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Synthesis of a 2'-Se-Uridine Phosphoramidite and Its Incorporation into Oligonucleotides for Structural Study

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SUPPORTING MATERIAL

General Section

Most solvents and reagents were purchased from Sigma, Fluka, or Aldrich (p.a.) and used without purification unless mentioned otherwise. Triethylamine (TEA) was dried over KOH (s) and distilled under argon. When necessary, solid reagents were dried under high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on Merck 60 F254 plates (0.25 mm thick; Rf values in the text are for the title products), and visualized under UV-light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; Ce(SO₄)₂·4H₂O, 10 g; H₂SO₄, 60 mL, conc.; H₂O, 940 mL) with heating. Preparative TLC was performed using Merck 60 F₂₅₄ pre-coated plates (2 mm thick). Flash chromatography was performed using Fluka silica gel 60 (mesh size 0.040-0.063 mm) using a silica gel:crude compound weight ratio of ca. 30:1. ¹H, ¹³C and ³¹P-NMR spectra were recorded using Bruker-300 or 400 (300 or 400 MHz). All chemical shifts (δ) are in ppm relative to tetramethylsilane and all coupling constants (J) are in Hz. High resolution mass spectrum (HRMS) analysis was performed at Scripps Center for Mass Spectrometry, California.

2'-deoxy-2'-Se-methyl-uridine (2).

Starting material **1** (1.26 g, 2.0 mmol) was placed in a round flask (25 mL) and 80% acetic acid (4 mL) was added to it. The detritylation reaction was monitored by

silica gel TLC (10% MeOH/CH₂CH₂, product $R_f = 0.46$). The reaction was completed in 2 h at room temperature. After a solution of toluene:ethanol (9:1, 10 mL) was added into the flask, the solution was evaporated to approximately 2 mL under reduced pressure. Another portion of toluene:ethanol (9:1, 10 mL) was then added to the flask; the solution was evaporated to approximately 1 mL under reduced pressure. Another portion of toluene:ethanol (9:1, 10 mL) was again added to the flask; the solution was evaporated to approximately 1 mL under reduced pressure. Another portion of toluene:ethanol (9:1, 10 mL) was again added to the flask; the solution was evaporated to the minimum volume under reduced pressure. The crude product was dissolved in methanol (4 mL), and the solution was dropwise added to petroleum ether (100 mL) under vigorous stirring. The precipitate was collected and redissolved in methanol (4 mL), and the solution was reprecipitated by dropwise adding it to petroleum ether (100 mL) under vigorous stirring. The purified compound **2** was collected as a white solid (0.62 g, 96% yield) with satisfied quality.

¹<u>H-NMR</u> (300 MHz, CDCl₃+DMSO- d_6) δ: 2.00 (s, 3H, CH₃Se), 3.46-3.51 (m, 1H, H-2'), 3.75-3.90 (m, 2H, H-5'), 4.04-4.08 (m, 1H, H-4'), 4.34-4.42 (m, 1H, H-3'), 4.76 (t, J = 4.8 Hz, CH₂-OH), 5.11 (br, 1H, OH), 5.67 (d, J = 8.1 Hz, 1H, H-5), 6.25 (d, J = 8.1 Hz, 1H, H-1'), 7.95 (d, J = 8.1 Hz, 1H, H-6), 10.96 (br, 1H, NH).

¹³<u>C-NMR</u> (100 MHz, CDCl₃+DMSO-*d*₆) δ: 8.21 (SeCH₃), 53.10 (C-2'), 67.28 (C-5'), 77.46 (C-3'), 91.48 (C-4'), 94.38 (C-1'), 107.39 (C-5), 145.51 (C-6), 155.66 (C-2), 168.51 (C-4).

HRMS (ESI-TOF): molecular formula, $C_{10}H_{14}N_2O_5Se$; $[M+Na]^+$: 344.995 (calc. 344.996).

5'-O-bis(trimethylsiloxy)benzhydroxysilyl-2'-deoxy-2'-Se-methyl-uridine (3).

2'-Deoxyuridine 2 (483 mg, 1.5 mmol) was placed in a round bottle flask (5 mL) and dried on high vacuum for one hour before addition of dry *N*,*N*-dimethylformamide (3.0 mL) and dry pyridine (403 μ L, 5 mmol). When the starting material was completely dissolved, benzhydroxy-bis(trimethylsilyloxy)silyl chloride (BzH-Cl, 0.98 g liquid, 2.3 mmol) was added dropwise over 30 minutes with stirring at 0°C. The reaction was then stirred under argon at room temperature and was monitored by TLC (1% MeOH/CH₂CH₂, product R_f = 0.40). After the reaction was completed in a few hours, methanol (0.5 mL) was added to quench the reaction. The reaction mixture was added to water (100 mL). The suspension was extracted with ethyl acetate (3x 100 mL). The combined organic layer was dried over anhydrous MgSO₄ for 30 min, and the organic solvent was evaporated under reduced pressure. The crude product was purified on a silica gel column (equilibrated with hexane/ethyl acetate = 8:2) and eluted with hexane/ethyl acetate gradient [8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8] to afford a white form compound **3** (86%, 0.92 g).

¹<u>H-NMR</u> (300 MHz, CDCl₃) δ : 0.06 and 0.07 (2x s, 18H, 2x (CH₃)₃Si), 1.99 (s, 3H, CH₃Se), 2.65 (m, 1H, OH), 3.25 (dd, $J_{2'-3'} = 4.8$ Hz, $J_{2'-1'} = 8.7$ Hz, 1H, H-2'), 3.82 (ddd, $J_{5'-3'} = 1.5$, $J_{5'-4'} = 11.4$ Hz, and $J_{5'-5'} = 11.4$ Hz, 2H, H-5'), 4.09-4.14 (m, 2H, H-3' and H-4'), 5.56 (dd, J = 2.4, 8.1 Hz, 1H, H-5), 5.91 (s, 1H, CH(Ph)₂), 6.16 (d, $J_{1'-2'} = 8.7$ Hz, 1H, H-1'), 7.23-7.32 (m, 10H, 2x C₆H₅), 7.74 (d, J = 8.1 Hz, 1H, H-6), 8.62 (br, 1H, NH).

¹³<u>C-NMR</u> (100 MHz, CDCl₃) δ: 1.56 and 1.59 (2x (CH₃)₃Si), 4.28 (SeCH₃), 50.18 (C-2'), 63.30 (C-5'), 71.96 (C-3'), 77.13 (CH(Ph)₂), 85.09 (C-4'), 87.07 (C-1'), 103.01

(C-5), 126.22, 126.28, 127.52, 127.54, 128.41 (CH of phenyls), 139.60 (C-6), 143.63, 143.67 (C of phenyls), 150.30 (C-2), 162.65 (C-4).

HRMS (ESI-TOF): molecular formula, $C_{29}H_{42}N_2O_8SeSi_3$; $[M+Na]^+$: 733.1306 (calc. 733.1306).

5'-O-bis(trimethylsiloxy)benzhydroxysilyl-2'-deoxy-2'-Se-methyl-uridine 3'-Omethyl*di*isopropylamino phosphoramidite (4).

5-(Benzylthio)-1H-tetrazole (192 mg, 1.0 mmol) and substrate 3 (0.71 g, 1.0 mmol) were placed in a round bottle flask (5 mL) and dried on high vacuum for one hour before injection of dry methylenechloride (2.0)mL), and bis(N.Ndiisopropylamino)methoxy phosphine (POMe, 393 mg liquid, 1.5 mmol). The reaction completion was indicated by TLC (hexanes:ethyl acetate =1:1, product $R_f = 0.51$] after 2 hours. The reaction mixture was then quenched with sodium bicarbonate (0.5 mL, sat.) by stirring for 5 min. After removal of the aqueous phase, the organic layer was dried over anhydrous MgSO₄ before evaporation. The crude product was purified on a silica gel column (equilibrated with hexane and 5% triethylamine) and eluted with an EtOAc/hexanes gradient [EtOAc in hexanes (5, 10, 15 and 20%)], to afford the desire product 4 (766 mg, 88%) as a colorless oil.

¹<u>H-NMR</u> (300 MHz, CDCl₃) δ : 0.06 and 0.08 (4x s, 36H, 4x (CH₃)₃Si), 1.15-1.25 (m, 24H, 8x CH₃-ipr), 1.91 and 1.95 (2x s, 6H, 2x CH₃Se), 3.15 and 3.30 (2x dd, $J_{2'-3'} = 5.4$ Hz, $J_{2'-1'} = 8.7$ Hz, 2H, 2x H-2'), 3.32 and 3.47 (2x d, $J_{CH3-OP} = 13.2$ Hz, 6H, 2x OCH₃), 3.53-3.68 (m, 4H, 4x CH-ipr), 3.74-3.88 (m, 4H, 2x H-5'), 4.21 (m, 2H, 2x H-4'), 4.44 (dd, J = 5.4, 9.6 Hz, 1H, H-3'), 4.53 (ddd, J = 2.1, 5.4, 9.6 Hz, 1H, H-3'), 5.47 and 5.49 (2x d, J = 8.1 Hz, 2H, 2x H-5), 5.91 (s, 2H, 2x CH(Ph)₂), 6.36 and 6.38 (2x d,

 $J_{1'-2'} = 9.0$ Hz, 2H, 2x H-1'), 7.18-7.36 (m, 20H, 4x C₆H₅), 7.69 and 7.73 (2x d, J = 8.1 Hz, 2H, 2x H-6), 8.02 (br, 2H, 2x NH).

¹³<u>C-NMR</u> (100 MHz, CDCl₃) δ: 1.53 and 1.57 (CH₃-Si), 3.44 and 3.68 (SeCH₃), 24.57, 24.65, 24.72, 24.82, 24.90 (CH₃-ipr), 43.04 and 43.17 (O-CH₃), 46.18, 46.25 and 46.95, 47.00, 49.86, 50.04 (CH-ipr), and 51.07, 51.24 (C-2'), 62.84 and 63.06 (C-5'), 74.86 and 75.02 (C-3'), 76.34 and 76.49 (CH(Ph)₂), 84.97 and 85.78 (C-4'), 89.10 and 89.35 (C-1'), 102.81 and 102.97 (C-5), 126.21, 126.30, 127.44, 127.48, 128.37, 128.39 (CH of phenyls), 139.91, 139.95 (C-6), 143.71, 143.75 (C of phenyls), 150.45, 150.47 (C-2), 163.04, 163.07 (C-4).

HRMS (ESI-TOF): molecular formula, $C_{36}H_{58}N_3O_9PSeSi_3$; $[M+H]^+$: 872.2448 (calc. 872.2456).



Figure A. Reversed-phase HPLC analysis of purified DMTr-off 2'-Se-DNA9mer (5'-GAGCU_{Se}CCAT-3'). The sample was analyzed on a Zorbax SB-C18 column (4.6 x 250 mm), eluted (1 mL/min) with a linear gradient from buffer A (20 mM triethylammonium acetate, pH 7.1) to 100% buffer B (50% acetonitrile, 20 mM triethylammonium acetate, pH 7.1) in 30 min. Its retention time is 17.1 min.



Figure B. MALDI-TOF MS analysis of the DMTr-off 2'-Se-RNA11mer (5'-CCUU_{Se}GACAAAG-3'; molecular formula: $C_{106}H_{133}N_{43}O_{72}P_{10}Se$), FW 3550.1; [M-H]: 3549 (calc. 3549.1).