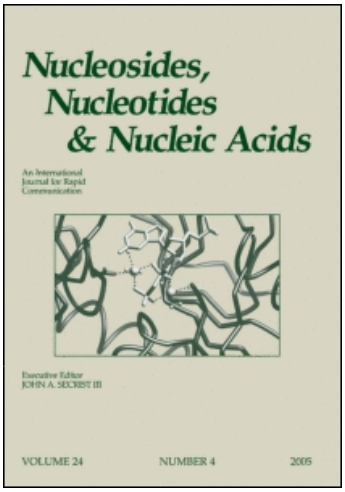

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Incorporation of 5''-Amino- and 5'-Mercapto-5'-Deoxy-2'-O-Methyl Nucleosides Into Hammerhead Ribozymes

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To cite this Article Matulic-Adamic, Jasenka , Haeberli, Peter , DiRenzo, Anthony B. , Mokler, Victor R. , Maloney, Lara , Beigelman, Leonid , Usman, Nassim and Wincott, Francine E.(1997) 'Synthesis and Incorporation of 5''-Amino- and 5'-Mercapto-5'-Deoxy-2'-O-Methyl Nucleosides Into Hammerhead Ribozymes', Nucleosides, Nucleotides and Nucleic Acids, 16: 10, 1933 — 1950
To link to this Article: DOI: 10.1080/07328319708002545
URL: http://dx.doi.org/10.1080/07328319708002545

NUCLEOSIDES & NUCLEOTIDES, 16(10&11), 1933-1950 (1997)

SYNTHESIS AND INCORPORATION OF 5'-AMINO- AND 5'-MERCAPTO-5'-DEOXY-2'-*O*-METHYL NUCLEOSIDES INTO HAMMERHEAD RIBOZYMES

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ABSTRACT. Novel 5'-amino-5'-deoxy-2'-*O*-methyl uridine, guanosine and adenosine 3'-*O*-phosphoramidites **5**, **11**, and **20**, as well as protected 5'-mercapto-5'-deoxy-2'-*O*-methyl uridine 3'-*O*-phosphoramidite **23** were synthesized from 2'-*O*-methyl nucleosides. These analogs were incorporated at the 5'-ends of hammerhead ribozymes to evaluate achiral bridging 5'-*N*-phosphoramidates and 5'-*S*-phosphorothioates as alternatives for non-bridging phosphorothioates commonly used for end stabilization against nucleases. Oligonucleotide synthesis and deprotection conditions were optimized for better yields of these modified ribozymes.

INTRODUCTION

Hammerhead ribozymes[1] are among the smallest catalytic RNAs with sequence-specific endoribonuclease activity. Their highly specific cleavage activity suggests their use as therapeutic agents for the inhibition of gene expression. For ribozymes to function as therapeutic agents, they must be either introduced exogenously or produced endogenously in afflicted cells. A major advantage of chemically synthesized ribozymes is that modifications may be introduced specifically at any desired position. This approach provides flexibility in designing more active and stable ribozymes. A variety of selective and uniform structural modifications have been applied to oligonucleotides to enhance nuclease resistance.[2-4] Improvements in the chemical synthesis of RNA[5,6] have led to the ability to similarly modify ribozymes containing the hammerhead ribozyme core motif.[7,8] In our

laboratory, a systematic study of the catalytic activity and nuclease stability of selectively modified 36-mer hammerhead ribozymes resulted in the identification of a generic motif containing 5 ribose residues, 30-32 2'-modified nucleotides and a 3'-3'-linked nucleotide "cap".[9] Several of these ribozymes had almost wild-type catalytic activity and demonstrated an overall increase in stability/activity of 53,000-80,000 fold compared to the all-RNA parent ribozyme.

In the course of the aforementioned studies we found that simultaneous "capping" of the ribozyme 5'-end with an inverted nucleotide, thereby providing a 5'-5'-linkage at the ribozyme terminus, or a simple hydroxy-alkyl chain in some cases resulted in a substantial decrease in catalytic activity (unpublished results). To increase 5'-end nuclease resistance, while preserving catalytic activity, we decided to investigate additional modifications; phosphodiester backbone modifications appeared to be the most attractive ones.

The changes in the backbone of DNA fragments often lead to an increased stability towards enzymatic hydrolysis by nucleases. The most prominent representatives of this class of modified oligonucleotides are non-bridging phosphorothioates and phosphoramidates, methylphosphonates or phosphotriesters.[2] One of the problems with using oligonucleotides bearing modifications at phosphorus is the generation of chirality at the phosphate group which leads to a complex mixture of diastereoisomers during the synthesis of such modified oligomers.

To overcome this problem we have chosen non-chiral phosphoramidate and phosphorothioate linkages (FIG. 1) for incorporation at the 5'-end of a hammerhead ribozyme. These linkages are electronically and sterically similar to their natural congener and we assumed that introduction of a single 3'-O-P(O)(O⁻)-NH-5' or 3'-O-P(O)(O⁻)-S-5' link at the 5'-end of the ribozyme would have little effect on its hybridization to a substrate and/or ribozyme cleavage activity. Letsinger and Mungall[10] reported the synthesis of a thymidine dimer and trimer possessing internucleotide phosphoramidate

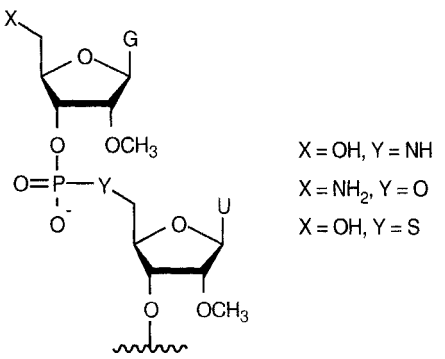

FIG. 1. 5'-end ribozyme modifications

bonds 3′-O-P(O)(O^-)-NH-5′ which were stable in neutral and alkaline conditions and showed increased stability against exonucleases. It is also reported[11] that introduction of a phosphoramidate 3′-NH-P(O)(O^-)-O-5′ leads to enhancement in thermal stability of the heteroduplex. While studies of 3'-*S*-modified oligodeoxynucleotides demonstrated complete resistance to cleavage by *Eco*-RV,[12] there are no related studies on 5'-*S*-modified oligonucleotides. Although there is great interest in the synthesis, chemical and biological properties of oligonucleotides with bridging 5'-*N*[10,13-16] and 5'-*S*[17-20] substitutions as well as 3'-*N*[11,13,21,22] and 3'-*S*[23-26] modified oligonucleotides, there are few reports[27-31] of the step-by-step elongation on solid support using 5'- or 3'-*N*(*S*)-modified nucleotide monomers.

Furthermore, during the course of our investigations we chose to explore the effect of placing a 5′-amine at the 5′-end of ribozyme. A terminal 5'-amino group of a thymidine dimer has been shown to efficiently inhibit the action of spleen phosphodiesterase.[10] If the stability observed in the dimer example could be effectively transferred to a ribozyme background, this moiety could be a good alternative to phosphorothioate linkages.

Because of the different chemical nature of N-R and S-R bonds compared to O-R bonds there is a requirement for the introduction of special protecting groups for the amino and thiol functions. In addition, special conditions are required for cleavage of these groups that, while considerably different from those routinely used in solid-phase DNA/RNA synthesis, still must be compatible with solid-phase phosphoramidite chemistry. Optimization of the synthetic cycle for the introduction of the modified monomers is usually necessary as well. All of the above makes the synthesis of these modified oligonucleotides a challenging goal.

Based on previous investigations in the 2′-deoxy series[28,29] we have chosen the 4-methoxytrityl (MMTr) group for the protection of the 5'-amine while the trityl (Tr) group was used for the protection of the 5'-mercapto functionality in modified monomers.

Here we report the synthesis of 5'-amino-5'-deoxy-2'-*O*-methyl uridine, guanosine and adenosine 3'-phosphoramidites **5**, **11** and **20** (FIG. 2), as well as 5'-mercapto-5'-deoxy-2'-*O*-methyl uridine 3'-phosphoramidite **23** (FIG. 3) and their incorporation into ribozymes. We previously demonstrated that extensive modification of hammerhead ribozyme with 2′-*O*-methyl nucleosides resulted in a catalytic motif with almost wild-type cleavage activity and considerably improved nuclease stability.[9] Another reason for

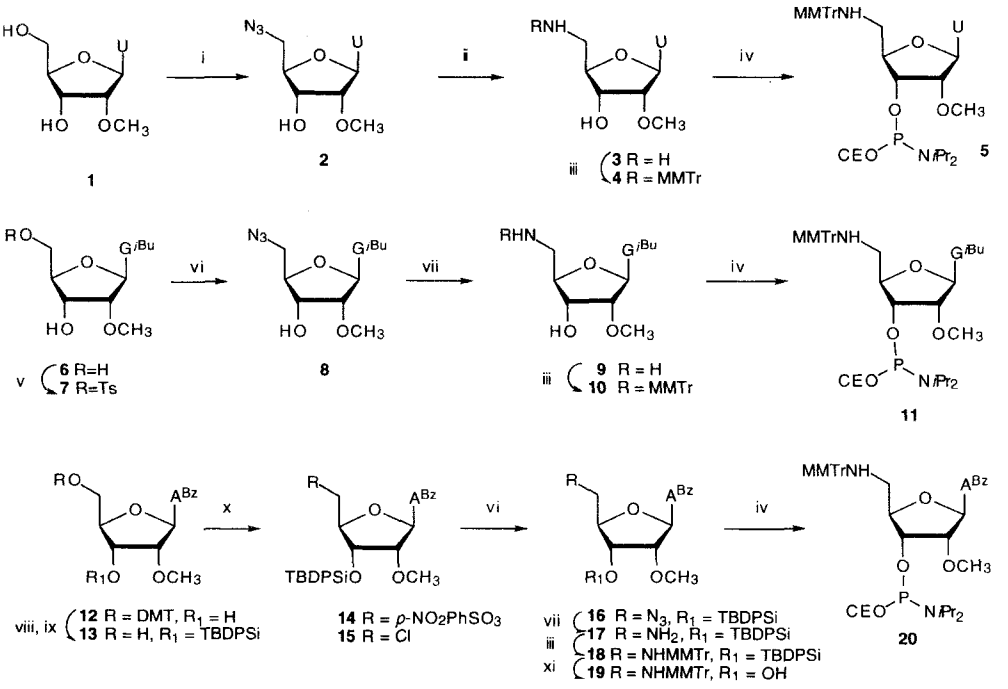

FIG. 2. Scheme for the preparation of 5′-amino-5′-deoxy building blocks **5**, **11** and **20**. Reagents: i, $Ph_3P/CBr_4/LiN_3/DMF$; ii, $Ph_3P/Pyr/NH_4OH$; iii, MMTrCl/DMAP/Et_3N/Pyr; iv, P(OCE)(N-*i*Pr_2)Cl/DIPEA/CH_2Cl_2; v, TsCl/Pyr; vi, LiN_3/DMF/55 °C; vii, H_2/10%Pd-C/EtOH; viii, TBDPSiCl/Im/DMF; ix, TFA/CH_2Cl_2; x, *p*-Nitro- benzenesulfonyl chloride/Pyr; xi, TBAF/THF.

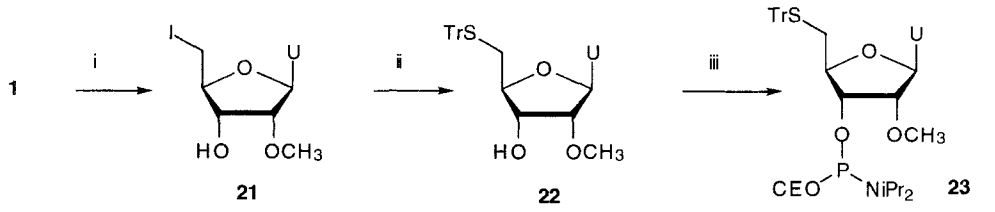

FIG. 3. Scheme for the preparation of 5′-mercapto-5′-deoxy building block **23**. Reagents: i, $CH_3P(OC_6H_5)_3I$/DMF; ii, TrSH/NaH/DMF; iii, P(OCE)(N-*i*Pr_2)Cl/DIPEA/CH_2Cl_2.

using 2'-*O*-methyl modified nucleotides is to prevent degradation of oligonucleotides by attack of the neighboring free 2'-hydroxyl on the phosphorus during deprotection, a well documented event in the case of 5'-*S*-modified ribonucleoside dimers.[17,20] We expected similar lability of the phosphoramidate internucleotide bond in the presence of a neighboring 2'-hydroxyl group.[32] Recently there have been two reports of the solid phase synthesis of oligonucleotides that contain a single bridging 5'-phosphorothioate at an RNA linkage.[33,34] Judicous choice of 2'-*O*-protecting group and deprotection reagents enabled the isolation of these oligonucleotides.

RESULTS AND DISCUSSION

Synthesis of monomer building blocks

The key intermediates for the synthesis of ribozymes containing bridging 5'-phosphoramidate and 5'-phosphorothioate linkages were 3'-*O*-phosphoramidites **5, 11, 20** and **23** synthesized according to FIG. 2 and 3.

5'-N-(4-Methoxytrityl)amino-5'-deoxy-2'-O-methyl uridine (5)

Uridine derivative **5** was synthesized in a manner similar to that reported by Mag and Engels[28] for the synthesis of a thymidine analog. 5'-Azido derivative **2** was synthesized in one step from 2'-*O*-methyl uridine (**1**) using the procedure of Yamamoto *et al.*[35] Ammonium hydroxide was used instead of water for the hydrolysis of intermediate 5'-phosphinimide during the conversion of **2** to **3**. It is well documented[36] that nucleoside phosphinimines are relatively stable in water compared to simple alkyl azides. Protection of the 5'-NH_2 group of **3** with a 4-methoxytrityl group, followed by standard phosphitylation[37] afforded 3'-*O*-phosphoramidite **5** in good yield.

5'-N-(4-Methoxytrityl)amino-5'-deoxy-N²-isobutyryl-2'-O-methyl guanosine (11)

Because the one-step procedure for the preparation of the 5'-azide described above does not work well for purine 2'-deoxynucleosides[28] we used a two-step procedure for the introduction of the azido group into the 5'-position of N^2-isobutyryl-2'-*O*-methyl guanosine (**6**).[38] Selective 5'-*O*-*p*-toluenesulfonation of **6** at 0 °C afforded the desired mono-substituted derivative **7** in 47% yield and the 3',5'-bis-substituted derivative in 15% yield. Attempts to improve the yield and selectivity of this reaction by the portionwise addition of *p*-toluenesulfonyl chloride had no effect.

Displacement of the OTs group of **7** with an N_3 group using LiN_3 in DMSO proceeded smoothly to yield **8** in 78% yield. As in the case of uridine derivative **2** attempts to use triphenylphosphine in water/pyridine[28] for reduction of **8** to **9** and thus avoid the simultaneous cleavage of the base labile N^2-isobutyryl group failed. Thus, catalytic hydrogenation of **8** using 10% Pd-C was utilized for the successful preparation of 5'-amino-5'-deoxy-2'-*O*-methyl derivative **9** (80% yield). It is worth noting that **9** underwent a gradual loss of the N^2-isobutyryl group when left in unbuffered aqueous solution for 16 h or longer. We attributed this unexpected deacylation to intramolecular base catalysis by the 5'-amino group of **9**. Protection of the free amino group of **9** with a 4-methoxytrityl group, followed by phosphitylation afforded 3'-*O*-phosphoramidite **11** in good yield.

5'-N-(4-Methoxytrityl)amino-5'-deoxy-N^6-benzoyl-2'-O-methyl adenosine (20)

The low selectivity in the tosylation of guanosine derivative **6** prompted us to use 3'-hydroxyl protection in the preparation of the adenosine analog. Thus, 5'-*O*-DMT derivative **12** was converted to the 3'-*O*-*t*-butyldiphenylsilyl (TBDPSi) derivative which was 5'-deprotected with TFA in CH_2Cl_2 to yield **13**. The reaction of **13** with a more reactive sulfonylating agent, *p*-nitrobenzenesulfonyl chloride, yielded unexpectedly a 2:1 mixture of 5'-*O*-*p*-nitrobenzenesulfonyl and 5'-chloro-5'-deoxy substituted derivatives **14** and **15**. It appears that **15** arose from the partial displacement of the sulfonyl group of **14** by chloride, a similar displacement has been described in the literature.[39] No attempt was made to separate these compounds and the mixture was treated with LiN_3 at 80 °C overnight to afford 5'-azido-5'-deoxy derivative **16** in 63% yield from **13**. Catalytic hydrogenation of **16** proceeded smoothly to afford 5'-amino derivative **17** which was, without purification, converted to 5'-*N*-MMTr protected derivative **18**. Cleavage of the 3'-*O*-TBDPSi group was achieved using tetrabutylammonium fluoride and the resulting **19** was phosphitylated under standard conditions to give the 3'-*O*-phosphoramidite **20** in 74% yield.

5'-Deoxy-5'-mercapto-2'-O-methyl uridine (23)

Synthesis of the 5'-deoxy-5'-mercapto-2'-*O*-methyl uridine monomer **23** started with selective iodination of 2'-*O*-methyl uridine **(1)** using methyltriphenoxyphosphonium iodide as described previously.[40] The iodo compound **21** was converted in 68% yield into the 5'-(*S*-triphenylmethyl)-mercapto compound **22** using the sodium salt of triphenylmethyl mercaptan in DMF as described by Sproat *et al.*[41] Introduction of an aqueous $Na_2S_2O_3$ wash into the work up step was beneficial in reducing the cleavage of the *S*Tr

group and formation of intermolecular disulfide bonds by any iodine present in the reaction mixture.[42] Phosphitylation of **22** under standard conditions[37] yielded 3'-*O*-phosphoramidite **23**.

Oligonucleotide synthesis

Bridging 5'-phosphoramidate

There are a number of issues that must be addressed when synthesizing oligomers containing bridging 5'-phosphoramidate linkages:

Coupling of the 5'-amine containing phosphoramidite to the growing chain.

Coupling of the following amidite to the 5'-amine.

The effect of ancillary reagents on the stability of the phosphoramidate linkage.

The effect of deprotection conditions on the stability of the phosphoramidate linkage.

Removal of the MMTr protecting group from the 5'-amine.

The optimal coupling conditions were ascertained in an extensive study on incorporation of 5'-amino modified monomers into Ribozyme **1**, directed at a site in the c-*myb* mRNA[43] (see TABLE 1). This ribozyme contains the sequence; 5'-$g_N$uu uuc ccU Gau Gag gcc gaa agg ccG aaA uuc ucc iT, wherein lower case letters represent 2'-*O*Me nucleotides, uppercase letters represent ribonucleotides, underlined letter is 2'-C-allyl substituted nucleotide and iT represents an inverted thymidine residue that is attached to the following nucleotide through a 3'-3'-phosphodiester linkage. The subscript, N, represents the amidate linkage in question, all other internucleotidic linkages are phosphodiester.

We found that a coupling time of 300 s for **5** and 300 s for the following 2'-*O*-Me nucleotide provided the best results. Furthermore, during this investigation we determined that exposure of the modified oligonucleotide to the HF/TEA desilylation solution caused degradation of the phosphoramidate linkage. As a result, the ribozyme was desilylated with TBAF.

We suspected that the lability of the phosphoramidate linkage was due to the mildly acidic nature of the HF/TEA solution. This led us to speculate that our syntheses could be similarly affected by the acidic nature of various ancillary reagents used during solid-phase oligonucleotide synthesis. We devised an experiment to study the influence of extended exposure of the modified oligonucleotides to the detritylation solution (TCA/CH_2Cl_2) and

TABLE 1: 5'-Amino-5'-deoxynucleotide incorporation

Experiment	Coupling of 5 (s)	Coupling of 2'-OMe-G (s)	Desilylating Reagent	Crude A_{260} units	% FLP
Rz1.A	600	600	HF/TEA	355	14.8
Rz1.B	600	600	TBAF	387	22.2
Rz1.C	600	900	TBAF	401	21.4
Rz1.D	600	450	TBAF	447	23.8
Rz1.E	**300**	**300**	**TBAF**	**455**	**27.3**

activator (*S*-ethyl tetrazole). Following completion of the synthesis, we exposed one oligomer to four "dummy cycles" of detritylation solution and another to four "dummy cycles" of activator. Although no impact upon full length product was observed with the extended detritylation exposure, there did appear to be a detrimental effect to protracted exposure to activator. This result correlates with those obtained from the coupling experiment (TABLE 1) wherein the greatest amount of full-length product was acheived when the 5'-amino amidite and the following monomer were coupled for the shortest amount of time.

Finally we investigated the removal of the MMTr protecting group. We had discovered, from an unrelated experiment (DiRenzo, A.B., unpublished results), that unless the detritylation reagent was delivered in a "flow through" process, wherein the acid is delivered to the column in pulses separated by wait steps, complete removal of MMTr group could not be effected. Therefore, detritylation was effected using four 10 s pulses of TCA with 7 s wait steps between each pulse. This was followed by 30 s of acetonitrile and then the four 10 s pulses of TCA were repeated. The incoming amidite was then coupled for 300 s to complete the synthesis.

5'-Amino group at the 5'-end

In the process of synthesizing ribozymes containing phosphoramidate linkages at the 5'-end, we also synthesized Ribozyme **2**, which contains a 5'-amine at the 5'-terminus of the ribozyme. This ribozyme, directed against a site in the VEGF receptor mRNA, contains the following sequence; **u**ua uaa ac**U** GA**U** Gag gcc gaa agg ccG aaA ucc cgg iB, upper and lower case letters represent ribo- and 2'-OMe nucleotides, respectively, while bold lower case and bold upper case represent 5'-amino and 2'-amino nucleotides,

respectively. Finally, iB stands for an inverted abasic residue that is attached to the following nucleotide through a 3'-3'-phosphodiester linkage.

The standard synthetic protocols were modified slightly to optimize synthesis. To ensure complete removal of the MMTr protecting group on the 5'-amine, the final detritylation step was adjusted as in the previous example. The final modification to the synthesis protocol was made in the choice of capping reagent. We had observed the formation of a side product, identified by MALDI-TOF MS as the 5'-*N*-acetylated ribozyme, when acetic anhydride was the capping agent. We postulated that this product arose from a transacetylation reaction that occurred during base deprotection. As a result, *t*-butylphenoxyacetic anhydride was used in place of acetic anhydride as a capping reagent and no side product resulted.

Bridging 5'-phosphorothioates

As mentioned previously, bridging 5'-phosphorothioates have been synthesized by Kuimelis *et al.*[33] and our own laboratory.[34] In the course of our work, which described the synthesis of an RNA substrate containing a single bridging 5'-phosphorothioate, we encountered difficulties when coupling the incoming amidite to the 5'-thiol. Synthesis was further complicated by the formation of disulfide dimers. Following a careful study of various coupling conditions, we found that reducing the coupling times from the previous studies[34] for both the 5'-thiol containing amidite and the incoming monomer, provided improved results. Furthermore, incorporation of an acetonitrile (containing a few drops of acetic acid) wash prior to coupling of the incoming amidite (Burgin, A.B., unpublished results) eliminated coupling difficulties and reduced dimer formation.

A single bridging 5'-phosphorothioate linkage was incorporated into the 5'-end of Ribozyme **3**. This ribozyme, with the sequence 5'-g_S**u**u uuc ccU Gau Gag gcc gaa agg ccG aaA uuc ucc iT-3', is a variation of Ribozyme **1**, wherein the four phosphorothioates at the 5'-end have been replaced with a single bridging 5'-phosphorothioate at the terminal internucleotidic linkage. The 5'-thiol phosphoramidite **23** was coupled for 300 s and the following phosphoramidite coupled for 400 s. The ribozyme was base deprotected as usual[6] and then treated with TEA/HF at 65 °C for 0.5 h rather than 1.5 h. Using the latter reagent we have not observed substantial cleavage of the P-S bond as observed when TBAF was used.[17]

EXPERIMENTAL

Materials and methods

2'-*O*-Methyl uridine, N^2-isobutyryl-2'-*O*-methyl guanosine and 5'-*O*-(4,4'-dimethoxytrityl)-N^6-benzoyl-2'-*O*-methyl adenosine were obtained from

ChemGenes Corporation (Waltham, MA). All NMR spectra were recorded on a Varian Gemini 400 spectrometer operating at 400.075 MHz for proton and 161.947 MHz for phosphorus. Chemical shifts in ppm refer to TMS and H_3PO_4, respectively. Coupling constants are in Hz. The solvent was $CDCl_3$ if not stated otherwise. The standard workup consisted of partitioning of the residue after removal of solvents between 5% aqueous $NaHCO_3$ and CH_2Cl_2 followed by washing of the organic layer with brine, drying over Na_2SO_4 and removal of solvents *in vacuo*. Analytical thin-layer chromatography (TLC) was performed with Merck Art. 5554 Kieselgel 60 F_{254} plates and column chromatography using Merck 0.040-0.063 mm Silica gel 60. Melting temperatures were determined on the Electrothermal Model IA 9200 apparatus and are uncorrected.

The general procedures for RNA synthesis and deprotection have been described previously.[6] Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μmol scale protocol with a 5 min coupling step for 2'-*O*-TBDMS protected nucleotides and 2.5 min coupling step for 2'-*O*-methyl nucleotides. A 6.5-fold excess of a 0.1 M solution phosphoramidite and a 24-fold excess of *S*-ethyl tetrazole relative to polymer-bound 5'-hydroxyl was used in each coupling cycle.

All analytical HPLC analyses were performed on a Hewlett Packard 1090 HPLC with a Dionex NucleoPac® PA-100 column, 4 x 250 mm, at 50 °C, as reported previously.[6]

CGE analyses were performed on a Hewlett Packard ^{3D}CE with a J & W μPAGE™-5 (5% T, 5% C) polyacrylamide gel-filled column, 75 μm I.D. x 75 cm, 50 cm effective length, 100 mM Tris-Borate, 7 M Urea, pH = 8.3, and J & W μPAGE™ Buffer (100 mM Tris-Borate, 7 M Urea, pH = 8.3). Samples were electrokinetically injected using -13 kV for 3-10 sec, run at -13 kV and detected at 260 nm.

MALDI-TOF mass spectra were determined on a PerSeptive Biosystems Voyager spectrometer. The instrument was run in linear mode with a matrix consisting of; 300 mM 3-hydroxy picolinic acid, 33.8 mM picolinic acid, 18.4 mM dibasic ammonium citrate in 1:1 CH_3CN/H_2O and 0.8% TFA. An internal standard was used to calibrate the instrument for each sample.

Electrospray mass spectrometry was run on a Fison Instruments VG Quattro-SG quadrupole mass spectrometer. Desalted and ammonium-exchanged samples were suspended in deionized water (1 nmol/μL). The ribozyme solution was added to an acetonitrile solution (17 μL, 80% ACN/2.5 mM CDTA/0.1% TEA, CDTA = 1,2 diaminocyclohexane-N,N,N',N'-tetraacetic acid). The ribozyme/acetonitrile solution was injected through a fused silica

loop (10 μL) at 4 μL/min. The instrument was run in ES(-) mode (cone voltage = 42 V) on a mass range of 400-1500.

Synthesis of monomer building blocks

5'-Azido-5'-deoxy-2'-O-methyl uridine (2) was synthesized from 2'-*O*-methyl uridine (**1**) in 79% yield (white foam) according to the procedure of Yamamoto *et al.*[35] for the preparation of 5'-azido-5'-deoxythymidine. ^{1}H NMR δ 9.16 (br s, 1H, NH), 7.70 (d, $J_{6,5}$=8.2, 1H, H6), 5.97 (d, $J_{1',2'}$=2.2, 1H, H1'), 5.87 (d, $J_{5,6}$=8.2, 1H, H5), 4.21 (m, 1H, H3'), 4.08 (m, 1H, H2'), 3.92 (dd, $J_{5',4'}$=2.2, $J_{5',5''}$=13.4, 1H, H5'), 3.87 (dd, $J_{4',5'}$=2.2, $J_{4',3'}$=5.6, 1H, H4'), 3.82 (dd, $J_{5'',4'}$=3.3, $J_{5'',5'}$=13.4, 1H, H5''), 3.67 (s, 3H, OMe).

5'-Amino-5'-deoxy-2'-O-methyl uridine (3) was synthesized from **2** according to a modification of the procedure of Mag and Engels[28] for the preparation of 5'-amino-5'-deoxythymidine: **2** (680 mg, 2.27 mmol) was dissolved in dry pyridine (5 mL) and triphenylphosphine (Ph_3P) (890 mg, 3.39 mmol) was added. The mixture was stirred for 2 h at rt at which time all the starting material had reacted. Concentrated NH_4OH (2 mL) was then added and the mixture stirred at rt for 2 h. Solvents were removed at reduced pressure, water was added (20 mL) and precipitate removed by filtration. The filtrate was extracted with benzene and ether and then evaporated to dryness. The residue was dissolved in isopropanol from which the amorphous solid precipitated on cooling (480 mg, 82%). ^{1}H NMR (DMSO-d_6) δ 8.01 (d, $J_{6,5}$=8.1, 1H, H6), 5.90 (d, $J_{1',2'}$=5.2, 1H, H1'), 5.71 (d, $J_{5,6}$=8.1, 1H, H5), 4.16 (app t, $J_{3',4'}$=5.0, 1H, H3'), 3.91 (app t, $J_{2',1'}$=5.2, 1H, H2'), 3.84 (q, $J_{4',3'}$=5.0, 1H, H4'), 3.43 (s, 3H, OMe), 2.88 (dd, $J_{5',4'}$=4.5, $J_{5',5''}$=13.7, 1H, H5'), 2.83 (dd, $J_{5'',4'}$=5.0, $J_{5'',5'}$=13.7, 1H, H5''). FAB-MS: 258 $[M+H]^+$.

5'-N-(4-Methoxytrityl)amino-5'-deoxy-2'-O-methyl uridine (4) was synthesized from **3** using 4-methoxytrityl chloride/DMAP/Et_3N/Pyr according to the procedure of Mag and Engels.[28] The product was obtained in 63% yield. ^{1}H NMR δ 8.25 (br s, 1H, NH), 7.54-6.88 (m, 15H, aromatic, H6), 5.96 (s, 1H, H1'), 5.70 (d, $J_{5,6}$=7.9, 1H, H5), 4.13 (m, 1H, H3'), 4.01 (m, 1H, H2'), 3.86 (s, 3H, TrOMe), 3.77 (m, 1H, H4'), 3.69 (s, 3H, OMe), 2.82 (dd, $J_{5',4'}$=2.9, $J_{5',5''}$=12.9, 1H, H5'), 2.66 (d, $J_{NH,5'}$=8.8, 1H, 5'NH), 2.42 (dd, $J_{5'',4'}$=6.8, $J_{5'',5'}$=12.9, 1H, H5'').

5'-N-(4-Methoxytrityl)amino-5'-deoxy-2'-O-methyl uridine-3'-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (5). To the solution of **4** (520 mg, 0.98 mmol) and *N,N*-diisopropylethylamine (DIPEA) (0.34 mL, 1.95 mmol) in CH_2Cl_2 (10 mL), under argon was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.30 mL, 1.34 mmol) dropwise, stirring was continued for 3 h at rt. The reaction mixture was then cooled to 0 °C, dry

MeOH (3 mL) was added and stirring continued for 5 min. The mixture was evaporated to dryness *in vacuo* (40 °C bath temp) and the residue chromatographed on a silica gel column using 20-70% gradient EtOAc in hexane (1% Et_3N) to afford **5** as a colorless foam (0.60 g, 83%). ^{31}P NMR δ 148.97 (s) and 148.67 (s).

5'-O-p-Toluenesulfonyl-N^2-isobutyryl-2'-O-methyl guanosine (**7**). N^2-Isobutyryl-2'-*O*-methyl guanosine (**6**)[38] (1.6 g, 4.36 mmol) was dissolved in dry pyridine (25 mL) and the solution was cooled to 0 °C while protected from moisture. *p*-Toluenesulfonyl chloride (1.0 g, 5.23 mmol) was added and the reaction mixture was left at 0-3 °C for 48 h. MeOH (10 mL) was added and the mixture evaporated to a syrup. After standard workup and column chromatography using 1-2% MeOH in CH_2Cl_2, **7** was obtained as a colorless foam, 1.06 g (47%). ^1H NMR δ 12.25 (br s, 1H, NH), 9.55 (br s, 1H, NH), 7.83 (d, $J_{H,H}$=8.3, 2H, Ts), 7.78 (s, 1H, H8), 7.42 (d, $J_{H,H}$=8.3, 2H, Ts), 5.83 (d, $J_{1',2'}$=6.2, 1H, H1'), 4.82 (app t, $J_{2',3'}$=5.7, 1H, H2'), 4.64 (m, 1H, H3'), 4.37 (dd, $J_{5',4'}$=2.2, $J_{5',5''}$=10.3, 1H, H5'), 5.23 (dd, $J_{4',5''}$=2.9, $J_{4',3'}$=5.2, 1H, H4'), 4.29 (dd, $J_{5'',4'}$=2.9, $J_{5'',5'}$=10.3, 1H, H5''), 3.47 (s, 3H, OMe), 2.76 (m, 1H, *CH*$(CH_3)_2$), 2.51 (s, 3H, Ts-Me), 1.29 (m, 6H, 2 x Me).

The 3',5'-di-*O*-*p*-toluenesulfonyl derivative was also isolated (0.45 g, 15%) from the reaction mixture along with 20% of the unreacted starting material.

5'-Azido-5'-deoxy-N^2-isobutyryl-2'-O-methyl guanosine (**8**). The 5'-*O*-Ts derivative **7** (780 mg, 1.5 mmol) was dissolved in dry DMSO (7 mL) and LiN_3 (370 mg, 7.56 mmol) was added under argon. The mixture was heated at 50 °C for 16 h and then evaporated to a syrup (oil pump, 50 °C). The residue was partitioned between water (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (4 x 20 mL), organic layers combined, dried (Na_2SO_4) and evaporated to dryness. Flash column silica gel chromatography using 2-25% MeOH in CH_2Cl_2 afforded **8**, 430 mg (78%), mp 107-109 °C (H_2O). ^1H NMR (DMSO-d_6) δ 12.17 (br s, 1H, NH), 11.68 (br s, 1H, NH), 8.36 (s, 1H, H8), 6.01 (d, $J_{1',2'}$=6.1, 1H, H1'), 5.52 (d, $J_{OH,3'}$=5.1, 1H, 3'OH), 4.47 (app t, $J_{2',3'}$=5.5, 1H, H2'), 4.37 (m, 1H, H3'), 4.12 (m, 1H, H4'), 3.75 (dd, $J_{5',4'}$=6.8, $J_{5',5''}$=13.2, 1H, H5'), 3.65 (dd, $J_{5'',4'}$=4.2, $J_{5'',5'}$=13.2, 1H, H5''), 3.43 (s, 3H, OMe), 2.86 (m, 1H, *CH*$(CH_3)_2$), 1.22 (s, 3H, Me), 1.20 (s, 3H, Me).

5'-Amino-5'-deoxy-N^2-isobutyryl-2'-O-methyl guanosine (**9**) To the solution of **8** (350 mg, 0.95 mmol) in 96% EtOH (30 mL) was added 10% Pd/C catalyst (60 mg). The mixture was hydrogenated under 35 psi of H_2 for 24 h. More EtOH was added and heated to get the partially crystallized product completely into solution. Then the catalyst was filtered off. On cooling, crystals formed which were filtered off and dried to give 260 mg in two crops

(80%), mp 197-199 °C. ^{1}H NMR (D_2O) δ 8.16 (s, 1H, H8), 6.15 (d, $J_{1',2'}$=4.6, 1H, H1'), 4.66 (app t, $J_{3',2'}$=5.4, $J_{3',4'}$=5.4, 1H, H3'), 4.57 (app t, $J_{2',1'}$=4.6, $J_{2',3'}$=5.4, 1H, H2'), 4.34 (m, 1H, H4'), 3.50 (s, 3H, OMe), 3.49 (m, 2H, H5', H5''), 2.82 (m, 1H, $CH(CH_3)_2$), 1.26 (s, 3H, Me), 1.24 (s, 3H, Me). FAB-MS: 367 $[M+H]^+$.

*5'-N-(4-Methoxytrityl)amino-5'-deoxy-N^2-isobutyryl-2'-O-methyl guanosine (**10**)* was synthesized from **9** using 4-methoxytrityl chloride/DMAP/Et_3N/Pyr according to the procedure of Mag and Engels,[28] in 80% yield. ^{1}H NMR δ 12.11 (br s, 1H, NH), 7.95 (br s, 1H, NH), 7.70 (s, 1H, H8), 7.53-6.86 (m, 14 H, aromatic), 5.92 (d, $J_{1',2'}$=4.9, 1H, H1'), 4.55 (app t, $J_{3',4'}$=5.0, 1H, H3'), 4.35 (app t, $J_{2',1'}$=4.9, 1H, H2'), 3.84 (s, 3H, Tr-OMe), 3.55 (s, 3H, OMe), 2.82 (br s, 1H, 3'OH), 2.78 (dd, $J_{5',4'}$=3.0, $J_{5',5''}$=12.4, 1H, H5'), 2.65 (br s, 1H, NH), 2.43 (dd, $J_{5'',4'}$=5.4, $J_{5'',5'}$=12.4, 1H, H5''), 1.09 (m, 6H, 2 x Me).

*5'-N-(4-Methoxytrityl)amino-5'-deoxy-N^2-isobutyryl-2'-O-methyl guanosine-3'-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**11**).* Using the same procedure as for the preparation of **5**, phosphoramidite **11** was obtained as a colorless foam in 80% yield after column chromatography using 1% EtOH in CH_2Cl_2 (1% Et_3N). ^{31}P NMR δ 148.74 (s) and 148.06 (s).

*3'-O-t-Butyldiphenylsilyl-N^6-benzoyl-2'-O-methyl adenosine (**13**).* 5'-O-(4,4'-Dimethoxytrityl)-N^6-benzoyl-2'-O-methyl adenosine **12** (5g, 7.3 mmol) was dissolved in DMF (20 mL), imidazole (1.5 g, 22 mmol) and *t*-butyldiphenylsilyl chloride (2.8 mL, 10.8 mmol) were added. The mixture was stirred at rt overnight. Methanol (10 mL) was added and the solution evaporated to a syrup. After standard work up the resulting syrup was dissolved in CH_2Cl_2 (100 mL) and cooled in an ice-bath. 3% TFA in CH_2Cl_2 (v/v, 100 mL) was added and the mixture was stirred at 0 °C for 10 min. Methanol (20 mL) and toluene (50 mL) were added and the solution concentrated to a syrup *in vacuo* (40 °C). The residue was coevaporated twice with toluene and then purified by column chromatography using 1-5% MeOH in CH_2Cl_2 for elution to yield **13** as a white foam (4.3 g, 95% yield). ^{1}H NMR δ 8.98 (br s, 1H, NH), 8.73 (s, 1H, H2), 8.13 (s, 1H, H8), 8.02-7.39 (m, 15H, 3 x Ph), 6.06 (d, $J_{1',2'}$=7.4, 1H, H1'), 5.86 (d, $J_{OH,5'}$=10.2, 1H, 5'OH), 4.55 (m, 2H, H2', H3'), 4.20 (br s, 1H, H4'), 3.70 (d, $J_{5',5''}$=12.9, 1H, H5'), 3.14 (d, $J_{5'',5'}$=12.9, 1H, H5''), 3.10 (s, 3H, OMe), 1.15 (s, 9H, *t*-Bu).

*5'-O-(4-Nitrobenzenesulfonyl)-3'-O-t-butyldiphenylsilyl-N^6-benzoyl-2'-O-methyl adenosine (**14**) and 5'-chloro-5'deoxy-3'-O-t-butyldiphenylsilyl-N^6-benzoyl-2'-O-methyl adenosine (**15**).* To a solution of **13** (4.3 g, 6.9 mmol) in dry pyridine (70 mL) was added 4-nitrobenzenesulfonyl chloride (2.47 g, 11 mmol) and the solution was left at rt overnight. Water (2 mL) was added and the solution concentrated to a syrup *in vacuo*. After standard workup the reaction mixture was purified by column chromatography using 1-5%

gradient MeOH in CH_2Cl_2 to yield 4.7 g of the inseparable mixture of **14** and **15** in 2:1 ratio. ^{1}H NMR for **14** δ 8.89 (br s, 1H, NH), 8.58 (s, 1H, H2), 8.16-7.36 (m, 20H, H8, aromatic), 6.00 (d, $J_{1',2'}$=3.8, 1H, H1'), 4.56 (app t, $J_{3',4'}$=5.1, 1H, H3'), 4.33 (m, 1H, H4'), 4.27 (dd, $J_{5',4'}$=2.8, $J_{5',5''}$=11.2, 1H, H5'), 4.14 (dd, $J_{5'',4'}$=5.3, $J_{5'',5'}$=11.2, 1H, H5''), 4.09 (app t, $J_{2',1'}$=3.8, 1H, H2'), 3.20 (s, 3H, OMe), 1.11 (s, 9H, *t*-Bu). ^{1}H NMR for **15** δ 8.92 (br s, 1H, NH), 8.71 (s, 1H, H2), 8.16-7.36 (m, 20H, H8, aromatic), 6.15 (d, $J_{1',2'}$=3.9, 1H, H1'), 4.51 (app t, $J_{3',4'}$=5.1, 1H, H3'), 4.42 (m, 1H, H4'), 4.06 (app t, $J_{2',1'}$=3.9, 1H, H2'), 3.82 (dd, $J_{5',4'}$=4.3, $J_{5',5''}$=12.1, 1H, H5'), 3.54 (dd, $J_{5'',4'}$=3.9, $J_{5'',5'}$=12.1, 1H, H5''), 3.25 (s, 3H, OMe), 1.13 (s, 9H, *t*-Bu).

*5'-Azido-5'-deoxy-3'-O-t-butyldiphenylsilyl-N^6-benzoyl-2'-O-methyl adenosine (**16**).* The above mixture of **14** and **15** (3.9 g) was dissolved in dry DMSO (30 mL) and LiN_3 (1.18 g, 24 mmol) was added. The reaction mixture was stirred at 80 °C overnight, then concentrated *in vacuo* (oil pump). After standard work up and column chromatography using 1-2% gradient MeOH in CH_2Cl_2, **16** was obtained as a colorless foam (2.55 g, 63% yield from **13**). ^{1}H NMR δ 8.92 (br s, 1H, NH), 8.72 (s, 1H, H2), 8.15 (s, 1H, H8), 8.02-7.36 (m, 15H, 3 x Ph), 6.14 (d, $J_{1',2'}$=3.4, 1H, H1'), 4.44 (app t, $J_{3',4'}$=5.1, 1H, H3'), 4.27 (m, 1H, H4'), 4.01 (app t, $J_{2',1'}$=3.4, $J_{2',3'}$=4.9, 1H, H2'), 3.53 (dd, $J_{5',4'}$=3.2, $J_{5',5''}$=13.3, 1H, H5'), 3.37 (dd, $J_{5'',4'}$=4.5, $J_{5'',5'}$=13.3, 1H, H5''), 3.29 (s, 3H, OMe), 1.13 (s, 9H, *t*-Bu).

*5'-Amino-5'-deoxy-3'-O-t-butyldiphenylsilyl-N^6-benzoyl-2'-O-methyl adenosine (**17**).* Using the same procedure as for the preparation of the guanosine analog **9**, **16** (2.5 g, 3.9 mmol) was converted into **17** (2.25 g, 94%) which resisted crystallization and was used crude in the next step. ^{1}H NMR δ 8.90 (br s, 1H, NH), 8.72 (s, 1H, H2), 8.23 (s, 1H, H8), 8.02-7.36 (m, 15H, aromatic), 6.13 (d, $J_{1',2'}$=4.4, 1H, H1'), 4.72 (app t, $J_{2',1'}$=4.4, $J_{2',3'}$=5.0, 1H, H2'), 4.17 (m, 2H, H3', H4'), 3.27 (s, 3H, OMe), 2.88 (dd, $J_{5',4'}$=3.2, $J_{5',5''}$ =13.8, 1H, H5'), 2.65 (dd, $J_{5'',4'}$=5.0, $J_{5'',5'}$=13.8, 1H, H5''), 1.12 (s, 9H, *t*-Bu).

*5'-N-(4-Methoxytrityl)amino-5'-deoxy-3'-O-t-butyldiphenylsilyl-N^6-benzoyl-2'-O-methyl adenosine (**18**).* Using the same procedure as for the preparation of **10**, **17** was converted into **18**. The product was then purified by column chromatography using 1-2% MeOH gradient in CH_2Cl_2 to provide a colorless foam (2.37 g, 76%). ^{1}H NMR δ 8.90 (br s, 1H, NH), 8.02 (s, 1H, H2), 7.95 (s, 1H, H8), 8.00-6.71 (m, 29H, aromatic), 6.04 (d, $J_{1',2'}$=6.4, 1H, H1'), 4.72 (app t, $J_{2',1'}$=6.4, $J_{2',3'}$=4.4, 1H, H2'), 4.65 (m, 1H, H3'), 4.33 (m, 1H, H4'), 3.80 (s, 3H, Tr-OMe), 3.20 (s, 3H, OMe), 3.03 (br s, 1H, NH), 2.26 (d, $J_{5',5''}$ =11.7, 1H, H5'), 2.15 (dd, $J_{5'',4'}$=4.3, $J_{5'',5'}$=11.7, 1H, H5''), 1.12 (s, 9H, *t*-Bu).

*5'-N-(4-Methoxytrityl)amino-5'-deoxy-N^6-benzoyl-2'-O-methyl adenosine (**19**).* To the solution of **18** (2.7 g, 3 mmol) in THF (30 mL) 1 M tetrabutylammonium fluoride (TBAF) in THF (6 mL) was added and the

mixture was stirred at rt 2 h. It was then concentrated to a syrup *in vacuo*. After standard work up and column chromatography using 10-30% gradient THF in CH_2Cl_2, **19** was obtained (1.6 g, 81%) as a colorless foam. ^1H NMR δ 8.90 (br s, 1H, NH), 8.14 (s, 1H, H2), 7.98 (s, 1H, H8), 8.02-6.79 (m, 19H, aromatic), 5.95 (d, $J_{1',2'}$=5.5, 1H, H1'), 4.91 (app t, $J_{2',1'}$=5.5, 1H, $J_{2',3'}$=5.2, H2'), 4.72 (m, 1H, H3'), 4.29 (m, 1H, H4'), 3.77 (s, 3H, Tr-OMe), 3.52 (s, 3H, OMe), 3.09 (br s, 1H, NH), 2.67 (d, $J_{OH,3'}$=3.4, 1H, OH3'), 2.60 (dd, $J_{5',4'}$=4.0, $J_{5',5''}$ =12.5, 1H, H5'), 2.48 (dd, $J_{5'',4'}$=2.08, $J_{5'',5'}$=12.5, 1H, H5''). FAB-MS: 657 $[M+H]^+$.

*5'-N-(4-Methoxytrityl)amino-5'-deoxy-N^6-benzoyl-2'-O-methyl adenosine-3'-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**20**).* Using the same procedure as for the preparation of **5**, **19** (1 g, 1.5 mmol) was converted into **20.** After column chromatography, using CH_2Cl_2 containing 1% Et_3N (v/v), a colorless foam (0.55 g, 74%) was obtained, ^{31}P NMR δ 151.2 (s), 151.8 (s).

*5'-Deoxy-5'-iodo-2'-O-methyl uridine (**21**).* This compound was prepared from **1** using the procedure of Verheyden and Moffatt[40] for selective iodination of thymidine. Following column chromatography using 1-5% MeOH in CH_2Cl_2 for elution, **21** was isolated in 59% yield. ^1H NMR (DMSO-d_6) δ 7.76 (d, $J_{6,5}$=8.1, 1H, H6), 5.94 (d, $J_{1',2'}$=5.4, 1H, H1'), 5.77 (d, $J_{5,6}$=8.1, 1H, H5), 5.52 (d, $J_{OH.3'}$=6.0, 1H, 3'OH), 4.11 (dd, $J_{3',2'}$=5.36, $J_{3',4'}$=10.2, 1H, H3'), 4.06 (app t, $J_{2',1'}$=5.4, 1H, H2'), 3.93 (m,1H, H4'), 3.63 (dd, $J_{5',4'}$=5.4, $J_{5',5''}$=10.6, 1H, H5'), 3.49 (dd, $J_{5'',4'}$=6.9, $J_{5'',5'}$=10.6, 1H, H5''), 3.42 (s, 3H, OMe).

*5'-(S-Triphenylmethyl)mercapto-5'-deoxy-2'-O-methyl uridine (**22**).* Sodium hydride (52 mg, 2.18 mmol) was suspended in dry DMF (1 mL) under argon at 0 °C. A solution of triphenylmethyl mercaptan (606 mg, 2.19 mmol) in dry DMF (7 mL) was added. The mixture was stirred for 10 min at rt, cooled in ice and a solution of **21** (690 mg, 1.80 mmol) in dry DMF (5 mL) was added. After 3 h at rt solvent was removed *in vacuo*, the residue dissolved in CH_2Cl_2 and washed with 5% aqueous $Na_2S_2O_3$ and water. The organic layer was dried (Na_2SO_4), evaporated to dryness and chromatographed using 1-2% MeOH in CH_2Cl_2 for elution to afford **22** (860 mg, 68%), mp 187-188 °C (EtOH-H_2O). ^1H NMR δ 8.43 (br s, 1H, NH), 7.51-7.29 (m, 16H, Tr, H6), 5.87 (d, $J_{1',2'}$=2.4, 1H, H1'), 5.78 (d, $J_{5,6}$=8.1, 1H, H5), 3.90 (m, 1H, H2'), 3.83 (m, 1H, H3'), 3.75 (dd, $J_{4',5'}$=2.4, $J_{4',3'}$=5.5, 1H, H4'), 2.81 (dd, $J_{5',4'}$=2.4, $J_{5',5''}$=13.0, 1H, H5'), 2.52 (dd, $J_{5'',4'}$=6.6, $J_{5'',5'}$=13.0, 1H, H5''). FAB-MS: 517 $[M+H]^+$.

*5'-(S-Triphenylmethyl)mercapto-5'-deoxy-2'-O-methyl uridine-3'-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (**23**)* Using the same procedure as for the preparation of **5**, 3'-phosphoramidite **23** was obtained as a white foam in 88% yield after flash chromatography purification using 50-75% gradient of EtOAc in hexane (1% Et_3N). ^{31}P NMR δ 149.1 (s) and 148.7 (s).

Ribozyme synthesis and purification

Ribozyme **1**, *Bridging 5'-phosphoramidate at the 5'-end.* Synthesis was performed as described[6] with a 300 s coupling time for the 5'-amino phosphoramidites **5, 11** and **20**. Detritylation was effected using a cycle that consisted of four 10 s pulses of TCA, each separated by 7 s wait steps, followed by 30 s of acetonitrile. This series was then repeated. Finally, the incoming phosphoramidite was coupled for 300 s to complete the synthesis. The ribozyme was base deprotected under standard conditions, however, desilylation was accomplished with TBAF in 24 h rather than HF/TEA solution.[6]

Ribozyme **2**, *5'-Amino group at the 5'-end.* The synthesis cycle was modified slightly from the usual protocol. The 5'-amino phosphoramidites **5, 11** and **20** were coupled for 300 s. The usual capping reagent, acetic anhydride, was replaced with *t*-butylphenoxyacetic anhydride. All ribozymes were synthesized trityl-on. The terminal MMTr group was then manually removed upon addition of four 10 s pulses of TCA, each separated by 7 s wait steps, followed by 30 s of acetonitrile. This series was repeated until no orange color was observed. The ribozyme was then deprotected under standard conditions. In the synthesis incorporating **5** (**Rz.2**), a total of 323 AU of crude material resulted with 41.8% full length product (135 AU). The ribozyme was purifed by anion exchange HPLC to provide 48 AU of purified ribozyme (MALDI-TOF MS calcd 12202.8, found 12202.4). Similar recoveries were obtained with monomers **11** and **20**.

Ribozyme **3**, *Bridging 5'-phosphorothioate at the 5'-end.* The oligomer (**Rz.3**) was synthesized using the 5'-thiol phosphoramidite **23,** coupled for 300 s, and the following amidite coupled for 400 s. Furthermore, following the addition of the 5'-thiol amidite, capping and oxidation, the column was removed from the synthesizer. The cap and frit were removed, the support was washed out of the column and into an empty syringe with 200 mM $AgNO_3$ in 1:1 CH_3CN:H_2O (10 mL). The syringe was capped, wrapped in foil and placed on a shaker for 1 h at rt. The mixture was then replaced into the column. The liquid was removed and the support was rinsed with 1:1 CH_3CN:H_2O (20 mL). The support was then treated with 50 mM DTT (10 mL) for 10 min at rt. The support was then washed with H_2O (20 mL), then CH_3CN that was acidified with a few drops of glacial acetic acid (20 mL). The column was placed on the synthesizer, washed with CH_3CN for 30 s then reverse flushed for 15 s, this procedure was repeated 4 times. The synthesis was then resumed, with the next phosphoramidite coupling for 400 s and the remaining phosphoramidites coupling for the standard times.

The ribozyme was deprotected with 40% aqueous methylamine for 10 min at 65 °C. The silyl groups were removed with TEA/HF solution in 30

min at 65 °C and the oligonucleotide was precipitated from the solution. The synthesis yielded 264 AU of crude material with 17.7% full length product and 8.5% disulfide dimer. Following purification using standard procedures a total of 26.5 AU of ribozyme (56.6% recovery) was isolated (ES-MS calcd 12250.86, found 12251.75) along with 9.2 AU of the disulfide linked 72-mer (ES-MS calcd 23781.1, found 23791.1).

CONCLUSION

5'-Amino-5'-deoxy and 5'-mercapto-5'-deoxy modified 2'-*O*-methyl nucleoside monomers for incorporation into oligonucleotides were synthesized and successfully incorporated into hammerhead ribozymes. Stability and activity of ribozymes containing bridging 5'-phosphoramidate and 5'-phosphorothioate linkages is currently under investigation in our laboratories. Ribozymes containing a 5'-amine at the 5'-end showed resistance to digestion by calf spleen 5'-exonuclease comparable to that observed with P=S backbone modifications,[44] making them attractive as potential therapeutics. 5'-Amino ribozymes are also useful synthons for postsynthetic conjugation to a variety of molecules aimed to deliver ribozymes into cells.

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Received April 10, 1997
Accepted June 27, 1997