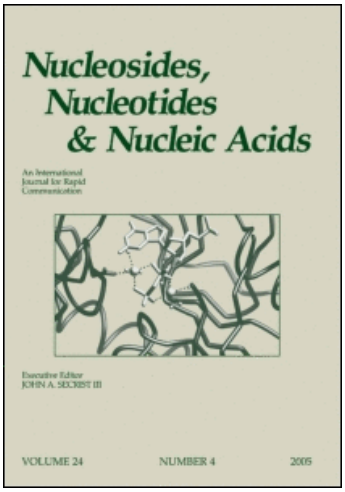

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Evaluation of RNA Transesterification Efficiency Using Stereospecific Serinol-Terpyridine Conjugates

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To cite this Article Putnam, William C. and Bashkin, James K.(2005) 'Synthesis and Evaluation of RNA Transesterification Efficiency Using Stereospecific Serinol-Terpyridine Conjugates', Nucleosides, Nucleotides and Nucleic Acids, 24: 9, 1309 — 1323
To link to this Article: DOI: 10.1080/15257770500230426
URL: http://dx.doi.org/10.1080/15257770500230426

Nucleosides, Nucleotides, and Nucleic Acids, 24 (9):1309–1323, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1080/15257770500230426

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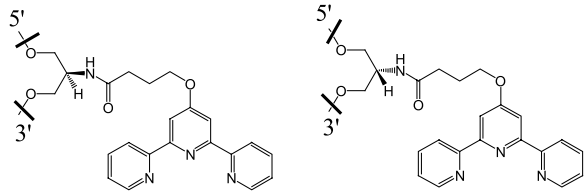
SYNTHESIS AND EVALUATION OF RNA TRANSESTERIFICATION EFFICIENCY USING STEREOSPECIFIC SERINOL-TERPYRIDINE CONJUGATES

William C. Putnam and James K. Bashkin ▫ *Department of Chemistry, Washington University, St. Louis, Missouri, USA*

▫ *Six novel artificial ribonucleases were synthesized employing a stereochemically pure abasic serinol backbone residue for attachment of the RNA transesterification agent copper(II) terpyridine. These stereochemically pure abasic residues were synthesized as phosphoramidite building blocks from the parent L-serine and D-serine starting building blocks and incorporated into oligonucleotides via solid-phase DNA synthesis. These artificial ribonucleases were constructed to determine if the stereochemistry of the alpha carbon of an abasic serinol residue has influence over RNA transesterification through selective placement of a pendant transesterification agent in either the major or minor groove. The novel artificial ribonucleases and previously synthesized artificial ribonucleases were challenged with a 28-mer and 159-mer RNA substrate. It was determined that the stereochemistry of the carbon atom derived from the α-carbon of serine did not influence the extent of cleavage in these studies using copper(II) terpyridine conjugated artificial ribonucleases.*



D-serinol-terpy (DST) L-serinol-terpy (LST)

Keywords Antisense, Artificial Ribonuclease, RNA Cleavage, Transesterification, Abasic Backbone, Serinol

Received 5 November 2004.

Acknowledgment for partial support of this research is made by to the donors of the Petroleum Research Fund, administered by the ACS and the National Science Foundation (CHE-9802660). Mass spectrometry was provided by the Washington University Mass Spectrometry Resource, an NIH Research Resource (Grant No. P41RR0954). We thank Dr. Lee Ratner of the Washington University Medical School (St. Louis, Missouri) for the plasmid containing the HIV *gag*-gene fragment.

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INTRODUCTION

The optimization of artificial ribonucleases for therapeutic applications has been a goal for over 15 years.[1–17] Artificial ribonucleases are synthetic analogues to ribonucleases. These artificial ribonucleases are conjugates of a nucleic acid (for molecular recognition) and an RNA transesterification catalyst (for RNA cleavage). They are also synthetic mimics of ribozymes (like the hammerhead) with the large catalytic domain replaced by a small molecule catalyst; thus, they are often termed ribozyme mimics. The use of a small molecule catalyst confers many advantages to these synthetic analogues that their natural counterparts do not possess. Two such advantages are that artificial ribonucleases can easily be modified by synthetic techniques to optimize activity and their small molecular weight may expedite in vivo delivery.[18–24]

Initially, our group synthesized and evaluated modified building blocks that were designed not to interfere with normal Watson-Crick base pairing (Figure 1a).[9] These initial artificial ribonucleases were not tremendously efficient at cleaving RNA.[11,12] In order to increase the transesterification efficiency, a number of optimization strategies were used. One of the optimization strategies taken employed the attachment of a transesterification catalyst to a serinol-based abasic backbone structure.[13,15,25] The use of the abasic structure directly removed the hydrogen bonds of one of the base pairs, resulting in increased local flexibility proximal to the catalyst. This molecular design was employed in response to reports that the rigidity of the RNA–DNA duplex suppresses transesterification of the RNA strand when compared to single-stranded RNA. This suppression is believed to result from the hindered formation of the 5-coordinate phosphorane that serves as the transition state for transesterification.[26] Serinol, the reduced form of serine, was chosen because the three-carbon diol structure also mimics the spacing of natural nucleotides. Using a copper(II)-terpyridine (Cu-terpy) RNA transesterification catalyst, Daniher and coworkers demonstrated a three-fold increase in transesterification efficiency using a artificial ribonuclease containing the

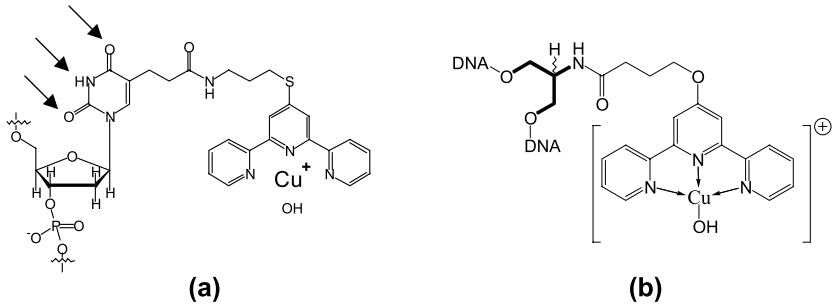


FIGURE 1 Active sites of serinol Cu(II)-terpy artificial ribonucleases: (a) Attachment at C5 position preserve hydrogen bonding sites (arrows). (b) Abasic residue attachment three carbon diol structure of serinol is shown in **bold**.

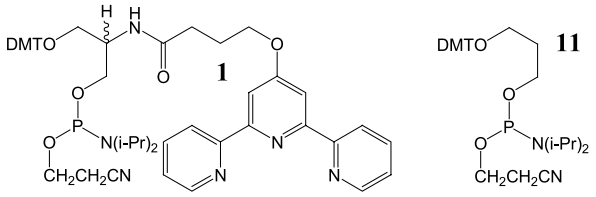


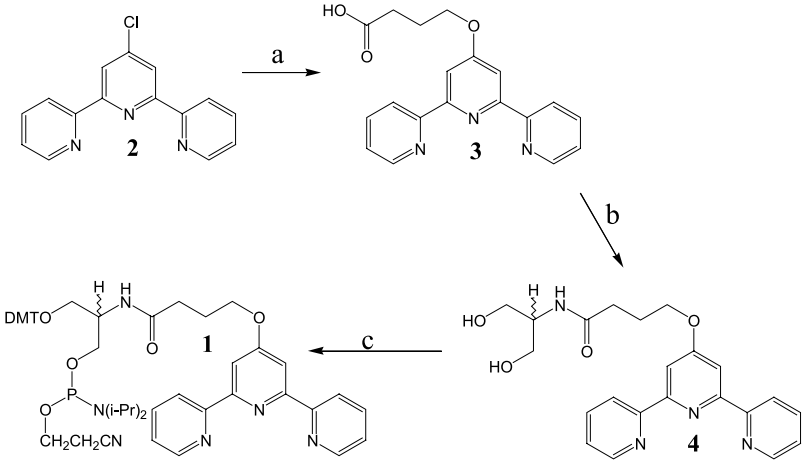
FIGURE 2 Phosphoramidite building blocks serinol-terpyridine ribozyme mimics: Phosphoramidite **1** and **Probe 11.ST**.

serinol residue (Figure 1b) when compared to a analogous fully duplexed ribozyme mimic.[13]

The use of serinol also greatly simplified the synthesis over previously modified native-nucleoside residues. This artificial ribonuclease with a serinol-terpy residue at the 11 position of a 17-mer was constructed using solid-phase automated DNA synthesis and phosphoramidite **1** (Figure 2).

The synthesis of the phosphoramidite building block **1** (Scheme 1) yielded two diastereomers, because the meso compound serinol was coupled to terpy acid **3** in the synthesis (step b) to yield the diol **4**. During the subsequent protection of one of the primary alcohols with a DMT (dimethoxytrityl) protecting group, the stereochemistry at the α-carbon was set (Figure 3). The carbon bearing the amino group of serinol is termed the α-carbon because it is derived from the α-carbon of serine.

One mechanism for RNA transesterification involves the general base deprotonation of the 2′-OH of RNA, thus, the 2′-OH of RNA is an essential moiety for RNA transesterification. The 2′-OH of RNA resides in the minor groove of the A-form RNA-DNA duplex. In these serinol-terpyridine oligonucleotides it is



SCHEME 1 Synthesis of the serinol-terpy building block **1**. a) 4-hydroxybutyric acid, KOH, DMSO; b) serinol, EDC-HCl, DMF; c) 1) DMT-Cl, Py; 2) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, methylene chloride, triethylamine.

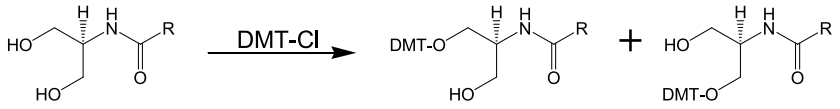


FIGURE 3 DMT protection of serinol-terpyridine (R = $CH_2CH_2CH_2$-O-Terpy): Production of enantiomers.

possible that only one of the stereoisomers geometrically allows the pendant transesterification agent to reach in the minor groove. In this case, only one of the two isomers generated from the phosphoramidite building block **1** would be active at RNA transesterification. To evaluate if the stereochemistry around the alpha carbon of a serinol based nonnucleoside backbone affects the transesterification of RNA, the two isomers were independently synthesized and tested.

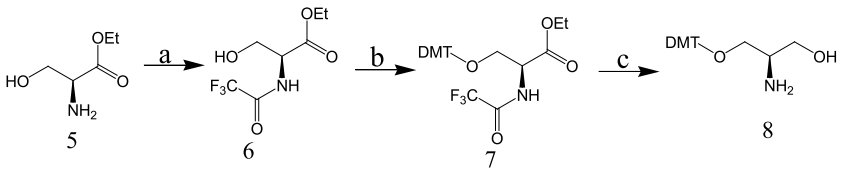
Groove discrimination by pendant ligands attached to abasic nucleic acid backbone residues was previously investigated by Fukui and coworkers.[27–29] In this study of intercalation, acridine was attached to the non-nucleoside residue threoninol and placed internally in a strand of DNA. It was determined that the (*R*,*R*)-isomer (*R* conformation at the α carbon) of threoninol places pendant ligands in the major groove.

MATERIALS AND METHODS

All synthetic reagents were purchased in the highest quality available from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used as received unless otherwise stated. All synthetic starting materials were co-evaporated from either toluene or pyridine to remove excess water. All solvents were dried over the appropriate agent (usually CaH_2). All synthetic reactions were carried out under an inert atmosphere of argon (Ar) or nitrogen (N_2). All DNA synthesis reagents were purchased from Oligos Etc. (Wilsonville, OR). All other reagents were purchased from the indicated vendors and used as received.

Synthesis of R-Serinol-Terpy Phosphoramidite Building Block (10R)

The *R*-serinol-terpy phosphoramidite building block **10R** was synthesized according to Schemes 2 and 3. *L*-serine ethyl ester **5R** was initially *N*-TFA protected by ethyl-trifluoroacetate. DMT-Cl was then used to protect the free hydroxyl **7R** (step b). A one-step deprotection-reduction sodium borohydride reaction was applied to give the DMT-protected stereo-specific serinol building block **8R** in a 75% yield over the 3 steps. The DMT-protected (*R*)-serinol **8R** was coupled to the terpyridine acid derivative **3** in the presence of the water-soluble coupling agent EDC and 1-hydroxybenzotriazole (HOBt) to yield the DMT-(R)-ser-terpy **9R** in a 60% yield. Reaction with the phosphoramidite reagent Cl-PA yielded the final building block **10R** in 82% yield. All yields presented as isolated yields.



SCHEME 2 Synthesis of DMT protected stereospecific serinol. (a) TFA-OEt, Et_3N, MeOH, 12 h, RT; (b) DMT-Cl, Py, 12 h, RT; (c) $NaBH_4$, LiCl, EtOH, 12 h, 0°C-RT.

Synthesis of S-Serinol-Terpy Phosphoramidite Building Block (10S)

Synthesis of the phosphoramidite building block based on (*S*)-serinol stereochemistry was constructed in a directly analogous fashion, with the coupling to the terpy acid derivative proceeding in a 63% yield and the phosphytilation yield of 85%.

Racemic Serinol-Terpy Phosphoramidite Building Block (1)

Phosphoramidite **1** was prepared and used in a similar fashion to previous studies (Scheme 1).[13,17,30]

Control Phosphoramidite Building Block (11)

The three carbon diol phosphoramidite (control) was purchased from Oligo's Etc. and used as received (Figure 2).[13,17,30]

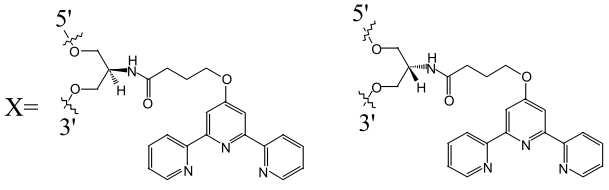
Synthesis of Artificial Ribonucleases Containing Stereospecific Serinol-Terpy Active Sites

Standard automated DNA synthesis was used for the production of DNA conjugates containing an abasic internal residue of stereospecific serinol terpy.

Oligonucleotides containing the *L*-serinol-terpy, **X** = ***L*-serinol-terpy**

Probe 6.LST–5′-CTA CA**X** AGT CTC TAA AG-3′
Probe 9.LST–5′-CTA CAT AG**X** CTC TAA AG-3′
Probe 11.LST–5′-CTA CAT AGT C**X**C TAA AG-3′

Oligonucleotides containing the *D*-serinol-terpy, **X** = ***D*-serinol-terpy**



D-serinol-terpy (DST) *L*-serinol-terpy (LST)

Probe 6.DST–5′-CTA CA**X** AGT CTC TAA AG-3′
Probe 9.DST–5′-CTA CAT AG**X** CTC TAA AG-3′
Probe 11.DST–5′-CTA CAT AGT C**X**C TAA AG-3′

These oligonucleotides were synthesized along with the racemic α-carbon serinol-terpy derived oligonucleotides (Probe 6.ST, Probe 9.ST, and Probe 11.ST) and the control oligonucleotides (Probe 6.CL, Probe 9.CL, and Probe 11.CL). These modified oligonucleotides were then gel purified and characterized. These procedures were conducted in a similar fashion to previous work.[13,17,30] Coupling yields were greater than 98% for the unmodified positions and greater than 90% for our modified building blocks. Both matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and direct-infusion electrospray ionization quadrupole mass spectrometry were used in the characterization and gave similar results. The masses found agreed well with the calculated values (± 2 amu).

Preparation of the 159-mer Substrate

The 159-mer RNA substrate was synthesized by runoff transcription using Ambion's MEGAshortscript™ T7 kit. In a total volume of 20 μL, the following were added at RT (the kit is stored at −20°C and the reagents were allowed to warm to RT for 45 min before use): 5 μL of 179-mer DNA template (from HIVHXB2r) (ca. 0.035 nmoles), 3 μL of nuclease-free water, 2 μL of 10X Buffer, 2 μL of each NTP (10 mM stock), and 2 μL of enzyme mix. The mixture was incubated at 37°C for 4 h (longer incubation times were also employed and good yields were also observed). 3 μL of DNase One was added to degrade the DNA template. The RNA was diluted by the addition of 115 μL of nuclease-free water and 15 μL of Ammonium Acetate (NH_4OAc) solution. The resultant solution was extracted twice with 150 μL of (24:25:1) phenol/chloroform/iso-amylalcohol. The aqueous layer was retained, spun through a S200 (Sephadex) column for desalting, and precipitated by the addition of 300 μL of EtOH (−70°C overnight). The ethanolic supernatant was removed after centrifugation (14,000 rpm for 15 min) and the pellet was dried by vacuum centrifugation (10 min).

The 5′-end was dephosphorylated using Ambion's KinaseMAX™ kit. The RNA was resuspended in 16 μL of nuclease-free water and 2 μL of 10 × dephosphorlation buffer was added, followed by the addition of 2 μL of calf-intestine-alkaline phosphatase (CIAP). The solution was mixed thoroughly and incubated in a heating block for 1 h at 37°C. The solution was extracted as before (with a third extraction of chloroform above). The RNA containing a 5′-OH was then ethanol-precipitated and dried as before.

The RNA was 5′-end labeled with Ambion's KinaseMAX™ kit. The RNA was reconstituted in 9 μL of nuclease-free water, 5 μL of γ-^{32}P ATP (Amersham), 4 μL of 5 × reaction buffer, and 2 μL of T4 polynucleotide kinase (T4 PNK) was added

to give a total solution volume of 20 μL. The reaction was incubated at 37°C for 1 h. The RNA was extracted, precipitated using 3 M NaOAc (pH 5.2), and dried as before.

The RNA was resuspended in 15 μL of nuclease-free water and 15 μL of 1X loading buffer (LB). It was then loaded on a 6% polyacrylamide gel and run for 3.5 h at 2000 V. This separated the desired product, the 159-mer, from failure sequence oligos (of shorter length) in the mixture. A film autoradiogram was taken of the gel. Using the film as a guide, the desired RNA band, the topmost band in the lane, was excised from the gel and the gel fragment transferred to a 1.5 mL Eppendorf tube. The gel fragment was crushed in the tube, and 200 μL of gel elution buffer (0.5 M ammonium acetate, 10 mM magnesium sulfate, 1 mM EDTA, 0.1% sodium dodecyl sulfate) were added to the tube. The solution was sealed with Parafilm and stirred or shaken overnight to elute the RNA from the gel.

After this overnight incubation, the RNA was desalted by EtOH precipitation as before. The RNA was resuspended in nuclease-free water and the UV absorbance at 260 nM was measured to evaluate the concentration.

Preparation of the 28-mer RNA Substrate

The 28-mer substrate was purchased (Oligos Etc.) and 5′-end labeled in a similar fashion to the 159-mer. The RNA was resuspended in 15 μL of nuclease-free water and 15 μL of 1X loading buffer (LB). It was then loaded on a 20% polyacrylamide gel and run for 3.5 h at 1400 V. This separated the desired product, the 28-mer, from failure sequence oligos (of shorter length) in the mixture. A film autoradiogram was taken of the gel. Using the film as a guide, the desired RNA band, the top-most band in the lane, was excised from the gel and the gel fragment transferred to a 1.5 mL Eppendorf tube. The gel fragment was crushed in the tube, and 200 μL of gel elution buffer (0.5 M ammonium acetate, 10 mM magnesium sulfate, 1 mM EDTA, 0.1% sodium dodecyl sulfate) was added to the tube. The solution was sealed with Parafilm and stirred or shaken overnight to elute the RNA from the gel.

After this overnight incubation, the RNA was purified by EtOH precipitation as before. The RNA was resuspended in nuclease-free water and the UV absorbance at 260 nM was taken to evaluate the concentration.

RNA Cleavage Reactions

RNA cleavage reactions were carried out in 10 mM (*N*-[2-Hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]) (HEPES) buffer (pH = 7.4), with 0.1 M $NaClO_4$, the respective RNA-mer [0.1 μM RNA (28-mer) and 0.01 μM RNA (159-mer)], 5 μM probe, and 5 μM metal (total reaction volume = 10 μL). The reactions were heated to the desired temperature and time. The reactions were quenched by the addition of 5 μL loading buffer and loaded on a denaturing polyacrylamide gel (20% gel for the 28-mer and 6% gel for the 159-mer). The gel image was quantified

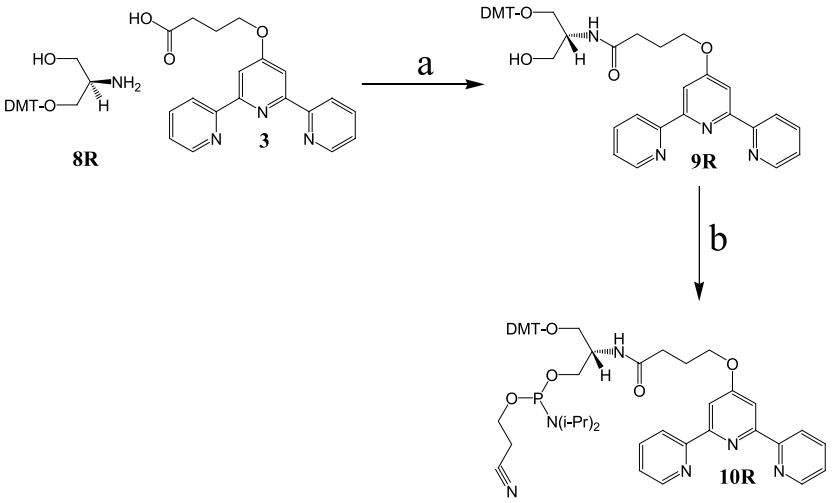
using a Molecular Dynamics PhosphorImager and the ImageQuant© (Version 3.3) software package.

RESULTS AND DISCUSSION

Synthesis of Stereospecific Serinol-Terpy Phosphoramidite Building Blocks

The stereospecific serinol-terpy phosphoramidite building blocks were synthesized according to Scheme 2. The production of the modified stereospecific serinol phosphoramidites was based on the chemistry of Benhida et al.[31] who studied the effects of abasic residues on duplex and triplex stability by melting temperature methods. Our synthetic strategy employed Benhida and coworkers'[31] stereospecific synthesis of the TFA-protected serinol **8**. That building block was the conjugated to the terpyridine-acid derivative **3** prior to phosphoramidite production and incorporation into an oligonucleotide.

The stereochemistry of the α-carbon of serinol in the stereospecific phosphoramidites is derived from the chemistry of the parent amino acid serine. Following trifluoroacetate (TFA) protection of the amine of serine ethyl ester, the free hydroxyl of the R-group is protected with a DMT group. This protection strategy avoids the direct use of the meso compound serinol, where there would be no direct stereoselectivity between the hydroxyls. In a subsequent deprotection-reduction step with lithium borohydride, the second hydroxyl is generated from the acid moiety and the amine is deprotected. This is functionally a double



SCHEME 3 Synthesis of the stereo-specific serinol-terpy phosphoramidite building block. (a) EDC-HCl, HOBt, DMF, 12 h, RT, (b) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, methylene chloride, triethyl amine, 1 h, RT.

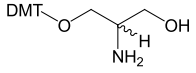
deprotection step because we are using the acid moiety as a protecting group for hydroxyl. The amine is then coupled via an amide bond to the ligand. In the final step, the free hydroxyl is reacted with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to give the appropriate stereospecific serinol phosphoramidite building block.

Nomenclature Note: the numbering scheme will denote the compound and the isomer of serine from which it came. The DMT-protected (*R*)-serinol will be called compound **8R** and the DMT-protected (*S*)-serinol will be called compound **8S** so there will be a series of compounds labeled as derived from the L-isomer of serine and **R** series of compounds labeled **S** that are derived from the D-isomer.

The DMT protected stereo-specific serinol was synthesized as detailed in Scheme 2 where the ethyl ester of the amino acid *L*-serine is reacted with ethyl trifluoroacetate (TFA-OEt) in methanol with triethylamine as a base to give the *N*-TFA protected *L*-serine ethyl ester **6R**. After overnight reaction, TLC (silica; staining with *p*-anisaldehyde) illustrated that all of the starting material had been consumed and a single spot of higher mobility (R_f) had been formed. After removal of the MeOH, the *N*-TFA protected intermediate was reacted with DMT-Cl in pyridine to give **7R**. TLC (UV silica) after stirring overnight showed that all of the starting material had been consumed to give predominately one spot of higher mobility. After removal of pyridine, the reaction mixture was subjected to the deprotection-reduction conditions of sodium borohydride in the presence of ethanolic lithium chloride. TLC once again revealed a single spot, this time of lower mobility. This product, **8R**, was isolated by column chromatography (silica; 10% methanol-methylene chloride). Spectral characterization of the product proved that it was the desired **8R** DMT-protected (*R*)-serinol. A similar route with the ethyl ester of *D*-serine yielded **8S** the DMT-protected (*S*)-serinol.

To determine if two isolated DMT-protected intermediates (**8R** and **8S**) were enatiomers with opposing stereochemistry, each of the two intermediates was coupled to a stereochemically-pure amino acid FMOC-*L*-histidine(BOC)-OH and subjected to TLC. The racemic DMT intermediate, **8RS**, was also synthesized and coupled to the same *L*-histidine building block (Figure 4).

Two DMT active peaks were formed with the *L*-His-derivative of the racemic-building block **8RS**, demonstrating that the enantiomers could be separated by TLC. The TLC of the DMT-(*S*)-serinol had one DMT active spot that corresponded to the higher R_f, and the DMT-(*R*)-serinol contained a single



8RS

FIGURE 4 Racemic DMT-serinol.

DMT active spot that corresponded to the lower R_f. The spots were then isolated by column chromatography to determine their structure and they were demonstrated to be the coupled products of the DMT intermediates and the *L*-His building block. This proved the stereochemistry of the DMT protected intermediates was opposite in nature, and the stereochemistry was derived from the α-carbon of serinol.

Scheme 3 details the synthesis of the stereospecific serinol-terpy phosphoramidite building blocks from the DMT-protected intermediates **8R** and **8S**. In order to obtain comparable RNA cleavage results, the synthesis of the phosphoramidites was conducted in a similar fashion to the original serinol-terpy building block. These conserved molecular design principles include the 4-ether substituent on terpy (keeping stereoelectronic effects on the metal constant) and the length of the tether to the metal complex.

Evaluation of the Cleavage Efficiencies of Stereospecific Serinol-Terpyridine Based Ribozyme Mimics

The cleavage efficiencies of our ribozyme mimics were evaluated with two RNA substrates. The first target is a 159-mer substrate that is derived from the mRNA of the HIV *gag*-gene, and the second substrate is a 28-mer, both of which contain the same 17-mer recognition sequence. The 28-mer RNA target that allowed ready identification of the cleavage products as true transesterification products, and a 159-mer fragment of the HIV *gag*-gene mRNA that would indicate the precise specificity of cleavage in the presence of many competing sites. Because of the shared recognition sequence, cleavage differences between the two substrates should predominately derive from either the differences in 1) molecular weight, 2) charge, or 3) the presence of secondary/tertiary structure.

RNA Transesterification 28-mer Substrate

Figure 5 is a typical audioradiogram of the sequence-specific cleavage of the 5′-end labeled 28-mer RNA substrate by ribozyme mimics modified at the 11 position. In the presence of EDTA, no sequence-specific RNA cleavage was observed with any of the probes. In accordance with previous reports, EDTA treatment is necessary in the case of oligonucleotides with pendant terpy ligands because of terpy's high binding constant for copper, which allows it to scavenge trace amounts of Cu(II) from commercial buffer salts.[13] For every RNA cleavage reaction, background cleavage was found at all of the 5′-UA-3′ sites, in keeping with their high natural propensity for scission.[32–34]

In the presence of copper, both 11.LST and 11.DST form active ribozyme mimics. The products of all of the cleavage reactions co-migrate with alkaline hydrolysis and Ribonuclease T_1 (3′-G specific) digestion products. This demonstrates that, in the presence of copper, these ribozyme mimics react via a

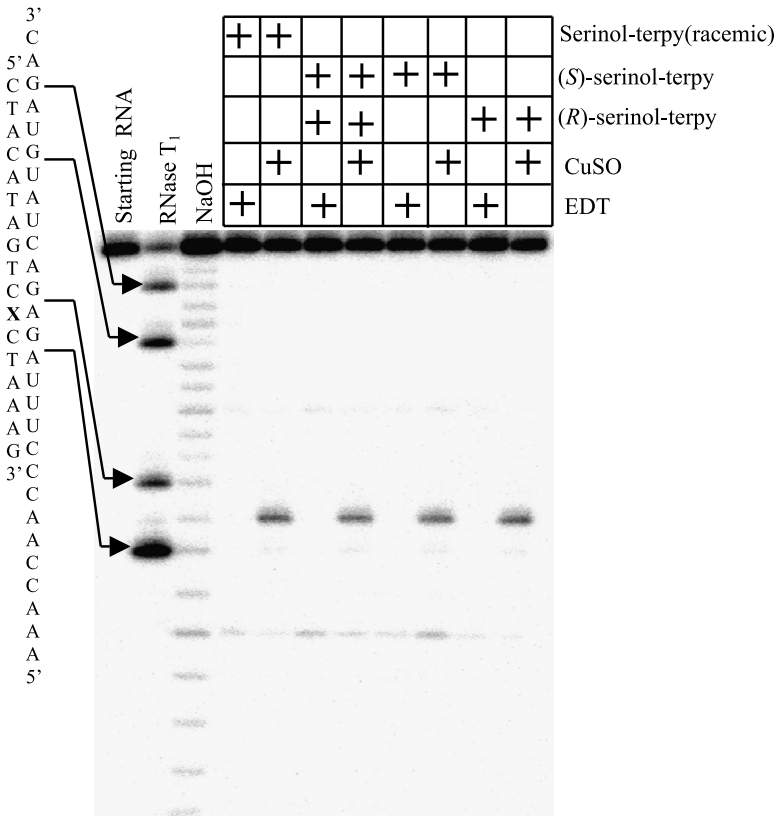


FIGURE 5 Audioradiogram of a 20% polyacrylamide gel (8 M Urea) of the site-specific cleavage of the 5′-end labeled 28-mer RNA ([0.1 μM]) by ribozyme mimics (reaction conditions: [$CuSO_4$] = 5 μM, [RNA] ~60 nM, [$NaClO_4$] = 0.1 M, [HEPES] = 10 mM pH 7.4, [EDTA] = 50 μM, 15 h, 37°C). First lane: unreacted RNA starting material. Second lane: Ribonuclease T_1 digest (G specific). Third lane: NaOH digest. Lanes 4–11: Treatment with stereospecific serinol-terpy artificial ribonucleases ([5 μM]) both in the absence and presence of copper and EDTA (EDTA treatment to remove trace contamination of copper).

biomimetic pathway of transesterification and hydrolysis and not via oxidative cleavage pathways.

Reactions were conducted in triplicate. The extent of sequence-specific RNA cleavage was statistically equivalent for both 11.LST and 11.DST (Table 1). The

TABLE 1 Sequence-Specific Cleavage of 28-mer and 159-mer RNA by Cu(II)-Terpy Artificial Ribonucleases

Artificial ribonuclease	Cleavage % 28-mer 68 h, 45°C	Cleavage site 28-mer	Cleavage % 159-mer 68 h, 45°C	Cleavage site 159-mer
11.CL	0	n/a	0	n/a
11.ST	65 ± 5	A^{16}	79 ± 8	A^{108}
11.DST	63 ± 7	A^{16}	80 ± 4	A^{108}
11.LST	64 ± 10	A^{16}	80 ± 6	A^{108}

Reaction conditions for reactions of 28- and 159-mer substrates can be found in Figures 5 and 6. Note differences in time and temperature. Percentages are given with standard deviations from the triplicate analyses.

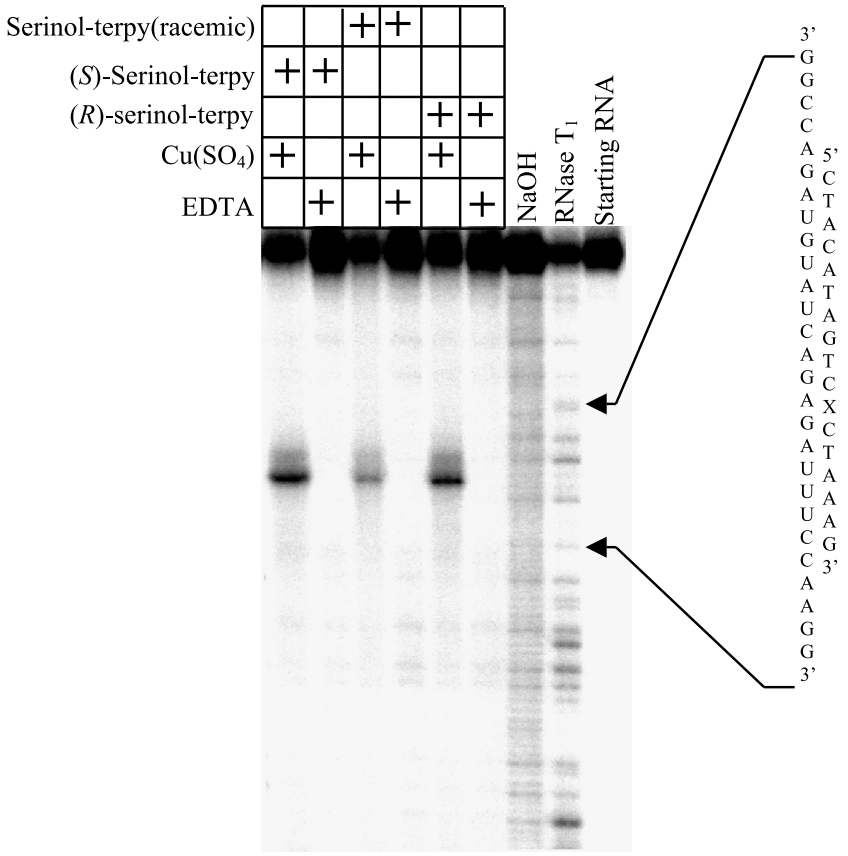


FIGURE 6 Audioradiogram of 6% polyacrylamide gel (8 M Urea) of the site-specific cleavage of the 159-mer RNA ([0.01 μM]) by artificial ribonucleases (reaction conditions: [Probe] = 5 μM, [$CuSO_4$] = 5 μM, [$NaClO_4$] = 0.1 M, [HEPES] = 10 mM pH 7.4, [EDTA] = 50 μM, 30 h, 37°C). Last lane: unreacted RNA starting material. Second to-last lane: Ribonuclease T_1 digest (G specific). Third to-last lane: NaOH digest. Lanes 1–6: Treatment with stereospecific serinol-terpy artificial ribonculeases ([5 μM]) both in the absence and presence of copper and EDTA (EDTA treatment to remove trace contamination of copper).

amount of sequence-specific RNA cleavage also was equivalent to the amount of cleavage observed when a mix of 11.LST and 11.DST was used as well as the amount of cleavage observed when 11.ST was used. Effort was made to ensure that there are not minor differences between the two isomers and their ability to cleave RNA. Different concentrations of artificial ribonuclease and metal were examined. Under all conditions attempted no statistical difference was observed. The results are similar for the ribozyme mimics that are modified at the 6 and 9 positions, although the overall extent of cleavage is less in accordance with previous results (data not shown). Probe 11.CL, the control oligonucleotide containing the abasic site and no pendant ligand, is completely inactive for RNA cleavage in the presence of Cu(II); therefore, no site-specific cleavage is derived solely from the presence of an abasic site. Similar results were obtained in the artificial ribonucleases modified in the 6 and 9 positions.

The equivalence of the extent of sequence-specific cleavage demonstrates that the cleavage of RNA by serinol-terpy derived ribozyme mimics is independent of the absolute conformation of the α-carbon of serinol under these conditions.

RNA Transesterification 159-mer Substrate

A representative image of an audioradiogram of the cleavage of the 159-mer substrate is depicted in Figure 5. The extent of cleavage by the different isomers of serinol-terpy containing oligonucleotides (11.LST or 11.DST) is equivalent. Furthermore, the amount of cleavage by any one isomer (11.LST or 11.DST) is equivalent to the racemic serinol-terpy containing oligonucleotide (11.ST). The equivalence of the extent of sequence-specific cleavage demonstrates that the cleavage of high-molecular-weight RNA substrates by serinol-terpy derived ribozyme mimics is independent of the absolute conformation of the α-carbon of serinol, as it was with the low-molecular weight 28-mer substrate.

CONCLUSIONS

Two novel phosphoramidites were synthesized based on stereo-specific serinol-terpyridine chemistry. They were designed to investigate the possibility of groove control at an abasic site to optimize the cleavage of RNA by ribozyme mimics. In the presence of copper(II) these terpyridine containing oligonucleotides derived from the chemistry of *L*-serine and *D*-serine transesterified RNA in equivalent amounts. The stereochemistry of the α-carbon of serine does not influence the amount of cleavage when using serinol-terpy-derived ribozyme mimics. We conclude that the flexibility of the serinol-based abasic site allows both conformations of serinol in Cu(II)-serinol-terpy ribozyme mimics to equally reach the 2′-OH of RNA; therefore, the extent of cleavage is equivalent.

REFERENCES

1. Cech, T.R. Ribozymes and their medical implications. JAMA **1988**, *260*, 3030–3034.
2. Cohen, J.S. *Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression*; CRC Press: Boca Raton, FL, 1989.
3. Haener, R.; Hall, J.; Pfutzer, A.; Huesken, D. Development of artificial ribonucleases. Pure Appl. Chem. **1998**, *70*(1), 111–116.
4. Morrow, J.R.; Buttrey, L.A.; Shelton, V.M.; Berback, K.A. Efficient catalytic cleavage of RNA by lanthanide(III) macrocyclic complexes: toward synthetic nucleases for in vivo applications. J. Am. Chem. Soc. **1992**, *114*(5), 1903–1905.
5. Beloglazova, N.; Fabani, M.; Zenkova, M.; Bichenkova, E.; Polushin, N.; Sil'nikov, V.; Douglas, K.; Vlassov V. Sequence-specific artificial ribonucleases. I. Bis-imidazole-containing oligonucleotide conjugates prepared using precursor-based strategy. Nucleic Acids Res. **2004**, *32*(13), 3887–3897.
6. Petersen, L.; de Koning, M.; van Kuik-Romeijn, P.; Weterings, J.; Pol, C.; Platenburg, G.; Overhand, M.; van der Marel, G.; van Boom, J. Synthesis and in vitro evaluation of PNA-peptide-DETA conjugates as potential cell penetrating artificial ribonucleases. Bioconjug. Chem. **2004**, *15*(3), 576–582.
7. Bashkin, J.K.; Gard, J.K.; Modak, A.S. Synthesis and characterization of nucleoside peptides: toward chemical ribonucleases. J. Org. Chem. **1990**, *55*, 5125.

8. Bashkin, J.K.; McBeath, R.J.; Modak, A.S.; Sample, K.R.; Wise, W.B. Synthesis and characterization of oligonucleotide peptides. J. Org. Chem. **1991**, *56*, 3168.
9. Bashkin, J.K.; Frolova, E.I.; Sampath, U. Sequence-specific cleavage of HIV mRNA by a ribozyme mimic. J. Am. Chem. Soc. **1994**, *116*, 5981–5982.
10. Bashkin, J.K.; Jenkins, L.A. The role of metals in the hydrolytic cleavage of DNA and RNA. Comments Inorg. Chem. **1994**, *16*, 77–93.
11. Bashkin, J.K.; Xie, J.; Daniher, A.T.; Jenkins, L.A.; Yeh, G.C. Ribozyme mimics for catalytic antisense strategies. NATO ASI Ser., Ser. C **1996**, *479*, 355–366.
12. Bashkin, J.K.; Xie, J.; Daniher, A.T.; Sampath, U.; Kao, J.L.-F. Building blocks for ribozyme mimics: conjugates of terpyridine and bipyridine with nucleosides. J. Org. Chem. **1996**, *61*, 2314–2321.
13. Daniher, A.T.; Bashkin, J.K. Precise control of RNA cleavage by ribozyme mimics. Chem. Commun. **1998**, 1077–1078.
14. Trawick, B.N.; Daniher, A.T.; Bashkin, J.K. Inorganic mimics of ribonucleases and ribozymes: from random cleavage to sequence-specific chemistry to catalytic antisense drugs. Chem. Rev. **1998**, *98*(3), 939–960.
15. Trawick, B.N.; Osiek, T.A.; Bashkin, J.K. Enhancing sequence-specific cleavage of RNA within a duplex region: incorporation of 1,3-propanediol linkers into oligonucleotide conjugates of serinol-terpyridine. Bioconjug. Chem. **2001**, *12*(6), 900.
16. Inoue, H.; Furukawa, T.; Tamura, T.; Komatsu, Y.; Ohtsuka, E. Two-terpyridine. Cu(II) complexes-containing antisense systems for rapid and highly site-specific RNA cleavage. Nucleic Acids Symp. Ser. **2000**, *44*, 279–280.
17. Putnam, W.; Daniher, A.; Trawick, B.; Bashkin, J. Efficient new ribozyme mimics: direct mapping of molecular design principles from small molecules to macromolecular, biomimetic catalysts. Nucleic Acids Res. **2001**, *29*(10), 2199–2204.
18. Eckstein, F. Structure, activity and application of chemically modified hammerhead ribozymes. NATO ASI Ser., Ser. C **1996**, *479*, 291–294.
19. Heidenreich, O.; Pieken, W.; Eckstein, F. Chemically modified RNA: approaches and applications. FASEB J. **1993**, *7*(1), 90–96.
20. Olsen, D.B.; Benseler, F.; Aurup, H.; Pieken, W.A.; Eckstein, F. Study of a hammerhead ribozyme containing 2′-modified adenosine residues. Biochemistry **1991**, *30*(40), 9735–9741.
21. Pieken, W.A.; Olsen, D.B.; Aurup, H.; Williams, D.M.; Heidenreich, O.; Benseler, F.; Eckstein, F. Structure-function relationship of hammerhead ribozymes as probed by 2′-modifications. Nucleic Acids Symp. Ser. **1991**, *24*, 51–53.
22. Usman, N.; Beigelman, L.; McSwiggen, J.A. Hammerhead ribozyme engineering. Curr. Opin. Struct. Biol. **1996**, *6*(4), 527–533.
23. Matulic-Adamic, J.; Karpeisky, A.M.; Gonzales, C.; Burgin, A.B., Jr.; Usman, N.; McSwiggen, J.A.; Beigelman, L. Modified nucleosides for ribozyme structure-activity studies. Collect. Czechoslov. Chem. Commun. **1996**, *61*, S271–S275.
24. Burgin, A.B., Jr.; Gonzalez, C.; Matulic-Adamic, J.; Karpeisky, A.M.; Usman, N.; McSwiggen, J.A.; Beigelman, L. Chemically modified hammerhead ribozymes with improved catalytic rates. Biochemistry **1996**, *35*(45), 14090–14097.
25. Huesken, D.; Goodall, G.; Blommers, M.J.J.; Jahnke, W.; Hall, J.; Haener, R.; Moser, H.E. Creating RNA bulges: cleavage of RNA in RNA/DNA duplexes by metal ion catalysis. Biochemistry **1996**, *35*(51), 16591–16600.
26. Kolasa, K.A.; Morrow, J.R.; Sharma, A.P. Trivalent lanthanide ions do not cleave RNA in DNA-RNA hybrids. Inorg. Chem. **1993**, *32*(19), 3983–3984.
27. Fukui, K.; Iwane, K.; Shimidzu, T.; Tanaka, K. Oligonucleotides covalently linked to an acridine at artificial abasic site: influence of linker length and the base-sequence. Tetrahedron Lett. **1996**, *37*(28), 4983–4986.
28. Fukui, K.; Tanaka, K. The acridine ring selectively intercalated into a DNA helix at various types of abasic sites: double strand formation and photophysical properties. Nucleic Acids Res. **1996**, *24*(20), 3962–3967.
29. Fukui, K.; Morimoto, M.; Segawa, H.; Tanaka, K.; Shimidzu, T. Synthesis and properties of an oligonucleotide modified with an acridine derivative at the artificial abasic site. Bioconjug. Chem. **1996**, *7*, 349–355.
30. Putnam, W.C.; Bashkin, J.K. De novo synthesis of artificial ribonucleases with benign metal catalysts. Chem. Commun. **2000**, *9*, 767–768.
31. Benhida, R.; Devys, M.; Fourrey, J.-L.; Lecubin, F.; Sun, J.-S. Incorporation of serinol derived acyclic

nucleoside analogs into oligonucleotides: influence on duplex and triplex formation. Tetrahedron Lett. **1998**, *39*(34), 6167–6170.

32. Kierzek, R. Nonenzymic hydrolysis of oligoribonucleotides. Nucleic Acids Res. **1992**, *20*(19), 5079–5084.
33. Kierzek, R. Hydrolysis of oligoribonucleotides: influence of sequence and length. Nucleic Acids Res. **1992**, *20*, 5073–5077.
34. Magda, D.; Miller, R.A.; Sessler, J.L.; Iverson, B.L. Site-specific hydrolysis of RNA by europium(III) texaphyrin conjugated to a synthetic oligodeoxyribonucleotide. J. Am. Chem. Soc. **1994**, *116*, 7439–7440.