12-14-2016

Synthesis of 2SeU-RNAs and RNA Squares

Xinghua Chen
xchen29@student.gsu.edu

SYNTHESIS OF ^{2Se}U-RNAs AND RNA SQUARES

by

XINGHUA CHEN

Under the Direction of Professor Zhen Huang, PhD

ABSTRACT

In this paper, an improved method [1] for the chemical synthesis of ^{2Se}U-RNA was reported using a streamlined strategy employs 2'-O-Thiomopholine-4-carbothioate protecting group. And single step deprotection of the resulting oligoribonucleotide product using 1,2-diamines/toluene under anhydrous conditions would retain the Selenium atom introduced on the 2-possiton of the modified Uracil. The process is doable with most standard heterobase protection and deprotection, it greatly simplifies the synthesis of ^{2Se}U-RNAs and can be applied to other Selenium modified RNAs synthesis. It makes the synthesis of RNA become as simple and efficient as the chemical synthesis of DNA. Furthermore, the design and synthesis of self-assembling ^{2Se}U-RNA square are reported which enable further structure studies and application of unique ^{2Se}U-RNAs.

INDEX WORDS: ^{2Se}U-RNA synthesis, Self-assembling, RNA square

SYNTHESIS OF ^{2Se}U-RNAs AND RNA SQUARES

by

XINGHUA CHEN

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in the College of Arts and Sciences

Georgia State University

2016

SYNTHESIS OF ^{2Se}U-RNAs AND RNA SQUARES

by

XINGHUA CHEN

Committee Chair: Zhen Huang

Committee: Ming Luo

Jun Yin

Electronic Version Approved:

Office of Graduate Studies

College of Arts and Sciences

Georgia State University

December 2016

DEDICATION

To my parents, my sisters, my uncle, Jamie and all the people whom I love and those who love me.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my committee for allowing me the opportunity to present my work and give a good reflection of the help that they have given me. I would especially like to thank Dr. Zhen Huang, who has not only been my PI for 2 years, but a mentor and a leader in research. I would like to thank Dr. Ming Luo and Dr. Jun Yin for advising me for my research. A giant thank you to my lab mates and group members: Dr. Salon Jozef, Cen Chen, Ziyuan Fang, Fukang Yang, Lin Qin, Lingrui Zheng, Maliha Anjum and Edwin. I am truly honored and grateful that you guys keep me around and that you helped me through my Master's degree. Thank you to all of my friends in chemistry. To Han Zhou, Li Zhou from Dr. Jun Yin's lab for helping me with my Gel electrophoresis and students from Dr. Ming Luo's lab for helping me with my crystal growth. I am truly honored and grateful that you guys keep me around and that you helped me through my Master's degree. You are some of the best friends someone could ask for and you were only right around the corner at all times. To my family: There aren't enough words to say in this language, but thank you so much for the love and support shown to me. I do this so you can say "my son/brother/nephew" is doing something with his life. And finally to Jamie Chen, you encouraged and supported me all the time, whenever I'm frustrated your warm words would make me positive and confident.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

1 INTRODUCTION

1.1 Purpose of the Study

To develop a new streamlined synthesis for Selenium modified RNA and structure study of the designed ^{2Se}U-RNA squares.

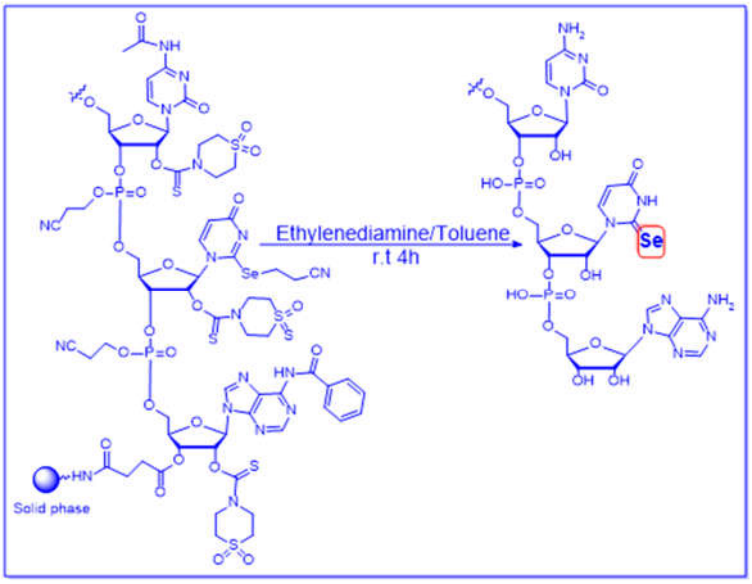


Figure 1 Single step deprotection by 1,2-diamines/toluene

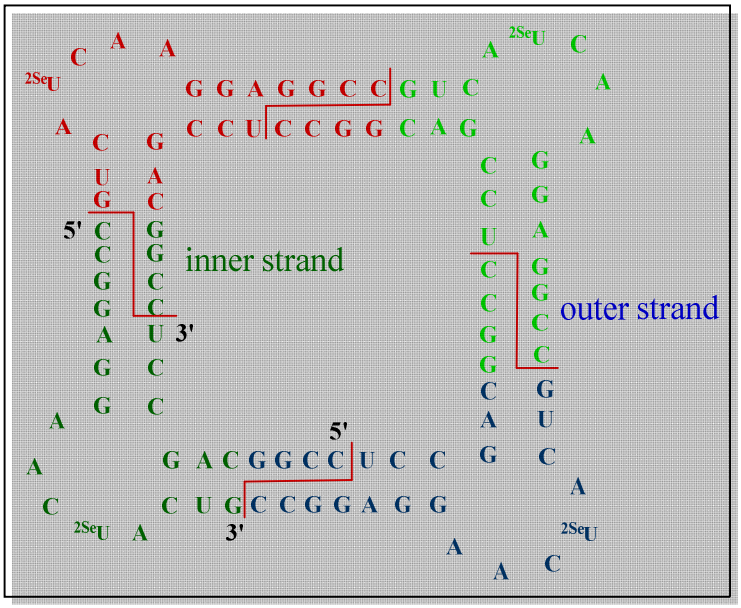


Figure 2 Secondary structure of the RNA square

1.1.1 Displacing one atom, solving a bunch of problems

In Huang Lab's previous research [2,3,4,5], it has been proved that systematic replacement of Oxygen with Selenium [6] can facilitate the crystallization of nucleic acids, help solve both phase determination and abnormal U/G wobble pair problems. Furthermore, the Se-modification will provide a useful tool in X-ray crystal structure studies of RNAs and their protein complexes. Today, the most effective method for making sequence-defined DNA oligonucleotides is based upon the solid-phase phosphoramidite method [7,8].

1.1.2 The unique hydroxyl group on RNA makes it more difficult for RNA synthesis

As we know, one unique difference between DNA and RNA is that RNA has a 2'-OH group, this extra 2'-OH group and its modifications play essential roles in structure and function diversities of noncoding RNAs. However, this additional hydroxyl in the 2'-position compelling

the presence of an extra protective group which makes the monomer synthesis of RNA much more difficult. The vicinal cis-diol of the 2'- and 3'-hydroxyls on the RNA nucleoside is difficult to be regiospecifically protected because the protective group migration between the two hydroxyl group.

The most widely used 2'-protective group for RNA synthesis is the fluoride-labile TBDMS ether, but this protective group is usually placed on the nucleoside non-regiospecifically, both 2'- and 3'-OH would be protected simultaneously, and the isolation of the two isomers would be tough. And TBDMS is unstable to basic conditions used to remove t-butylphenoxyacetyl protecting group, the loss of TBDMS would lead to phosphodiester chain cleavage and 3'- to 2'-phosphate migration.

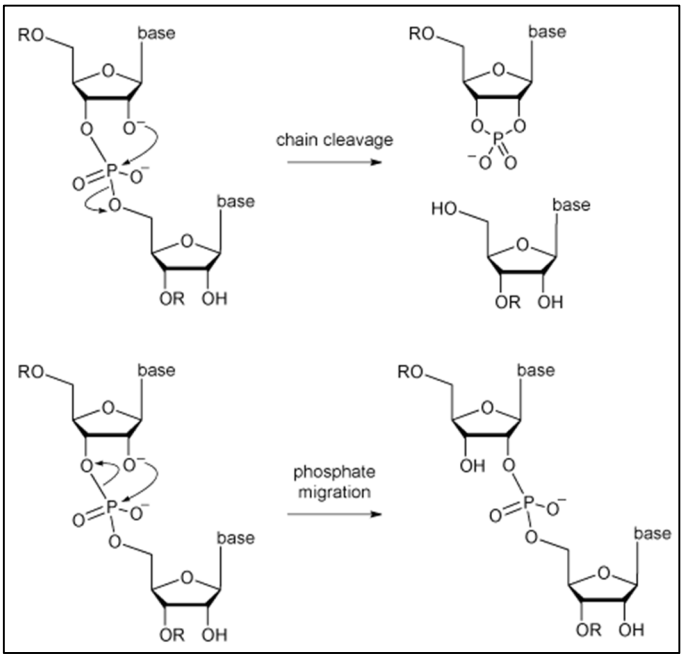


Figure 3 RNA chain cleavage and phosphate migration

Further, the fluoride ion used for the removal of TBDMS may remove the Se atom introduced at the 2-position of U base which disrupt the functionality. Thus, the TBDMS technique is not practical in applications such as beads or microarrays [9], residue of TBAF used to remove TBDMS PG would negatively influence the HPLC analysis. And extra processing and purification required to remove the fluoride reagent greatly increases the complexity of performing high-throughput RNA synthesis. It' is reported [10] that RNA monomers that do not require fluoride ion to remove the 2'-hydroxyl protective group can utilize the convenient regiospecific synthesis methods originally developed by Markiewicz (Figure 4).

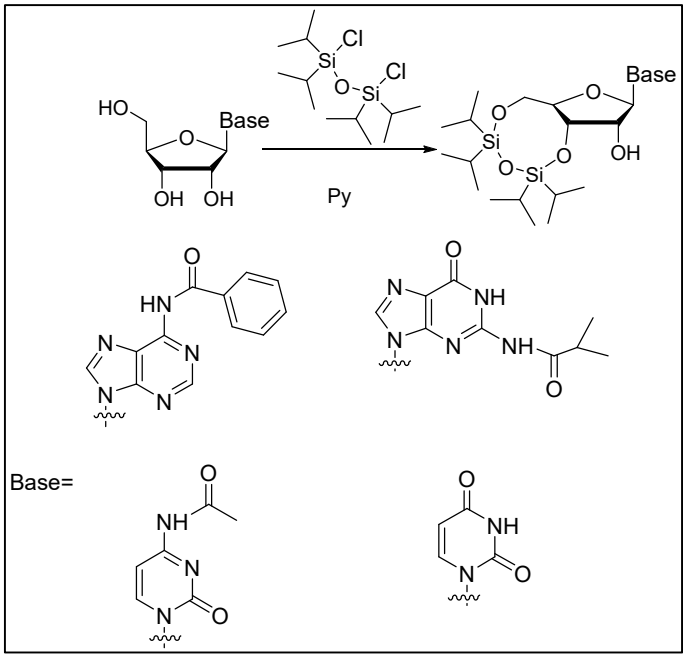


Figure 4 Markiewicz method for the protection of 3'-OH and 5'-OH groups

1.1.3 Our streamlined 2'-O-Thiomopholine-4-carbothioate strategy

In our research, we utilized the 2'-O-Thiomopholine-4-carbothioate strategy [1] developed by Dr. Marvin H. Caruthers and coworkers to protect the 2'-OH group, and the 2-position of uracil is modified with Selenium [4]. Using ethylenediamine/toluene, the cleavage from the beads and

removal of all the protecting groups for the bases, the 2'-OH group and ^{2Se}U can be simultaneously removed in a single step without disrupting the ^{2Se}U-RNA functionality.

As our previous research revealed, the biophysical and structural studies of ^{2Se}U-RNAs indicate that this single atom replacement can indeed create a novel U/A base pair higher specificity than the natural one and the ^{2Se}U/A pair maintains a structure virtually identical to the native U/A pair while discriminating against U/G wobble pair. This oxygen replacement with selenium offers a unique chemical strategy to enhance the base pairing specificity at the atomic level. The ^{2Se}U-RNA can serve as a powerful tool for crystallographic studies of RNA nanostructures and RNA/drug and RNA/protein complexes through crystallization facilitation and phase determination.

1.1.4 Structure study of ^{2Se}U-RNA squares

The 3D structures of noncoding RNA molecules reveal recurring architectural motifs that have been exploited for the design of artificial RNA nanomaterials. Structural characterization of artificial RNA Nano-objects has been limited to low-resolution microscopy studies by the size and complexity of RNA. The folding of RNA is governed by recurring structural motifs [11], the most common one is the double helix that involves consecutively stacked pairs of complementary nucleobases interacting via hydrogen bonds. Approaches toward artificial RNA architectures have relied on the ability of RNA strands to hybridize via complementary base sequences [12,13]. Inspired by the work [14] of Dr. Thomas Hermann, we designed and synthesized ^{2Se}U-RNA sequences for self-assembling RNA square (Figure 5.) to increase the resolution for artificial RNA Nano-objects development.

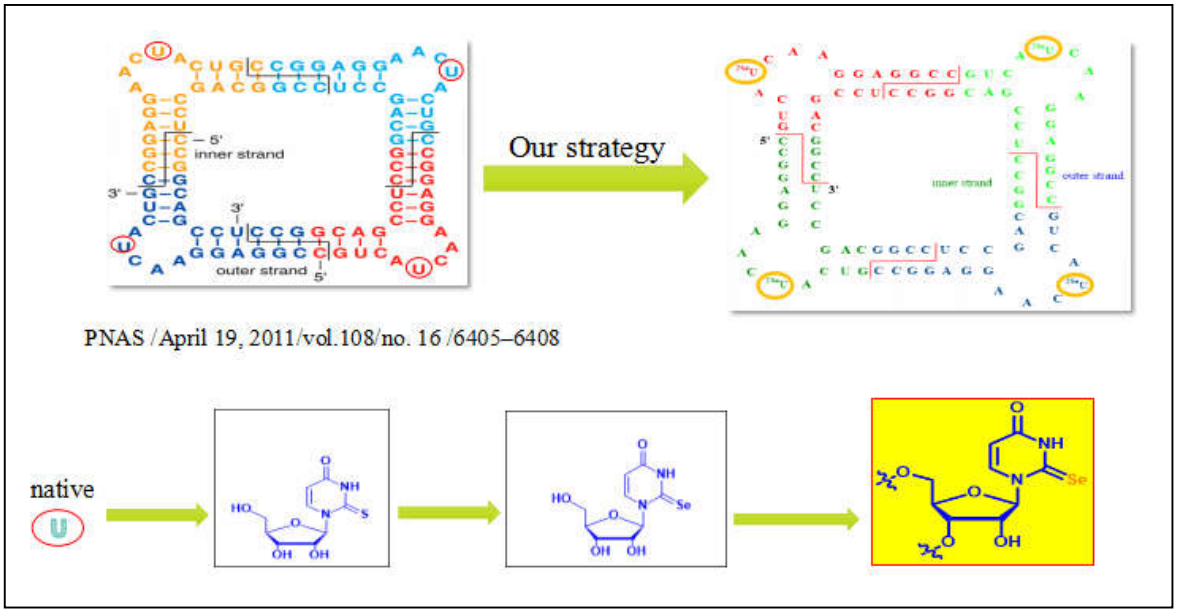
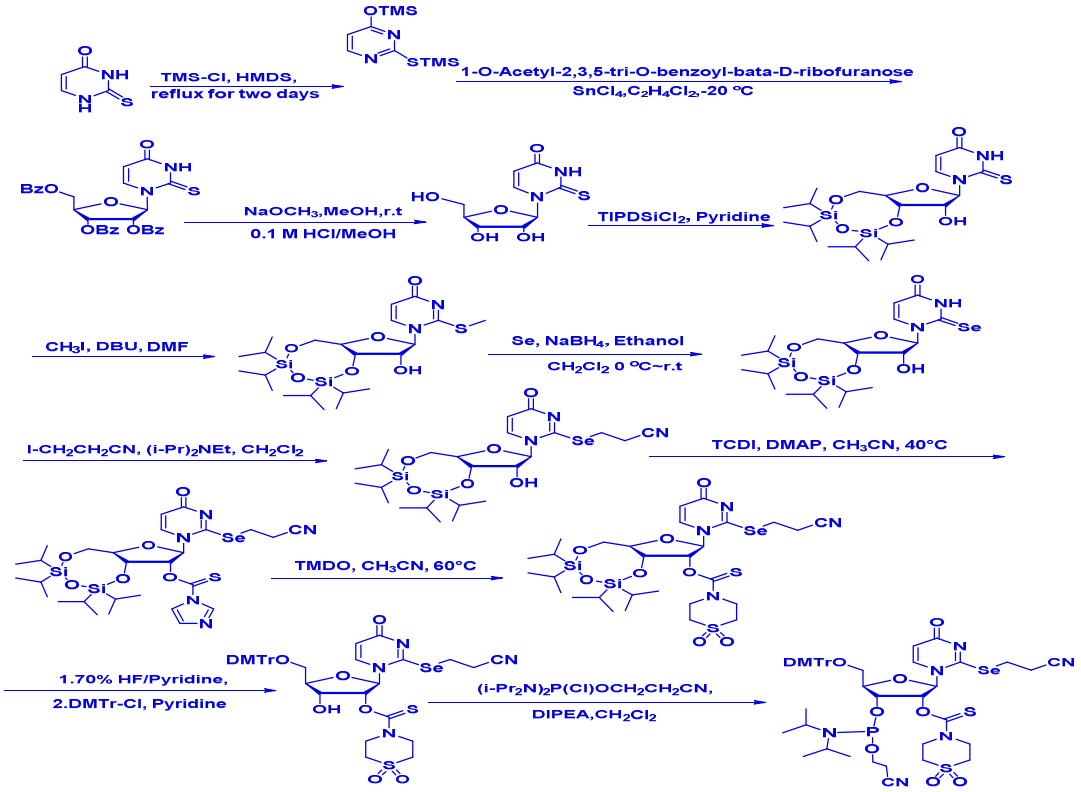


Figure 5. Our strategy for 2SeU-RNA square



Scheme 1 The synthesis of 2SeU phosphoramidite monomer

Reagents and conditions:

(1) $TIPDSiCl_2$, Pyridine, r.t; (2) CH_3I, DBU, DMF; (3) Se, $NaBH_4$, Ethanol, CH_2Cl_2; (4) ICH_2CH_2CN, $(i\text{-}Pr)_2NEt$, CH_2Cl_2; (5) TCDI, DMAP, CH_3CN, r.t overnight; (6) TMDO, CH_3CN, 60°C; (7) 70% HF/Pyridine in 2-methyltetrahydrofuran, (8) DMTr-Cl, Pyridine; (9) $(iPr_2N)_2P(Cl)OCH_2CH_2CN$, 4-methylpholine, CH_2Cl_2.

1.2 Expected Results

The streamlined process using 2'-O-Thiomopholine-4-carbothioate protecting group provides rational strategies for the chemical synthesis of ^{2Se}U phosphoramidite, these products would be comparable to the products produced from tert-butyldimethylsilyl protecting strategy. Se atom incorporated in the oligonucleotides could survive well when use the ethylenediamine/toluene (v:v=1:1 at r.t) strategy for the cleavage and de-protection.

And, self-assembling of ^{2Se}U-RNA squares would be obtained.

2 EXPERIMENT

2.1 Chemical synthesis of phosphoramidite monomer

2.1.1 3,5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-thiouridine

Compound **1** (10.0 g, 38.4 mmol) was dissolved in anhydrous pyridine (100 mL) in a 250 mL round bottom flasks, then the solution was set in ice bath and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (20 ml, 21.8 mmol) was injected gradually with stirring, after that the reaction was left run overnight. Then the reaction was monitored by TLC (5%MeOH in CH_2Cl_2, Rf=0.8) which indicated the reaction was completed. Then, 100 mL cool water was added into the reaction and left stirring for 20 min, after that, ethyl acetate (40 ml*3) was added for extraction of our desired product. The organic collections were combined and washed with saturated NaCl solution (50 mL*3), then the organic solution was dried over anhydrous $MgSO_4$, $MgSO_4$ was removed by filtration and the solvent was removed via rotary evaporator under reduced pressure. The crude was purified by silica gel chromatography column (50% CH_2Cl_2 in hexane to 0.8% MeOH in CH_2Cl_2) to afford a kind of white foam. Yield: 17.6 g was obtained as pure (91.2%).

^{1}H-NMR ($CDCl_3$): δ 1.0-1.15 (m, 28H, 4 x *i*Pr); 2.91 (s, 1H, 2'-OH); 4.03 (dd, *J* = 2.8 and 13.6 Hz, 1H, H-5'); 4.1 (d, *J* = 4.8 Hz, 1H, H-2'); 4.15 (dd, *J* = 2.8 and 9.2 Hz, 1H, H-4'); 4.29 (d, *J* = 13.6 Hz, 1H, H-5'); 4.35 (m, *J* = 4.8 and 9.2 Hz, 1H, H-3'); 5.83 (s, 1H, H-1'); 6.07 (d, *J* = 8.0 Hz, 1H, H-5); 7.9 (d, *J* = 8.0 Hz, 1H, H-6) ppm. HRMS (ESI-TOF), $C_{21}H_{38}N_2O_6SSi_2$, $[M+H^+]^+$= 503.8011 (calc. 503.7782).

2.1.2 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-methylthiouridine

To a stirred solution of 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-uridine **2** (15.0 g, 29.8 mmol) in dry DMF (150 mL) cooled at 0 °C in a 500 ml round bottom flask, methyl iodide

(30.0 mL, 7.0 eq.) and 1,8-diazabicyclo [5.4.0]-undec-7-ene (8.7 mL, 2.0 eq.) were added sequentially with stirring. The reaction mixture was stirred for 2h at 0-5 °C and monitored by TLC (5% MeOH in CH_2Cl_2, Rf=0.5). When the reaction was completed, 100 ml cool water was added into the reaction, then ethyl acetate (80 mL*3) was used for extraction, the organic collections were combined and washed with t water (50 mL*3). The organic layer was dried over anhydrous $MgSO_4$, $MgSO_4$ was removed by filtration and the solvent was removed via rotary evaporator under reduced pressure. The crude product was puri ed by silica gel chromatography column (CH_2Cl_2 to 2% MeOH in CH_2Cl_2). Yield: 12.6 g of colorless foam. Yield: 81.8%.

^{1}H-NMR ($CDCl_3$): δ 1.0-1.15 (m, 28H, 4 x *i*Pr); 2.66 (s, 3H, SCH_3); 2.91 (s, 1H, 2'-OH); 4.03 (dd, *J* = 2.8 and 13.6 Hz, 1H, H-5'); 4.1 (d, *J* = 4.8 Hz, 1H, H-2'); 4.15 (dd, *J* = 2.8 and 9.2 Hz, 1H, H-4'); 4.29 (d, *J* = 13.6 Hz, 1H, H-5'); 4.35 (m, *J* = 4.8 and 9.2 Hz, 1H, H-3'); 5.83 (s, 1H, H-1'); 6.07 (d, *J* = 8.0 Hz, 1H, H-5); 7.9 (d, *J* = 8.0 Hz, 1H, H-6) ppm. ^{13}C-NMR ($CDCl_3$): δ 168.51, 161.69, 137.26, 109.42, 91.26, 82.11, 77.35, 76.07, 68.31, 59.53, 17.41, 17.32, 17.25, 17.17, 16.96, 16.94, 16.85, 16.77, 14.61, 13.37, 12.91, 12.85, 12.49 ppm. HRMS (ESI-TOF), $C_{22}H_{40}N_2O_6SSi_2$, $[M+H^+]^+$= 517.2249 (calc. 517.2224).

2.1.3 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-selenouridine

To a clear solution of NaSeH generated by the addition of absolute ethanol (100 ml) to selenium (6.1 g, 4.0 eq) and sodium borohydride (3.5g, 4.8 eq) at 0 °C, solution of compound **3** (10g, 19.3 mmol) in dry CH_2Cl_2 (100.0 mL) was injected at room temperature. The reaction mixture was stirred overnight under argon. After completion, CH_2Cl_2 (70 mL) was added to the reaction mixture. The resulting yellow solution was washed with water (50 ml*3) and the organic layer dried over anhydrous $MgSO_4$, $MgSO_4$ was removed by filtration and the solvent was removed via rotary evaporator under reduced pressure. Purification was performed by pre-

equilibrated (1% N, N-Dimethylethylamine in 50% CH_2Cl_2/Hexane) silica gel chromatography column (50% CH_2Cl_2/Hexane to 0.8% MeOH in CH_2Cl_2). Yield: 8.6 g of slightly yellow foam. Yield: 81.1%.

^{1}H-NMR ($CDCl_3$): δ 1.04-1.14 (m, 28H, 4 x iPr); 2.64 (br, 1H, 2'-OH); 4.03 (d, J = 12.8 Hz, 1H, H-5'); 4.27-4.33 (m, 3H, H-2',3',5'); 4.39 (d, J = 2.8 Hz, 1H, H-4'); 6.13 (d, J = 8.0 Hz, 1H, H-5); 6.43 (s, 1H, H-1'); 8.05 (d, J = 8.0 Hz, 1H, H-6); 10.8 (s, 1H, NH) ppm. ^{13}C-NMR ($CDCl_3$): δ 175.65, 158.85, 139.95, 107.88, 95.80, 82.68, 75.66, 68.23, 59.47, 17.23, 17.21, 17.05, 17.03, 16.75, 16.71, 16.66, 16.55, 13.40, 12.99, 12.85, 12.47 ppm. HRMS (ESI-TOF), $C_{21}H_{38}N_2O_6SeSi_2$, $[M+H^+]^+$= 551.1504 (calc. 551.1512).

2.1.4 3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2cyanoethylselanyluridine

To a solution of the 2-selenouridine nucleoside 4 (8.0 g, 14.6 mmol) in dry CH_2Cl_2 (80 mL) cooled at 0 °C, freshly distilled 3-iodopropionitrile (14 mL, 6 eq.) and diisopropylethylamine (7.6 mL, 3 eq.) were added sequentially. The reaction was stirred overnight at r.t, monitored by TLC (5% MeOH in CH_2Cl_2, Rf=0.4), when the reaction was completed, 50 ml cool water was added into the reaction and stirred at r.t for 20 min, then the mixture was partitioned, the aqueous phase was extracted with CH_2Cl_2 (25 ml*3), the organic collections were combined and washed with saturated brine (30 ml*4), then the organic collection was dried over anhydrous $MgSO_4$, $MgSO_4$ was removed by filtration and the solvent was removed via rotary evaporator under reduced pressure, the crude mixture was purified by silica gel chromatography column (50% CH_2Cl_2 in hexanes to 3% MeOH in CH_2Cl_2). Yield: 5.8 g a kind of colorless foam was obtained. Yield: 65.2%.

^{1}H-NMR ($CDCl_3$): δ 1.03-1.13 (m, 28H, 4 x iPr); 2.97 (s, 1H, 2'-OH); 3.03-3.12 (J = 6.4 and 12.8 Hz, 2H, CH_2CN); 3.42-3.57 (m, J = 6.4 and 12.8 Hz, 2H, $SeCH_2$); 4.04 (dd, J = 2.8 and 13.6 Hz, 1H, H-5'); 4.09 (d, J = 4.8 Hz, 1H, H-3');4.15 (d, J = 9.0 Hz, 1H, H-2'); 4.29 (d, J = 13.6 Hz, 1H, H-5'); 4.36 (dd, J = 4.8 and 9.0 Hz, 1H, H-4'); 5.63 (s, 1H, H-1'); 6.11 (d, J = 8.0 Hz, 1H, H-5); 7.90 (d, J = 8.0 Hz, 1H, H-6) ppm. ^{13}C-NMR ($CDCl_3$): δ 167.70, 156.97, 137.79, 118.63, 110.18, 92.71, 76.19, 68.37, 59.56, 23.76, 18.72, 17.40, 17.33, 17.24, 17.19, 16.98, 16.94, 16.87, 16.77, 13.37, 12.91, 12.85, 12.50 ppm. HRMS (ESI-TOF), $C_{24}H_{41}N_3O_6SeSi_2$, $[M+H^+]^+$= 604.1795 (calc. 604.1777).

2.1.5 2'-O-(1H-imidazole-1-thiocarbonyl)-3',5'-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-cyanoethylselanyluridine

To a solution of 2-Selenoprotected nucleoside **5** (5.5 g, 9.1 mmol) in dry acetonitrile (55.0 mL), 1,1'-thiocarbonyldiimidazole (1.9 g, 1.2 eq) and 4-(dimethylamino) pyridine (111.1mg, 0.1 eq.) were added. The reaction was stirred overnight at room temperature. Then monitored by TLC (5% MeOH in CH_2Cl_2, Rf=0.6), the solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (50 mL). The organic phase was washed with water (25ml*3), and the organic layer was dried over anhydrous $MgSO_4$, $MgSO_4$ was removed by filtration and the solvent was removed via rotary evaporator under reduced pressure. The crude was purified by silica gel chromatography column (75% CH_2Cl_2 in hexane to 4% MeOH in CH_2Cl_2) to afford 4.3 g of the slightly yellow target product. Yield: 78.2%.

^{1}H-NMR ($CDCl_3$): δ 0.89-1.11 (m, 28H, 4 x iPr); 3.05 (t, J = 6.4 Hz, 2H, CH_2CN); 3.39-3.46 (m, J = 6.4 and 12.8 Hz, 1H, $SeCH_2$); 3.55-3.61 (m, J = 6.4 and 12.8 Hz, 1H, $SeCH_2$); 4.07 (d, J = 13.6 Hz, 1H, H-5'); 4.15 (d, J = 9.2 Hz, 1H, H-4'); 4.34 (d, J = 13.6 Hz, 1H, H-5'); 4.58-4.61 (m, J = 5.2 and 9.2 Hz, 1H, H-3'); 5.87 (s, 1H, H-1'); 6.15-6.19 (m, J = 5.2 and 8.0 Hz, 2H, H-2',5); 7.12, 7.68, 8.38 (3 s, 3H, H-Imidazole); 7.91 (d, J = 8.0 Hz, 1H, H-6) ppm. ^{13}C-NMR ($CDCl_3$): δ 181.81,

167.11, 156.62, 137.31, 131.44, 118.38, 118.17, 110.69, 90.75, 83.36, 83.15, 67.95, 59.09, 54.59, 24.32, 18.74, 17.37, 17.32, 17.20, 17.17, 16.89, 16.87, 16.69, 16.59, 13.35, 12.95, 12.81, 12.47 ppm. HRMS (ESI-TOF), $C_{29}H_{43}N_5O_6SSeSi_2$, $[M+H^+]^+$= 714.1718 (calc. 714.1716).

2.1.6 2'-O-(1,1-Dioxo-1λ⁶-thiomorpholine-4-carbothioate)-3',5'-O-(1,1,3,3 tetraisopropyl- disiloxane-1,3-diyl)-2-cyanoethylselanyluridine.

A mixture of 2'-imidazolthiocarbonyl activated nucleoside **6** (4.0 g, 5.6 mmol) and thiomorpholine-1,1-dioxide (0.9 g, 1.2 eq.) in dry acetonitrile (40.0 mL) was heated at 60 °C for 4 h. The reaction was monitored by TLC (5% MeOH in CH_2Cl_2, Rf=0.5), when the reaction was completed, the solvent was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (40 mL). The organic phase was washed with water (20ml*3), then the organic layer was dried over anhydrous $MgSO_4$, $MgSO_4$ was removed by filtration and the solvent was removed via rotary evaporator under reduced pressure. Purification was done by silica gel chromatography column (75% CH_2Cl_2 in hexane to 3% MeOH in CH_2Cl_2) to afford a kind of 3.2g colorless foam. Yield: 74.4%.

^{1}H-NMR ($CDCl_3$): δ 1.0-1.13 (m, 28H, 4 x iPr); 3.05 (t, J = 6.4 Hz, 2H, CH_2CN); 3.09-3.19 (m, 4H, 2 x CH_2); 3.38-3.45 (m, J = 6.4 and 12.8 Hz, 1H, $SeCH_2$); 3.59-3.65 (m, J = 6.4 and 12.8 Hz, 1H, $SeCH_2$); 4.0-4.09 (m, 3H, CH_2 and H-5'); 4.22-4.31 (m, 2H, CH_2); 4.52 (dd, J = 5.2 and 9.2 Hz, 1H, H-3'); 4.57-4.6 and 4.93-4.96 (m, m, 2H, H-5'); 5.78 (s, 1H, H-1'); 6.15 (d, J = 8.0 Hz, 1H, H-5); 6.20 (d, J = 5.2 Hz, 1H, H-2'); 7.85 (d, J = 8.0 Hz, 1H, H-6) ppm. ^{13}C-NMR ($CDCl_3$): δ 186.64, 167.13, 156.79, 137.38, 118.58, 110.67, 91.40, 83.46, 82.21, 67.83, 59.27, 51.72, 51.29, 48.78, 44.11, 24.36, 18.89, 17.40, 17.32, 17.22, 17.16, 16.91, 16.86, 16.78, 16.66, 13.36, 13.00, 12.87, 12.18 ppm. HRMS (ESI-TOF), $C_{29}H_{48}N_4O_8S_2SeSi_2$, $[M+Na^+]^+$= 803.1515 (calc. 803.1515).

2.1.7 2'-O-(1,1-Dioxo-1λ⁶-thiomorpholine-4-carbothioate)-5'-O-(4,4'-Dimethoxytrityl)-2 cyanoethylselanyluridine

The TC-modified nucleoside **7** (3.0 g, 3.8 mmol) was dissolved in dry 2-methyltertrafuran (30.0 mL) with 2 mL of anhydrous pyridine and the mixture was set in ice bath. To this mixture, 70% HF/pyridine (6.0 mL, 3 eq) was added dropwise with stirring. After addition, the reaction was allowed to proceed at room temperature until a colorless solid precipitated appeared and the reaction was monitored by TLC (10% MeOH in CH_2Cl_2, Rf=0.5). Then the reaction was quenched by 15 ml saturated sodium bicarbonate, the mixture was isolated by separatory funnel, the aqueous phase was extracted by 2-methyltertrafuran (10 ml*4), then the organic collections were combined and dried over anhydrous $MgSO_4$ and $MgSO_4$ was removed by filtration, the solvent was removed by evaporation under reduced pressure. The crude was purified with silica gel column (5% MeOH in CH_2Cl_2 to 8% MeOH in CH_2Cl_2). The collections were combined and dried by evaporation to afford a kind of white solid. The product was then combined with dry DMT-Cl (1.4 g, 1.1 eq) in a 100 ml round bottom flask, the mixture was dried under high vacuum oil pump overnight, then anhydrous pyridine (20.0 ml) was injected at 0°C with stirring. After addition, the reaction was left at r.t overnight. The reaction was monitored by TLC (5% MeOH in CH_2Cl_2, Rf=0.5). After completion, saturated $NaHCO_3$ (10 mL) was added with stirring, CH_2Cl_2 (20 ml*3) was used for extraction, the organic collections were combined and washed with brine (10 ml*3), then the organic layer was dried over anhydrous $MgSO_4$. The $MgSO_4$ was removed by filtration and the solvent was removed by evaporation under reduced pressure, keeping the water bath temperature lower than 30°C. The crude DMTr-nucleoside **8** was purified by pre-equilibrated (1% N, N-Dimethylethylamine in 50% CH_2Cl_2/Hexane) silica gel chromatography column (75% CH_2Cl_2 in hexane to 2% MeOH in CH_2Cl_2 containing 1% of pyridine). 1.3 g a kind of colorless foam was obtained. Yield: 39.8% over two steps.

^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.00 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 7.4 Hz, 2H), 7.40 – 7.33 (m, 7H), 6.91 (d, J = 2.5 Hz, 2H), 6.88 (d, J = 2.5 Hz, 2H), 6.03 (s, 2H), 5.71 (d, J = 7.8 Hz, 1H), 5.00 (s, 1H), 4.79 (d, J = 13.5 Hz, 1H), 4.55 (d, J = 13.3 Hz, 1H), 4.46 – 4.39 (m, 1H), 4.32 (d, J = 2.1 Hz, 1H), 4.26 – 4.18 (m, 1H), 3.81 (s, 6H), 3.58 (dd, J = 11.0, 2.1 Hz, 1H), 3.49 – 3.42 (m, 3H), 3.35 – 3.10 (m, 5H), 3.03 (dt, J = 9.9, 6.8 Hz, 2H).

^{13}C-NMR ($CDCl_3$): δ 186.56, 167.54, 158.90, 157.49, 144.07, 139.34, 135.08, 134.68, 130.18, 128.14, 128.12, 127.19, 118.86, 113.36, 110.24, 90.42, 87.54, 86.05, 82.78, 69.93, 63.15, 55.27, 51.59, 51.12, 48.91, 44.28, 24.24, 18.63 ppm. HRMS (ESI-TOF), $C_{38}H_{40}N_4O_9S_2Se$, $[M+H^+]^+$=841.1480 (calc. 840.1410).

2.1.8 2'-O-(1,1-Dioxo-1λ⁶-thiomorpholine-4-carbothioate)-5'-O-(4,4'-Dimethoxytrityl)-2-cyanoethylselanyluridine-3'-O-(β-cyanoethyl)-N,N-diisopropylphosphoramidite

The starting material **8** (0.3 g, 0.3 mmol) was placed in a 25 ml round bottom flask and dried on high vacuum overnight. Dry CH_2Cl_2 (5 mL), 4-Methylmorpholine (0.3 ml, 1.8 mmol) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite(100µL,1.5eq) were then added sequentially. The reaction mixture was stirred at room temperature under argon for 2h and monitored by TLC (5% MeOH in CH_2Cl_2, Rf=0.6). The reaction mixture was then quenched with saturated $NaHCO_3$ (10 ml) and extracted with CH_2Cl_2 (10 ml*2). The combined organic layers were dried over anhydrous $MgSO_4$ followed by filtration and solvent was removed by evaporation. The resulting mixture was purified on pre-equilibrated (10% ethyl acetate in hexane with 1% N,N-Dimethylethylamine) silica gel chromatography column. The phosphoramidite **9** was eluted using a gradient of EtOAc from 10% to 50% and evaporated to a colorless foam. Yield: 0.27 g of the target product was obtained as colorless foam. Yield:72.1%. HRMS (ESI-TOF), $C_{47}H_{57}N_6O_{10}PS_2Se$, $[M+H^+]^+$= 1041.2552 (calc. 1041.2558).

2.2 Oligonucleotide solid-phase synthesis and purification

2.2.1 Oligonucleotide solid-phase synthesis

The stoichiometry of native TC RNA Phosphoramidite followed the TC RNA Phosphoramidite User Guide from Sigma Aldrich.

Table 1 SAFC recommends TC Amidite Diluent for solution up to 0.1M concentration (from Sigma Aldrich)

a. TC A	*9.5mL/g*
b. TC C	*10.4mL/g*
c. TC G	*9.7mL/g*
d. TC U	*10.8mL/g*

The freshly made phosphoramidite **9** (0.12 g) was dried under high vacuum at r.t overnight, the following sequences were synthesized by 3400 DNA synthesizer.

Table 2. Designed sequences

No.	Sequences
1	5'-CCG GCA GCC U-3' (native inner)
2	5'-CCG GAG GAA CUA CUG-3' (native outer)
4	5'-CCG GAG GAA C^{2Se}UA CUG-3' (Se-modified outer)
5	5'-CCG GAG GAA CUA C^{2Se}UG-3' (Se-modified outer)
6	5'-CCG GAG GAA C^{2Se}UA C^{2Se}UG-3' (Se-modified outer)

2.2.2 Deprotection of the oligonucleotide using anhydrous ethylenediamine/toluene under conditions.

Table 3. Screening of the optimal conditions for the single step deprotection

Percentage of Ethylenediamine(in volume) r.t 4 h	
100% dry Ethylenediamine	50% dry Ethylenediamine with 50%anhydrous toluene is the optlmal cosolvent
75% dry Ethylenediamine with 25%anhydrous toluene	
50% dry Ethylenediamine with 50%anhydrous toluene	
25% dry Ethylenediamine with 75%anhydrous toluene	

By screening different anhydrous ethylenediamine/toluene conditions, we found that the 1:1 ratio of ethylenediamine/toluene (v:v) single step deprotection was the optimal one which selenium atom survived in maximum, we hypothesize that the aromatic toluene contributed to the stability of the aromatic base ring.

After all the protecting groups were removed and the samples were desalted, the samples were analyzed and purified by HPLC under the following conditions.

Table 4. Analysis HPLC conditions

DMTr-On	Time	B	flow	Max. Press
1	0 min	5%	1.0	400
2	8 min	60%	1.0	400
3	12 min	100%	1.0	400
4	20 min	100%	1.0	400
DMTr-Off	Time	B	flow	Max. Press
1	0 min	5%	1.0	400
2	15 min	40%	1.0	400
3	17 min	100%	1.0	400
4	20 min	100%	1.0	400

Buffers: A. 20 mM TEA-AC in water solution (2L) B. 20 mM TEA-AC in Water/Acetonitrile (1:1 in volume, 2L in total) C. 80% Methanol in water solution (2L)

Table 5. Pre-HPLC conditions

DMTr-On	Time	B	flow	Max. Press
1	0 min	5%	8.0	500
2	10 min	60%	8.0	500
3	15 min	100%	8.0	500
4	30 min	100%	8.0	500
DMTr-Off	Time	B	flow	Max. Press
1	0 min	5%	8.0	500
2	20 min	40%	8.0	500
3	30 min	100%	8.0	500

Buffers: A. 20 mM TEA-AC in water solution (2L) B. 20 mM TEA-AC in Water/Acetonitrile (1:1 in volume, 2L in total) C. 80% Methanol in water solution (2L)

2.3 Self-assembling ^{2Se}U-RNA squares

RNA and Buffer Preparation:

Take each of the TOF-MS determined RNA samples (25 uM)

Buffer: 10 mM sodium cacodylate buffer, 5 mM $MgCl_2$, pH 6.5.

For the single strand: 4 uL of each diluted sample

1 uL 10X Sodium cacodylate/$MgCl_2$ as buffer for each

5 uL DI water

For the assembling: 4 uL of diluted sample 2, 4, 5, 6 (outer strand)

4 uL of diluted sample 1 (inner strand)

1 uL 10X Sodium cacodylate/$MgCl_2$ for each

1 uL DI water

2.3 Gel Electrophoresis

Self-assembling RNA squares were analyzed on 13% native polyacrylamide gel in 40 mM MOPS buffer and 2.5 m $MgCl_2$ under power 300.0 V for 15 minutes. Visualization was performed under UV after ethidium bromide staining.

3 RESULTS

The desire for an efficient ^{2Se}U nucleoside monomer synthesis method inspired us to utilize the streamlined 2'-O-Thiomopholine-4-carbothioate protecting method, and all the analysis of compound 8 turned out to be positive as shown which reveals what we have synthesized is the desired product.

3.1 ^{1}H-NMR and ^{13}C-NMR analysis of compound 8

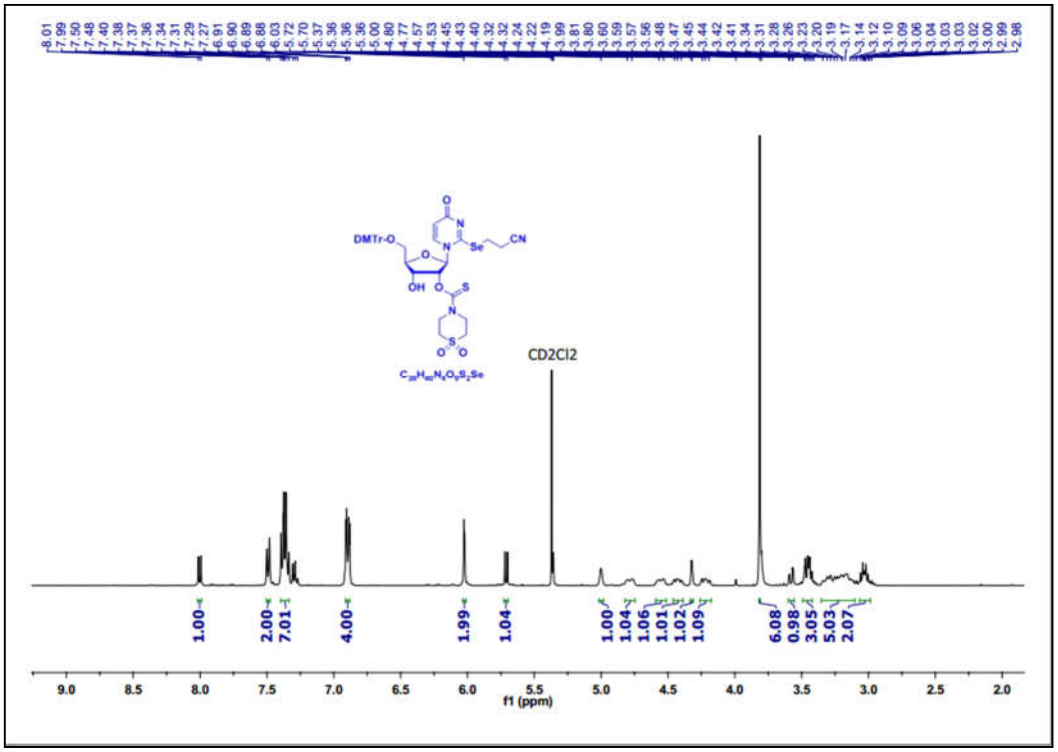


Figure 6. The 1H-NMR of compound 8

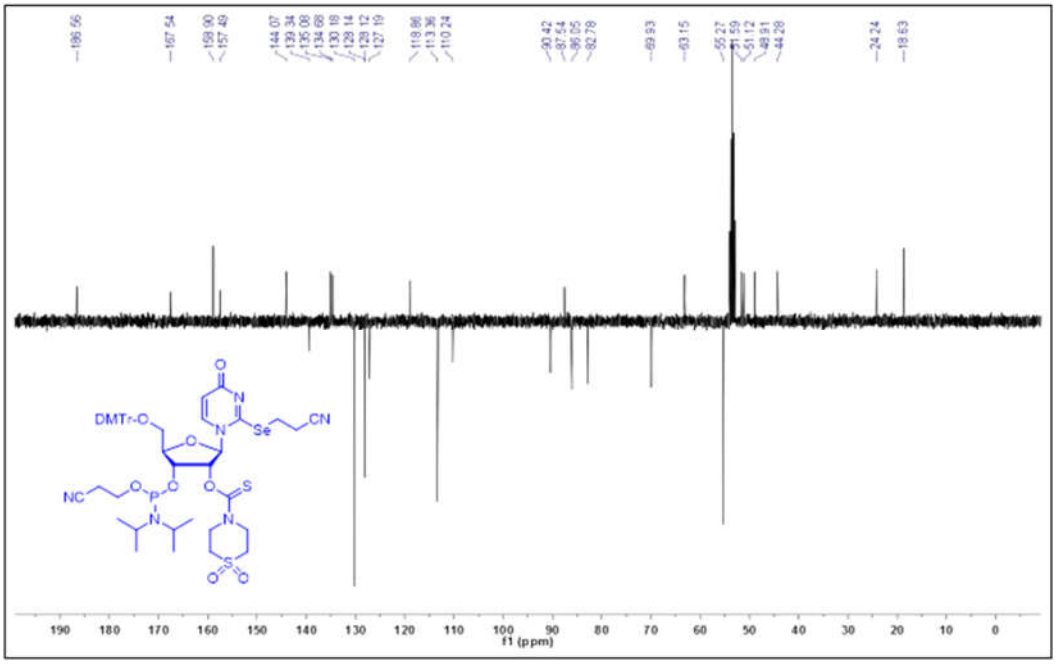


Figure 7. The 13C-NMR of compound 8

3.2 Mass analysis of compound 8

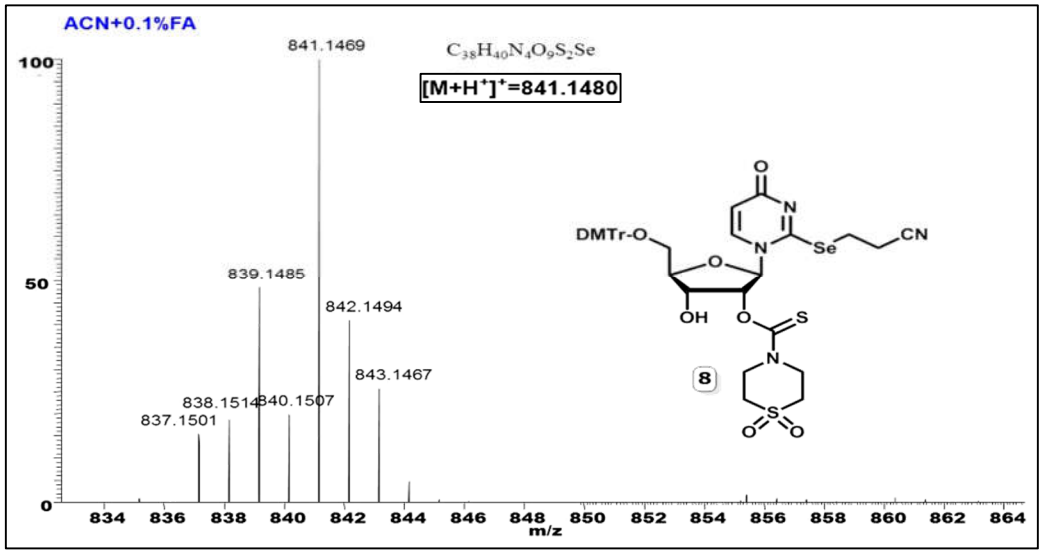


Figure 8. The mass analysis of compound 8

3.3 HPLC analysis of the DMTr-on oligonucleotide

The HPLC analysis (Figure 9 and Figure 10) and TOF-MS analysis (Table.4) indicated that selenium atom survived through the processes.

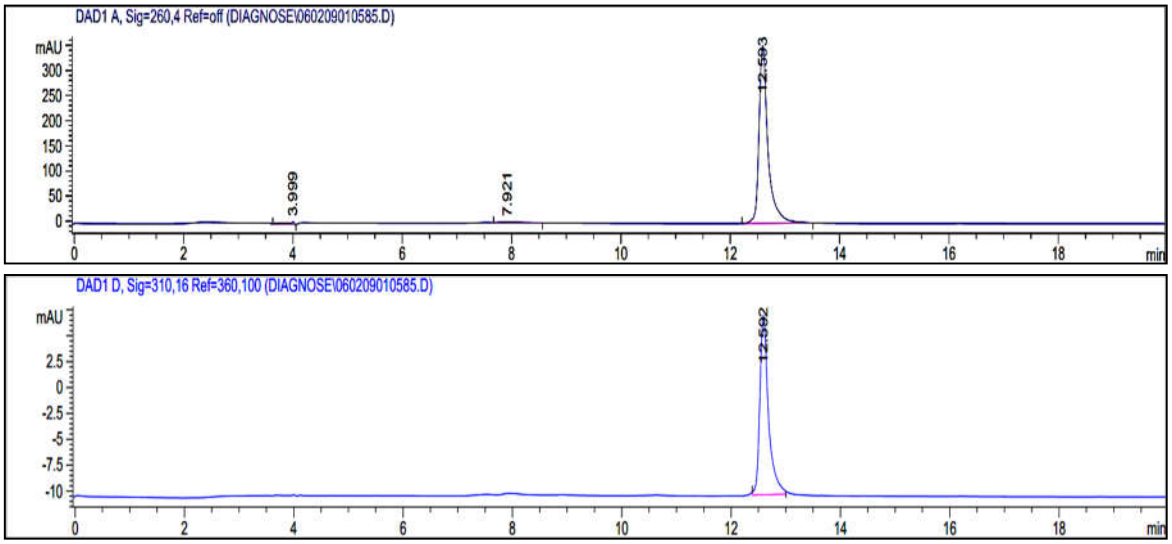


Figure 9. HPLC analysis of DMTr-on oligonucleotide

3.4 HPLC analysis of the DMTr-off oligonucleotide

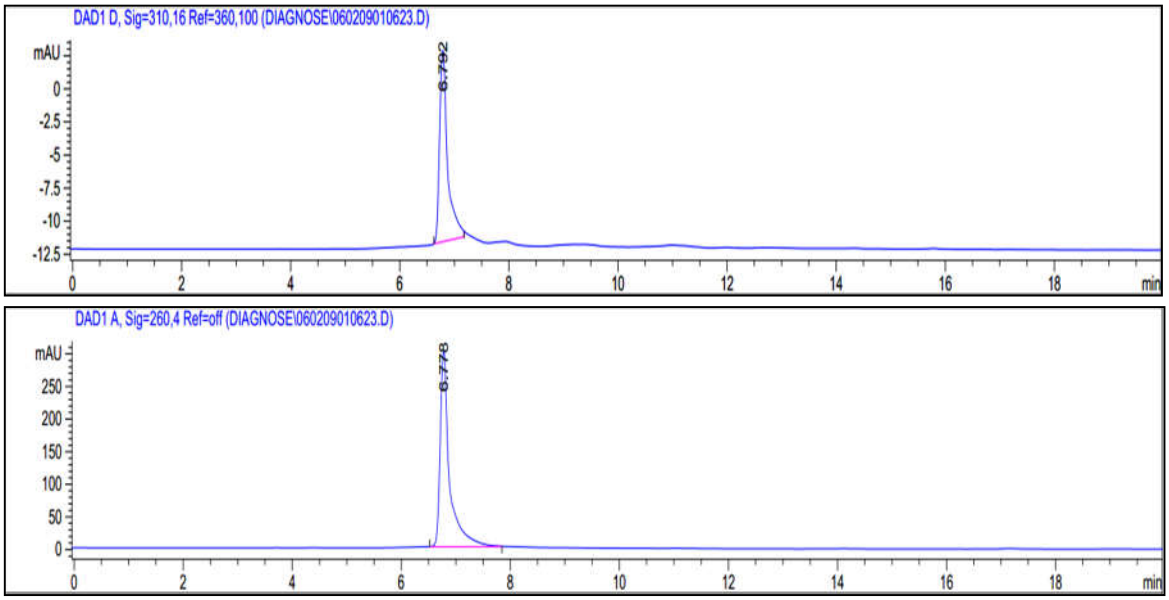


Figure 10. HPLC analysis of DMTr-off oligonucleotide

3.5 TOF-MS analysis of desired sequences

Table 6. TOF-MS analysis of desired sequences

Sequences	Calculated M/Z	Measured M/Z $[M+H^+]^+$
5'-CCG GCA GCC U-3' ($C_{94}H_{111}N_{37}O_{68}P_9$)	3134.9	3135.8
5'-CCG GAG GAA CUA CUG-3'($C_{144}H_{165}N_{61}O_{101}P_{14}$)	4813.9	4814.1
5'-CCG GAG GAA C^{2Se}UA CUG-3'($C_{144}H_{165}N_{61}O_{100}P_{14}Se$)	4877.0	4878.4
5'-CCG GAG GAA CUA C^{2Se}UG-3' ($C_{144}H_{165}N_{61}O_{100}P_{14}Se$)	4877.0	4878.3
5'-CCG GAG GAA C^{2Se}UA C^{2Se}UG-3' ($C_{144}H_{165}N_{61}O_{99}P_{14}Se_2$)	4940.1	4941.0

3.6 Native polyacrylamide gel electrophoresis of Self-assembling RNA square

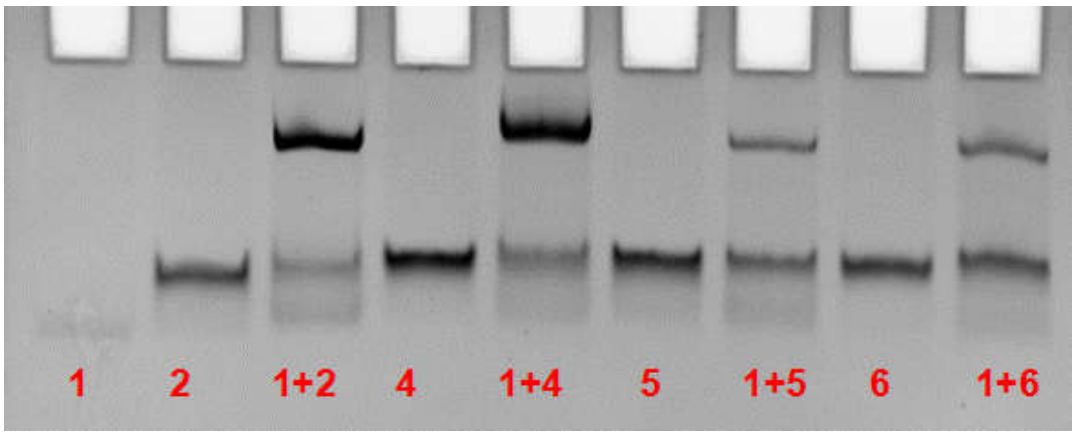


Figure 11. Native polyacrylamide gel electrophoresis of single strand and square RNA

4 CONCLUSIONS

The streamlined process using 2'-O-Thiomopholine-4-carbothioate protecting group provides rational strategies for the chemical synthesis of ^{2Se}U phosphoramidite, these products were comparable or superior to the products produced from TBDMS, and it's promising for the rapid and simplified synthesis of other selenium modified ribonucleoside phosphoramidites, which is crucial for the construction of high-qualified RNA sequences. By using ethylenediamine/toluene (v:v=1:1 at r.t) for the cleavage and deprotection of Selenium modified oligonucleotides, it's much more convenient, and the incorporated Se atom could survive well.

Self-assembling of ^{2Se}U-RNA squares can be obtained which offers a potential strategy for further structure studies and nanomaterial application development.

REFERENCES

1. Jeffrey R. Sampson; Marvin H. Caruthers. *J. Am. Chem. Soc. 2011, 133, 11540– 11556.*

2. Jiansheng Jiang; Jia Sheng; Nicolas Carrasco; Zhen Huang. *Nucleic Acids Research, 2007, Vol. 35, No. 2 477–485.*

3. Nicolas Carrasco; Yuri Buzin; Elizabeth Tyson; Eliezer Halpert; Zhen Huang. *Nucleic Acids Research, 2004, 32, 1638-1646.*

4. Huiyan Sun; Jia Sheng; Abdalla E. A. Hassan; Sibo Jiang; Jianhua Gan; Zhen Huang. "*Novel RNA base pair with higher specificity using single selenium atom", Nucleic Acids Research, 2012, Vol. 40, No. 11 5171–5179.*

5. Jozef Salon; Guexiong Chen; Yoani Portilla; Markus W. Germann; Zhen Huang. *"Synthesis of a 2'-Se-Uridine Phosphoramidite and Its Incorporation into Oligonucleotides for Structural Study",Org Lett. 2005 December 8; 7(25): 5645–5648.*

6. Lina Lin; Jia Sheng; Zhen Huang. *Chem.Soc.Rev. 2011, 40, 4591–4602.*

7. Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc. 1981,103, 3185–3191*.

8. Beaucage, S. L.; Iyer, R. P. *Tetrahedron 1992, 48, 2223–2311.*

9. Lackey, J. G.; Mitra, D.; Somoza, M. M.; Cerrina, F.; Damha, M. J. *J. Am. Chem. Soc. 2009, 131, 8496–8502.*

10. W.T Markiewicz; M. Wiewiórowski. *Nucl.Acids Res. (1978) 1 (suppl1): s185-s189. Doi: 10.1093/nar/1.suppl_1. s18*

11. Holbrook SR, Cheong C, Tinoco I, Jr, Kim SH (1991) *Crystal structure of an RNA double helix incorporating a track of non-Watson-Crick base pairs. Nature 353:579–581.*