Selenium modification of nucleic acids: preparation of oligonucleotides with incorporated 2′-SeMe-uridine for crystallographic phasing of nucleic acid structures

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Published online 22 March 2007; doi:10.1038/nprot.2007.75

This protocol describes a method for introducing an anomalously scattering atom into oligonucleotides at the 2′-position of uridine by conventional solid-phase synthesis. The 2′-SeMe ribose modification is particularly attractive for derivatization of RNA to facilitate crystal structure determination. The estimated time for the synthesis and HPLC purification of oligonucleotides with incorporated 2′-SeMe-uridine residues is approximately 46 h for 'trityl on' and approximately 32 h for 'trityl off' methods, respectively.

INTRODUCTION

Selenium has been widely used for derivatization of proteins in the form of selenomethionine (Se-Met)[1] that can be readily incorporated into proteins in place of Met[2,3]. The presence of selenium allows crystallographic phasing via multi- or single-wavelength anomalous dispersion techniques (MAD or SAD, respectively). A few years ago, Huang, Egli and co-workers reported the incorporation of selenium at the C2′-position of 2′-deoxyuridine (2′-SeMe-U) using a chemical synthetic approach which is fully compatible with automated oligonucleotide synthesis[4]. This protocol describes the synthesis of 2′-SeMe-U phosphoramidite (**Fig. 1**) and its incorporation into a DNA decamer sequence, whose X-ray structure was subsequently determined using the MAD technique[5]. Substitution at the C2′-position of the ribose typically locks the sugar in a C3′-*endo* conformation, thereby resulting in an A-form geometry of the duplex. Therefore, this approach is best suited for derivatization of A-DNAs and RNA[5]. (For a more general method for introducing selenium into DNA and nucleic acid analogs that is expected not to influence the backbone conformation, see the phosphoroselenoate (PSe) derivatization in this issue of *Nature Protocols*[6]). In addition to 2′-SeMe-U[4,5], syntheses for 2′-SeMe-C[7,8], -A[9] and -G[10] phosphoramidites have also been achieved. Ligation of chemically synthesized oligonucleotides bearing 2′-SeMe-modified residues with longer RNAs obtained through *in vitro* transcription has been used to produce selenoated RNAs of up to 100 residues length[8,9]. Recently, structure determination of a new RNA binding motif has been reported based on 2′-SeMe-U modified RNAs[11]. Readers are advised to also consult the protocol with a focus on the preparation of 2′-SeMe-containing RNAs published by Micura *et al.*[12]

Here, we outline the protocol for derivatizing oligonucleotides with a 2′-SeMe-modified residue, taking 2′-SeMe-U as an example. However, neither the syntheses of 2′-SeMe substituted phosphoramidites nor the oligonucleotide syntheses using the 2′-SeMe-C, -G, or -A phosphoramidites, are discussed; note as well that, throughout this protocol, the term oligonucleotides refers to oligodeoxynucleotides. For the generation of longer selenoated nucleic acids based on enzymatic methods, see refs. 8 and 10.

MATERIALS

REAGENTS

- 2′-SeMe-U phosphoramidite can be synthesized by following the reported procedures[4,8]. On the other hand, this material is available commercially and can be purchased from Glen Research Corporation, VA. *Note:* For syntheses of 2′-SeMe-C phosphoramidite, refer to the works by Carrasco *et al.*[7] and Höbartner *et al.*[8]; for that of 2′-SeMe-A and -G, refer to the recent works by Höbartner *et al.*[9] and Moroder *et al.*[10], respectively; for enzymatic production of 2′-SeMe-modified RNAs, see refs. 8 and 9
- Ammonium hydroxide, 30% (NH_4OH; J.T. Baker, cat. no. 9733)
- Drierite absorbents (Fisher Scientific, cat. no. 07-578-3A)
- Phosphorous pentoxide (P_2O_5; Acros; Fisher Scientific, cat. no. AC20089) **! CAUTION** Wear gloves and safety glasses while handling
- Calcium hydride (CaH_2; Acros; Fisher Scientific, cat. no. AC19996) **! CAUTION** Wear gloves and safety glasses while handling
- Calcium chloride ($CaCl_2$; Acros; Fisher Scientific, cat. no. AC30038)
- Acetonitrile, OmniSolv (CH_3CN; EMD Chemicals Inc., cat. no. AX0142-1) **! CAUTION** Wear gloves and safety glasses while handling
- De-blocking or detritylation reagent: 3% dichloroacetic acid/dichloromethane (Chem Genes Corp., MA, cat. no. RN-1468) **! CAUTION** Wear gloves and safety glasses while handling this reagent; follow this caution while handling all DNA synthesis reagents
- Activation reagent: 0.25 M 5-ethylthio tetrazole/CH_3CN (Chem Genes Corp., cat. no. RN-1466)
- Capping solution, CAP A: acetic anhydride/pyridine/tetrahydrofuran (THF) (Chem Genes Corp., cat. no. RN-1458)
- Capping solution, CAP B: 10% *N*-methylimidazole/THF (Chem Genes Corp., cat. no. RN-1481)
- Oxidation solution: 0.02 M iodine/pyridine/H_2O/THF (Chem Genes Corp., cat. no. RN-1455)
- Deoxyadenosine-*N*-benzoyl 2-cyanoethyl-*N*, *N*-diisopropyl (CED) phosphoramidite (Chem Genes Corp., cat. no. ANP-5551)
- Deoxycytidine-*N*-benzoyl CED phosphoramidite (Chem Genes Corp., cat. no. ANP-5552)
- Deoxyguanosine-*N*-isobutyrl CED phosphoramidite (Chem Genes Corp., cat. no. ANP-5553)
- Thymidine CED phosphoramidite (Chem Genes Corp., cat. no. ANP-5554)
- Deoxyguanosine-*N*-isobutyrl long-chain alkyl amino (lcaa) control pore glass (CPG) 500 Å (Chem Genes Corp., cat. no. N-5103-05)
- Trifluoroacetic acid (TFA; Sigma-Aldrich, cat. no. T62200) **! CAUTION** Toxic and highly corrosive. Wear gloves and safety glasses while handling
- Triethylamine (TEA; Sigma-Aldrich, cat. no. T0886)
- Hydrochloric acid solution, 1 N (HCl; EMD Chemicals Inc., cat. no. HX0603D-6)
- Triethylammonium acetate buffer, pH 7.0 (TEAAc; Fluka, cat. no. 90357)
- Molecular sieves 3 Å, 3–5 mm beads (Alfa Aesar, cat. no. L05359) (for drying of molecular sieves, see REAGENT SETUP)
- Molecular sieves 4 Å, 3–5 mm beads (Alfa Aesar, cat. no. L05466)

- Tris (hydroxymethyl) aminomethane (TRIS bone, J.T. Baker, cat. no. 4109)
- Methanol (MeOH; Mallinckrodt Chemicals, cat. no. 3041)
- Tris–HCl buffer (see REAGENT SETUP)

EQUIPMENT

- SpeedVac (Thermo Electron Corp., model no. SC110)
- DNA synthesizer (see EQUIPMENT SETUP)
- Plastic syringes and plastic hypodermic syringes (all polypropylene)
- Screw cap vials (2 ml capacity)
- Teflon tape
- pH paper (ColorpHast, pH 5–10; EMD Chemicals Inc., cat. no. M95883)
- Corning sterile filter system (nylon membrane; Fisher Scientific, cat. no. 09-761-8)
- Drying tube (Fisher Scientific, cat. no. 09-215A)
- Muffle furnace (Barnstead International, model no. FB1415M, 100–1,100 °C, 1,450 W)
- HPLC system with a UV detector
- HPLC reverse phase (C18) column (Hypersil ODS column, 4.6 × 250 mm^2; Thermo Electron Corp., cat. no. 30105-254630) (see EQUIPMENT SETUP)
- HPLC column strong anion exchange (SAX) column (Dionex DNAPac PA-100 analytical column, 4 × 250 mm; Dionex Corp., cat. no. 043010) (see EQUIPMENT SETUP)
- Sep-Pak cartridges (Waters Corp., cat. no. WAT051910)

REAGENT SETUP

Drying of Molecular Sieves Molecular sieves 3 and 4 Å, approximately same quantities (approximately 200 g each) are mixed in a beaker and heated at 350 °C in a Muffle furnace for 3 h. Switch off the heating, and when the temperature drops to approximately 200 °C, transfer the beakers into a vacuum desiccator (desiccator containing drying agent, drierite, placed below the desiccator plate), and allow to cool for several hours (typically 3 h or more). The molecular sieves activated in this fashion are active for approximately 2 weeks. **! CAUTION** Care should be taken while handling molecular sieves (search for material safety data sheet for cat. nos L05359 and L05466 at http://www.alfa.com/webapps/ec120w.pgm). Use thermal protection (insulated mittens) while handling the beakers at 200 °C, and wear safety glasses. Using insulated tongs is often helpful, with caution while transferring the beaker from the furnace to the desiccator. The vacuum desiccator is released with argon gas to take out the dried molecular sieves.

Dry CH_3CN should be used throughout the oligonucleotide synthesis. Dry CH_3CN (purity 98%), by distillation over P_2O_5, followed by reflux and distillation over CaH_2 pellets[13]. For the most part, 0.5–1% (wt/vol) P_2O_5 is added to the distilling flask containing CH_3CN to remove the water content. (Excess P_2O_5 should be avoided as it leads to the formation of an orange polymer.) To achieve this, to 3 l CH_3CN, approximately 30–40 g P_2O_5 is added, refluxed for 3–4 h and distilled. To the distillate, approximately 15 g CaH_2 pellets is added and the solvent is then refluxed for 3–4 h, followed by distillation. The dried CH_3CN can be transferred to 1 l bottles and sealed with Teflon tape. Prior to use, dried molecular sieves (mixture of molecular sieves 3 and 4 Å) are added, and the solvent can be used after 2–4 h. Alternatively, CH_3CN with low water content (10 p.p.m. or less) is considered suitable for oligonucleotide synthesis, and the traces of moisture present can be removed using activated molecular sieves. **! CAUTION** Always wear gloves and safety glasses while handling CH_3CN, P_2O_5 and CaH_2. (For P_2O_5 and CaH_2, see material safety data sheets available at https://fscimage.fishersci.com/msds/96386.htm and https://fscimage.fishersci.com/msds/95942.htm, respectively.) Take precaution to exclude atmospheric moisture during the distillation procedure using fresh $CaCl_2$ drying tubes.

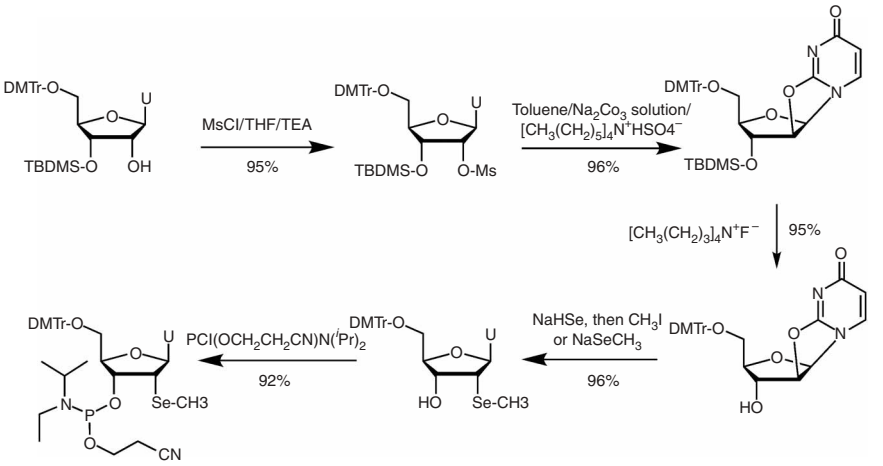

Figure 1 | Synthesis of 2′-SeMe-U phosphoramidite[4,8]. (Ref. 8 reports a four-step synthesis.)

Preparation of Tris–HCl buffer, pH 7.8 Tris buffer (pH 7.8) can be prepared by dissolving 60.57 g Tris base in 300 ml deionized (DI) water, adjusting the pH to 7.8 by adding HCl (1 N) dropwise with stirring, and making up the volume (using DI water) to 500 ml in a volumetric flask. The resulting buffer, which has a concentration of 1 M, is filtered through a Corning sterile filter system with nylon membrane, and stored at 4 °C.

EQUIPMENT SETUP

DNA synthesizer setup for synthesis For oligonucleotide synthesis, it is advised to charge the automated DNA synthesizer with freshly prepared phosphoramidite solutions and reagents. The phosphoramidite solutions can be further protected from moisture by adding the required amount of activated 3 and 4 Å molecular sieves (see Step 1 of the procedure).

In our laboratory, we use an ABI 381A DNA synthesizer and a concentration of 0.1 M for the phosphoramidite solutions. Other reagents used are listed in the MATERIALS section. Syntheses are normally performed on a 1-µmol scale, on CPG supports with 500 Å pore size with the nucleoside immobilized through an lcaa linker. Standard protocols for the synthesis of oligonucleotides are followed for all the steps namely (i) de-blocking, (ii) base condensation or coupling, (iii) capping and (iv) oxidation, following the manufacturer's recommendations except for a slightly longer wait period for the 2′-SeMe-U phosphoramidite coupling, which is 10 min. In accordance with the literature[14], we also found that use of 0.25 M 5-ethylthiotetrazole/CH_3CN gives better coupling efficiencies and overall yields and this reagent was therefore used as the activator.

HPLC setup for oligonucleotide purification (reversed phase HPLC purification) 5′-O-dimethoxytrityl (DMTr) oligonucleotides can be purified by reversed phase HPLC (C-18). Buffer A: 50 mM TEAAc, pH 7.0; buffer B: 50 mM TEAAc, 60% CH_3CN, pH 7.0 (filter the buffers through a Corning sterile filter system with nylon membrane, before use). Turn the HPLC on and equilibrate the column with 35 ml buffer A with a flow rate of 1 ml min^{-1} (approximately 8 column volumes). The UV lamp is also switched on, allowed to stabilize for 10 min and auto-zeroed before sample injection.

HPLC setup for oligonucleotide purification (anion exchange HPLC purification) Buffer A: 25 mM Tris–HCl (pH 7.8); buffer B: 25 mM Tris–HCl and 1.0 M NaCl (pH 7.8) (filter the buffers through a Corning sterile filter system with nylon membrane, before use). The initial setup and HPLC column equilibration (Dionex DNAPac PA-100 analytical column used for anion exchange HPLC) are similar to those described above for reverse phase purification, by equilibrating the anion exchange HPLC column with 30 ml buffer A (approximately 10 column volumes).

PROCEDURE

1| To 50 µmol 2′-SeMe-U phosphoramidite (41 mg), add 0.55 ml dry CH_3CN, and dried 3 and 4 Å molecular sieves (approximately 10–20 beads). The phosphoramidite is placed at position X (X-phosphoramidite position) on the machine. **▲ CRITICAL STEP** The oxidizing reagent used for the phosphite oxidation is 0.02 M iodine/pyridine/H_2O/THF [20 s (delivery time), 50 s (wait time)], under which conditions the 2′-SeMe function was found to be stable. It is worth noting the use of DL-DTT

(a reducing agent) for the synthesis of longer RNAs containing incorporated 2′-SeMe-modified residues[9]. A more elaborate description of the use of DTT for the synthesis of long 2′-SeMe-modified RNAs can be found elsewhere[9].

2| The oligonucleotide synthesis can be performed by making use of the trityl 'on' option, Method A below, where the 5′-O-DMTr group is left 'on' during the synthesis, or choosing the trityl 'off' option, Method B below, where the 5′-O-DMTr group is left 'off' during the synthesis.

▲ **CRITICAL STEP** The choice of trityl 'on' or 'off' mainly depends on the sequence. The synthesized oligonucleotide may have higher order structures (i.e., purine-rich sequences; multiple or broad peaks that are observed in an initial analytical HPLC chromatogram), which may interfere in the HPLC purification step. In such cases, a HPLC purification at elevated temperature (e.g., 40–80 °C range) is often the method of choice, as high temperatures will help disrupt these structures. Alternatively, one can carry out a trityl 'on' synthesis, separate the full-length, 5′-O-DMTr bearing oligonucleotide (at 254 nm) using a reversed phase HPLC purification [see below, Method 2A(vi–vii)] and carry out the detritylation of the DMTr group. Please note that trityl 'on' oligonucleotides should be purified with care as some detritylation of the product may occur, resulting in low yield of purified oligonucleotides. It is important to dissolve the oligonucleotide in aqueous buffer between pH 7.1 and 7.5 before and during trityl 'on' reversed phase HPLC purification. The HPLC purified 5′-O-DMTr bearing oligonucleotide can then be subjected to detritylation. After removing the DMTr-OH using hexanes, followed by anion exchange HPLC purification, one can obtain pure oligonucleotide [Method 2A(xi–xiv)]. Method B with 5′-O-DMTr 'off' (5′-OH) is appropriate for short oligonucleotide sequences (12 mer or shorter).

(A) Method A

(i) Synthesize the oligonucleotide with incorporated 2′-SeMe-U residue, with the trityl 'on' option (i.e., synthesize a 5′-O-DMTr oligonucleotide).

(ii) Take out the column (in this case, the solid support bears oligonucleotide with 5′-O-DMTr) from the synthesizer and transfer the solid support (CPG inside the column) to a 2-ml screw cap vial and add 1 ml NH_4OH. Cap the vial tightly, vortex the solution and keep it on a heating block at 60 °C for 8 h.

(iii) Cool the vial in an ice bath for 30 min.

■ **PAUSE POINT** Unscrew the cap and leave the vial in a fume hood overnight (12–16 h) so that the oligonucleotide solution becomes free of ammonia.

(iv) Spin the vial in a centrifuge at 4,000 r.p.m. (1,306*g*, 3 min) at room temperature (25–27 °C, throughout the procedure), pipette out the supernatant to a 2-ml Eppendorf tube and lyophilize the sample to dryness. Resuspend the oligonucleotide in 200 µl DI water, remove a 5 µl aliquot from the suspension and dilute it with 995 µl DI water. Measure the UV absorbance at 260 nm, and estimate the concentration of the oligonucleotide solution. Concentration of the oligonucleotide solution can be expressed as UV OD at a wavelength of 260 nm. The total OD is calculated using the formula

$$\mathrm{OD} = A_{260} \times d \times V,$$

where OD is total optical density, A_{260} is the UV absorbance at 260 nm, d is the dilution factor (in this case, 1,000/5 = 200) and V is the volume in milliliters in which the oligonucleotide was resuspended (in this case, 0.20).

(v) Switch on the HPLC, attach the reverse-phase column (4.6 mm × 250 mm, Hypersil ODS column) and equilibrate the column with buffer A (buffer A = 50 mM TEAAc, pH 7.0).

▲ **CRITICAL STEP** The HPLC purification is carried out on a C-18 column (reversed phase HPLC purification).

(vi) Based on the concentration estimate in Step 2A(iv), inject a measured volume containing 15 OD of oligonucleotide into the column and subject it to a gradient run. Typically use a gradient run of 0–75% buffer B (buffer A: 50 mM TEAAc, pH 7.0; buffer B: 50 mM TEAAc, 60% CH_3CN, pH 7.0) in 60 min with a 1 ml min^{-1} flow rate (approximately 15 column volumes). Monitor the UV absorbance continuously at 260 and 280 nm. Depending on the length of oligonucleotide, the gradient run needs to be altered by increasing or decreasing the percentage of buffer B to standardize the HPLC run.

? TROUBLESHOOTING

(vii) Once the HPLC run is standardized, repeat the HPLC purifications and collect the fractions containing the 5′-O-DMTr oligonucleotide.

(viii) Pool the fractions containing the 5′-O-DMTr oligonucleotide and lyophilize in a SpeedVac. The removal of volatile TEAAc is achieved by multiple evaporations with water (3 × 1 ml).

(ix) Dissolve the oligonucleotide in 800 µl DI water, and add 200 µl 10% (vol/vol) trifluoroacetic acid (TFA). After 5 min, add 50 µl triethylamine (TEA) and test the pH of the solution using a pH paper (make sure it is basic, pH greater than 8).

! CAUTION TFA is toxic and highly corrosive. Wear gloves and safety glasses while handling TFA.

(x) Transfer the solution from Step 2A(ix) into a separatory funnel, and extract the DMTr-OH from the aqueous phase using hexanes (3 × 5 ml). Discard the hexanes layer (upper layer), and lyophilize the aqueous layer to dryness. Dissolve the resulting dry oligonucleotide in 1 ml DI water, and estimate its concentration as in Step 2A(iv).

(xi) Switch on the HPLC, attach the anion exchange column (4 mm × 250 mm, Dionex, DNAPac PA-100) and equilibrate the column with buffer A (25 mM Tris–HCl, pH 7.8).

▲ CRITICAL STEP The HPLC purification is carried out on a strong anion-exchange column (ion-exchange HPLC purification).

(xii) Depending on the concentration [Step 2A(x)], a measured volume containing 15 OD oligonucleotide is injected into the column, and subjected to a gradient run. Typically a gradient run of 0–60% buffer B (buffer A: 25 mM Tris–HCl, pH 7.8; buffer B: 25 mM Tris–HCl, 1.0 M NaCl, pH 7.8) in 60 min with a 1 ml min^{-1} flow rate (approximately 20 column volumes) is used. The UV absorbance is monitored continuously at 260 and 280 nm.

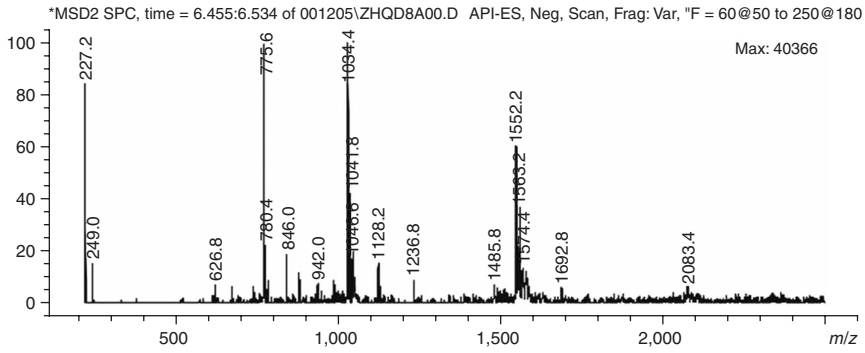

Figure 2 | Electrospray mass spectrum of 5′-GCGTA 2′-SeMe-U ACGC-3′, $C_{97}H_{123}N_{38}O_{58}P_9Se$, molecular mass = 3106.98 (including all isotopes). Measured (expected) M/e: $[M-2H^+]^{2-}$ = 1552.2 (1552.5); $[M-3H^+]^{3-}$ = 1034.4 (1034.7); $[M-4H^+]^{4-}$ = 775.6 (775.8). Reproduced with permission from the American Chemical Society (ref. 4, supplementary information).

(xiii) After standardizing the HPLC run, repeat the HPLC purifications and pool the fractions containing the 5′-OH oligonucleotide.

(xiv) Oligonucleotide purified using HPLC can be desalted on Sep-Pak C-18 cartridges (Waters Inc.). Attach the cartridge to a 10-ml syringe with a three-way Leur-lock in between. Flush the cartridge with 10 ml CH_3CN, and then with 10 ml DI water. Apply the oligonucleotide solution to the cartridge, and remove the salts by washing with 10 ml DI water. Elute the desalted oligonucleotide (which is now bound to the C-18 cartridge) with 5 ml 60% (vol/vol) MeOH in water and collect it in 2-ml Eppendorf tubes.

(xv) Lyophilize the fractions in a SpeedVac and resuspend in a measured amount of DI water for estimation of concentration, characterization (mass spectrometry), followed by crystallization experiments.

▲ CRITICAL STEP The oligonucleotides bearing 2′-SeMe-U need to be stored in the dark (e.g., wrapped in aluminum foil).

(B) Method B

(i) Synthesize the oligonucleotide with incorporated 2′-SeMe-U residue, with the trityl 'off' option (i.e., synthesize a 5′-OH oligonucleotide).

(ii) Follow Step 2A(ii–iv) with the solid support to obtain crude oligonucleotide (in this case, the solid support bears oligonucleotide with 5′-OH), and estimate the concentration of the oligonucleotide.

(iii) Follow Step 2A(xi–xiii) to get purified fractions containing the 5′-OH oligonucleotide.

? TROUBLESHOOTING

(iv) Desalting and lyophilization are achieved by following Step 2A(xiv–xv).

● TIMING

Step 1: 10 min; Step 2A(i): Approximately 15 min nt^{-1} (nucleotide denoted as 'nt'); Step 2A(ii): 8 h; Step 2A(iii): 12 h; Step 2A(iv): 10 min; Step 2A(v): 1 h; Step 2A(vi–vii): 1 h/HPLC run (5 h); Step 2A(viii): 8 h; Step 2A(ix–x): 20 min; Step 2A(xi): 1 h; Step 2A(xii–xiii): 1 h /HPLC run (5 h); Step 2A(xiv): 10 min; Step 2A(xv): 4 h; Step 2B(i): Approximately 15 min nt^{-1}; Step 2B(ii): 21 h; Step 2B(iii): 7 h; Step 2B(iv): 4 h

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 1**.

TABLE 1 | Troubleshooting table.

Step	Problem	Possible reasons	Solution
2A(vi)/2B(iii)	Lots of peaks and uncertainty in deciding the identity of individual oligonucleotide peaks	The reagents are of poor quality, and/or the phosphoramidite solutions are not dry	Repeat the synthesis with freshly prepared phosphoramidite solutions and add dried molecular sieves 3 and 4 Å. Also test the synthesizer for proper flow of reagents (flow test) and do the 'self-test' if available for the machine
2A(vi)/2B(iii)	Presence of many shorter oligonucleotide fragments (*n*–1, *n*–2, etc., where *n* is the length of the oligonucleotide synthesized) in the HPLC profile	Either the phosphoramidite solutions are not dry or the selenium moieties are getting oxidized during the phosphite-phosphate oxidation step by iodine	Try using freshly prepared phosphoramidite solutions, and include an additional step after capping-oxidation step namely the treatment with a chemical reducing agent (reduction step), *threo*-1,4-dimercapto-2,3-butanediol. [DTT (100 mm) in ethanol/H_2O (2/3).] More details of the reduction step are given in ref. 9, and the respective supporting information at http://pubs.acs.org

ANTICIPATED RESULTS

Yields

The yields of 2′-SeMe-U incorporated oligonucleotide syntheses are good for a short oligonucletide (20 mer or less), typically in the range of 30–50%, provided the coupling reactions are efficient.

Mass spectrometry

The oligonucleotides synthesized were characterized by MALDI-TOF or electrospray mass spectrometry (e.g., **Fig. 2**).

ACKNOWLEDGMENTS This work was supported by the U.S. National Institutes of Health (Grant R01 GM55237 to M.E.).

COMPETING INTERESTS STATEMENT The authors declare no competing financial interests.

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1. Hendrickson, W.A. Synchrotron crystallography. *Trends Biochem. Sci.* **25**, 637–643 (2001).
2. Doublié, S. Production of selenomethionyl proteins in prokaryotic and eukaryotic expression systems. in *Macromolecular Crystallography Protocols: Preparation and Crystallization of Macromolecules* Vol. 1 (ed. Doublié, S.) 91–108 (Humana Press, Totowa, New Jersey, 2006).
3. Hendrickson, W.A., Horton, J.R. & LeMaster, D.M. Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure. *EMBO J.* **9**, 1665–1672 (1990).
4. Du, Q. *et al.* Internal derivatization of oligonucleotides with selenium for X-ray crystallography using MAD. *J. Am. Chem. Soc.* **124**, 24–25 (2002).
5. Teplova, M. *et al.* Covalent incorporation of selenium into oligonucleotides for X-ray crystal structure determination via MAD: proof of principle. Multiwave length anomalous dispersion. *Biochimie* **84**, 849–858 (2002).
6. Pallan, P.S. & Egli, M. Selenium modification of nucleic acids: preparation of phosphoroselenoate derivatives for crystallographic phasing of nucleic acid structures. *Nat. Protoc.* **2**, 640–646 (2007).
7. Carrasco, N., Buzin, Y., Tyson, E., Halpert, E. & Huang, Z. Selenium derivatization and crystallization of DNA and RNA oligonucleotides for X-ray crystallography using multiple anomalous dispersion. *Nucleic Acids Res.* **32**, 1638–1646 (2004).
8. Höbartner, C. & Micura, R. Chemical synthesis of selenium-modified oligoribonucleotides and their enzymatic ligation leading to an U6 SnRNA stem-loop segment. *J. Am. Chem. Soc.* **126**, 1141–1149 (2004).
9. Höbartner, C. *et al.* Syntheses of RNAs with up to 100 nucleotides containing site-specific 2′-methylseleno labels for use in X-ray crystallography. *J. Am. Chem. Soc.* **127**, 12035–12045 (2005).
10. Moroder, H., Kreutz, C., Lang, K., Serganov, A. & Micura, R. Synthesis, oxidation behavior, crystallization and structure of 2′-methylseleno guanosine containing RNAs. *J. Am. Chem. Soc.* **128**, 9909–9918 (2006).
11. Serganov, A. *et al.* Structural basis for Diels-Alder ribozyme-catalyzed carbon-carbon bond formation. *Nat. Struct. Mol. Biol.* **12**, 218–224 (2005).
12. Micura, R. *et al.* Preparation of 2(-deoxy-2′-methylseleno-modified RNA. in *Current Protocols in Nucleic Acid Chemistry* 1.15.1–1.15.34 (John Wiley & Sons, Inc., Hoboken, New Jersey, 2006).
13. Sproat, B. *et al.* An efficient method for the isolation and purification of oligoribonucleotides. *Nucleosides & Nucleosides* **14**, 255–273 (1995).
14. Armarego, W.L.F. & Perrin, D.D. (Eds.) *Purification of Laboratory Chemicals* 4th Edn. (Butterworth-Heineman, The Bath Press, Bath, Great Britain, 1996).