41–7.20 (9H, m, DMT); 6.83 (2H, d, J=6.4 Hz, DMT); 6.81(2H, d, J=6.2 Hz, DMT); 5.89 (1H, d, J=6 Hz, H1′); 4.79 (1H, t, J=11.2 Hz, H4′); 4.30–4.27 (1H, q, J=12, H2′); 4.05–4.02 (1H, q, J=13.7, H3′); 3.95(3H, s, OCH_3); 3.79 (6H, s, DMT–OCH_3); 3.47 (3H, s, N^1–CH_3); 3.45–3.42 (1H, m, H5′), 3.36–3.32 (1H, m, H5″).

HRMS (glycerol & MeOH), m/z (MH^+) calcd for $C_{33}H_{34}N_4O_6S$ 615.22773, found 615.22637; Rf=0.54 ($CHCl_3$/MeOH, 95:5); Single spot by TLC.

5′-O-(4,4′-Dimethoxytrityl)-3′-O,1-dimethyl-6-thioinosine-2′-O-(cyanoethyl-N,N-di-*iso*propyl) phosophoramidite (**9**). To a vigorously stirring solution of **8** (2.46 g, 4 mmol) in acetonitrile (30 mL) was added diisopropylammonium tetrazolide (189.22 mg) and 2-cyanoethyl tetraisopropylphophorodiamidite (2 g, 3.22 mmol). The salt was prepared by adding diisopropylamine (2 mL, 14.28 mmol) to a solution of

tetrazole (412 mg, 5.88 mmol) in dry acetonitrile (22 mL) and isolated by filtering the precipitate that formed after 10 min of reaction time. The reaction mixture was stirred overnight under argon. The reaction mixture was diluted with one volume of saturated $NaHCO_3$ and extracted with ethyl acetate (three x 50 mL). The organic layer was washed twice with brine (2 x 25 mL), once with H_2O (25 mL), dried over anhydrous Na_2SO_4 and evaporated to dryness. The product was purified by flash chromatography under argon by gradient elution of ethyl acetate from 0 to 80% in hexanes in the constant presence of 1% triethylamine. Product was recovered at 70–80% ethyl acetate. Solvent was removed *in vacuo* by coevaporation with CH_2Cl_2 to give a white foam. Isolated yield was 2.6 g (80%). ^{31}P-NMR (400 MHz, $CDCl_3$): 152.42, 152.04. HRMS (3NBA & $CHCl_3$), m/z (MH^+) calcd for $C_{42}H_{51}N_6O_7PS$ 814.32776, found 814.32702. Rf=0.86 ($CHCl_3$/MeOH, 95:5); Rf=0.20, 0.26, R_P and S_P Rf values not assigned, (EtOAc/hexanes, 1:1); chromatographically homogeneous.

3′-O,1-Dimethyl-6-thioinosinic acid 32-mer, (**10**). The 32-mer oligonucleotide was synthesized on a 1 μmol scale using an ABI model 394 automated DNA synthesizer. As opposed to the standard protocol, coupling time was extended to 25 min, *tert*-butyl peroxide was used as the oxidizing solution and final product was cleaved from the support resin for only 1 hr in NH_4OH at room temperature. Crude DMTr-ON product was dried by speed evacuation at room temperature and purified by the Poly-Pac II cartridge purification scheme developed by Glen Research. Following cartridge preparation with acetonitrile (4 mL) and ammonium acetate (2.0 M, 4 mL), the cartridge was flushed three times with the DMTr-ON oligo solution (3 mL, 0.1 M triethyl ammonium acetate). The cartridge was washed with 6 mL NH_4OH (1:10) followed by 4 mL H_2O. Oligonucleotide was detritylated by flushing the column with 2% TFA (4 mL) and following a water wash (4 mL), the DMTr-OFF product was eluted with acetonitrile/water (1:1, 4 mL). Collected eluent was lyophilized and converted to Na^+ form by again using the Poly Pac-II cartridge. For Na^+ conversion, the cartridge was prepared with acetonitrile (4 mL) and sodium acetate (2.0 M, 4 mL) and the DMTr-OFF product was prepared by dissolution in 3 mL 0.1 M sodium acetate. DMTr-OFF oligonucleotide was loaded onto the cartridge by flushing three times with the solution. The cartridge was washed with 6 mL of sodium acetate (0.1 M) and the oligonucleotide was eluted as the Na^+salt with acetonitrile/H_2O (1:1, 4 mL). Collected product was lyophilized, dissolved in 5 mL H_2O and dialyzed against 0.1 M NaCl (24 hr, 8 L) and against H_2O (48 hr, 16 L). Lyophilized product yielded 53 % of a white fluffy solid. Product was collected for MALDI by reverse phase HPLC chromatography. UV: ε_{max} 16898 at 318 nm; MALDI (M + H) calcd 11807, found 11815; HPLC retention time 21.14 min (reverse phase protocol); 8.64 min (size exclusion protocol); homogeneous by HPLC.

Enzymatic Degradation of 10

To a solution of **10** in 0.1 M NaCl (100 μl, 2 mg/mL) was added 65 μl of a solution comprised of the following: Tris HCl (2M, pH 9.0, 33 μl); 0.1 M $MgCl_2$ (44 μl), venom phosphodiesterase (44 μl); and alkaline phosphatase (22 μl). The solution was incubated at 37°C with gentle rocking for 24 hours and then diluted to 5 mL with NaCl (0.1 M). TLC in DCM/MeOH (95:5) confirmed degradation to nucleoside **7** as confirmed by co-elution with the authentic material.

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Anti-HIV Evaluation

CEM-SS cells passaged in T-75 flasks were split (1:2) 24 hours prior to the time of infection. Just prior to infection, cells were washed twice with media, pelleted and resuspended with media to a concentration of 5×10^4 cells per mL. Cell viability was evaluated by trypan blue exclusion and any culture found to have less than 95% viability was discarded. For HIV analysis, 50 μL of the cell culture was added to drug containing wells and cell control wells in a 96 well plate.

For the standard HIV assay, HIV-1_{RF} initially acquired from the NIH AIDS Research and Reference Reagent Program were grown in CEM–SS cells for stock pools that were subsequently frozen at −80°C. Just prior to infection, the virus was slowly thawed, suspended in media and diluted to a concentration that would effect 85–95% cell kill in six days. For analysis, 50 μL was added to drug containing wells and virus control wells in a 96 well plate. Additional anti-HIV evaluations with other virus strains were conducted by the same protocol.

For HIV analysis, the test compound was brought up in either 0.1 M NaCl, PBS or H_2O to predetermined concentrations. Compound was aliquoted into wells at 0.32 μL, 1.0 μL, 3.2 μL, 10 μL, 32 μL and 100 μL. Drug was added to wells to provide duplicate measurements of cells + drug, drug + media, and triplicate measurements of cells + drug + media at each drug concentration.

Cell viability was determined at the end of the testing period by MTS staining. Briefly, 20 μL of MTS was added to all wells and incubated overnight. The sealed plates were inverted several times and dye absorbance was read at 490 nm with a plate reader. Calculations from the absorbance readings provided % CPE reduction, % cell viability, IC50 and TC50 values.

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REFERENCES

1. Player, M.R.; Torrence, P.F. The 2-5A system: Modulation of viral and cellular processes through acceleration of RNA degradation. Pharmacol. Ther. **1998**, *78*, 55–113.
2. Torrence, P.F.; Xiao, W.; Li, G.; Khamnei, S. Development of 2′,5′ Oligonucleotides as potential therapeutic agents. Curr. Med. Chem. **1994**, *1*, 176–191.
3. Sobol, R.W.; Fisher, W.L.; Reichenbach, N.L.; Kumar, A.; Beard, W.A.; Wilson S.H.; Charubala, R.; Pfleiderer, W.; Suhadolnik, R.J. HIV-1 reverse transcriptase: inhibition by 2′,5′-oligonucleotides. Biochemistry **1993**, *32*, 12112–12118.
4. Montefiore, D.C.; Sobol, R.W., Jr.; Li, W.W.; Reichenbach, N.L.; Suhadolnik, R.;

Charubala, R.; Pfleiderer, W.; Modiszewski, A.; Robinson, W.E., Jr.; Mitchell, W.M. Phosphorothioate and cordycepin analogues of 2′,5′-oligoadenylate: inhibition of human immunodeficiency virus type I reverse transcriptase and infection in vitro. Proc. Natl. Acad. Sci. U. S. A. **1989**, *86*, 7191–7194.

5. Schroder, H.C.; Kelve, M.; Schacke, H.; Pfleiderer, W.; Charubala, R.; Suhadolnik, R.J.; Muller, W.E. Inhibition of DNA topoisomerase I activity by 2′,5′-oligoadenylates and mismatched double stranded RNA in uninfected and HIV-1-infected H9 cells. Chem. Biol. Int. **1994**, *90*, 169–183.
6. Giannaris, P.A.; Damha, M.J. Oligoribonucleotides containing 2′,5′-phosphodiester linkages exhibit binding selectivity for 3′,5′-RNA over 3′,5′ DNA. Nucleic Acids Res. **1993**, *21*, 4742–4749.
7. Kariko, K.; Sobol, R.W., Jr.; Suhadolnik, L.; Li, S.W.; Reichenbach, N.L.; Suhadolnik, R.J.; Charubala, R.; Pfleiderer, W. Phosphorothioate analogs of 2′,5′-oligoadenylate. Enzymatically synthesized 2′.4′-phophorothioate dimer and trimer: unequivocal structural assignment and activation of 2′,5′-oligoadenylate-dependent endoribonuclease. Biochemistry **1987**, *26*, 7127–7135.
8. Kitade, Y.; Nakata, Y.; Hirota, K.; Maki, Y.; Pabuccuoglu, A.; Torrence, P.F. 8-Methyladenosine-substituted analogues of 2–5A: synthesis and their biological activities. Nucleic Acids Res. **1991**, *19*, 4103–4108.
9. Torrence, P.F.; Maitra, R.K.; Lesiak, K.; Khamnei, S.; Zhou, A.; Silverman, R.H. Targeting RNA for degradation with a (2′–5′)oligoadenylate antsense chimera. Proc. Natl. Acad. Sci. U. S. A. **1993**, *90*, 1300–1304.
10. Schmidt, A.; Chernajovsky, Y.; Shulman, L.; Federman, P.; Beressi, H.; Revel, M. An interferon-induced phosphodiesterase degrading (2′–5′)oligoisoadenylate and the C–C–A terminus of tRNA. Proc. Natl. Acad. Sci. U. S. A. **1979**, *76*, 4788–4792.
11. Herdewijn, P.; Charubala, R.; De Clercq, E.; Pfleiderer, W. Nucleotides. Part XXXII. Synthesis of 2′,5′ connected oligonucleotides. Prodrugs for antiviral and antitumoral nucleosides. Helv. Chem. Acta **1989**, *72*, 1739–1748.
12. Verheijen, J.C.; Chen, L.; Bayly, S.F.; Torrence, P.F.; Van der Marel, G.A.; Gijsbert, A.; Van Boom, J.H. Synthesis and RNAse L binding and activation of a 2–5A-(5′)-DNA-(3′)-PNA chimera, a novel potential antisense molecule. Nucleosides Nucleotides Nucleic Acids **2000**, *19*, 1821–1830.
13. Wasner, M.; Henderson, E.E.; Suhadolnik, R.J.; Pfleiderer, W. Nucleotides. Part XLIV. Synthesis, characterization and biological activity of monomerica nd trimeric cordycepin-cholesterol conjugates and inhibition of HIV-1 replication. Helv. Chem. Acta **1994**, *77*, 1757–1767.
14. Polak, M.; Manoharan, M.; Iamati, G.B.; Plavec, J. Tuning of conformational preorganization in model 2′,5′-and 3′,5′-linked oligonucleotides by 3′-and 2′-O-methoxyethyl modification. Nucleic Acids Res. **2003**, *31*, 2066–2076.
15. Sheppard, T.L.; Rosenblatt, A.T.; Breslow, R. Preparation of 2′-O-(β-cyanoethyl phosphoramidites) of 3′-deoxycytidine and 3′-deoxyguanosine and their use for solid phase synthesis of oligodeoxynucleotides containing 2′,5′-phosphodiester linkages. J. Org. Chem. **1994**, *59*, 7243–7248.
16. Rhie, S.-Y.; Pfleiderer, W. Nucleosides. LVI. Synthesis and chemical modification of 3′-deoxypyrimidine nucleosides. Nucleosides Nucleotides **1994**, *13*, 1425–1452.
17. Alul, R.; Hoke, G.D. (2′–5′)-Oligo-3′-deoxynucleotides: selective binding to single-stranded RNA but not DNA. Antisense Res. Dev. **1995**, *5*, 3.

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18. Jin, R.; Chapman, W.H.; Srinivisan, A.R.; Olson, W.K.; Breslow, R.; Breslauer, K.J. Comparative spectroscopic, calorimetric and computational studies of nucleic acid complexes with 2′,5′-vs. 3′,5′-phosphodiester linkages. Proc. Natl. Acad. Sci. U. S. A. **1993**, *90*, 10568–10572.
19. Jung, K.-E.; Switzer, C. 2′,5′-DNA containing guanine and cytosine form stable duplexes. J. Am. Chem. Soc. **1994**, *116*, 6059–6061.
20. Broom, A.D.; Agrawal, V.K.; Tutonda, M.G.; Fain, H.D.; Buckheit, R.W., Jr. Unusual single-stranded polyribonucleotides as potent anti-HIV agents. J. Med. Chem. **1995**, *38*, 3253–3257.
21. Buckheit, R.W., Jr.; Lackman-Smith, C.; Snow, M.J.; Halliday, S.M.; White, E.L.; Ross, L.J.; Agrawal, V.K.; Broom, A.D. PMTI, a broadly-active unusual single-stranded polyribonucleotide, inhibits human immunodeficiency virus replication by multiple mechanisms. Antivir. Chem. Chemother. **1999**, *10*, 23–32.
22. Tutonda, M.G.; Buckheit, R.W., Jr.; Agrawal, V.K.; Broom, A.D. Antiviral oligo- and polyribonucleotides containing selected triazolo[2.3-a]purines. J. Med. Chem. **1998**, *41*, 4958–4964.
23. Hyde, R.M.; Buckheit, R.W., Jr.; Broom, A.D. ''Senseless'' antiviral polyribonucleotides: Poly(1-propargylinosinic acid). Nucleosides Nucleotides Nucleic Acids **2002**, *21*, 45–54.
24. Miura, K.; Ueda, T. Nucleosides and nucleotides. XIII. Synthesis of thiopurine nucleosides from adenosine and guanosine derivatives by the sulfohydrolysis. Chem. Pharm. Bull. **1975**, *23*, 2064–2069.
25. Ratsep, P.C.; Mishra, N.C.; Broom, A.D. Thiopurine nucleosides: variations in hydrophobicity among N^1 substituents. Nucleosides Nucleotides **1991**, *10*, 1641–1655.
26. Kierzek, R.; Caruthers, M.H.; Longfellow, Ce.E.; Swinton, D.; Turner, D.H.; Freier, S.M. Polymer-supported RNA synthesis and its application to test the nearest-neighbor model for duplex stability. Biochemistry **1986**, *25*, 7840–7846.
27. Wasner, M.; Arion, D.; Borkow, G.; Noronha, A.; Uddin, A.H.; Parniak, M.A.; Damha, M.J. Physicochemical and biochemical properties of 2′.5′-linked RNA and 2′,5′-RNA:3′,5′-RNA ''hybrid'' duplexes. Biochemistry **1998**, *37*, 7478–7486.
28. Kumar, A.; Dass, D.; Atreyi, M.; Rao, M.V.R.; Katti, S.B. Conformational rigidity introduced by 2′,5′-phosphodiester links in DNA. Nucleosides Nucleotides Nucleic Acids **2001**, *20*, 1783–1796.
29. Sawai, H.; Kuroda, K.; Seki, J.; Ozaki, H. Conformational and stacking properties of 3′.5′ and 2′,5′ linked oligoribonucleotides studied by CD. Biopoymers **1996**, *39*, 173–182.
30. Tutonda, M.G.; Fain, H.D.; Buckheit, R.W., Jr.; Broom, A.D. The role of hydrophobic versus hydrophilic base character in the anti-HIV activity of purine-containing polyribonucleotides. Nucleosides Nucleotides **1997**, *16*, 173–182.
31. Hyde, R.M. ''Senseless'' Nucleic Acid Macromolecules. Synthesis, Characterization and Biological Studies. Ph.D. Dissertation; University of Utah, 2002.

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