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2′-Methylseleno-modified oligoribonucleotides for X-ray crystallography synthesized by the ACE RNA solid-phase approach

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ABSTRACT

Site-specifically modified 2′-methylseleno RNA represents a valuable derivative for phasing of X-ray crystallographic data. Several successful applications in three-dimensional structure determination of nucleic acids, such as the Diels–Alder ribozyme, have relied on this modification. Here, we introduce synthetic routes to 2′-methylseleno phosphoramidite building blocks of all four standard nucleosides, adenosine, cytidine, guanosine and uridine, that are tailored for 2′-*O*-*bis*(acetoxyethoxy)methyl (ACE) RNA solid-phase synthesis. We additionally report on their incorporation into oligoribonucleotides including deprotection and purification. The methodological expansion of 2′-methylseleno labeling via ACE RNA chemistry is a major step to make *Se*-RNA generally accessible and to receive broad dissemination of the *Se*-approach for crystallographic studies on RNA. Thus far, preparation of 2′-methylseleno-modified oligoribonucleotides has been restricted to the 2′-*O*-[(triisopropylsilyl)oxy]methyl (TOM) and 2′-*O*-*tert*-butyldimethylsilyl (TBDMS) RNA synthesis methods.

INTRODUCTION

Selenium-labeled oligonucleotides have become recognized to represent useful derivatives for phasing of X-ray crystallographic data in nucleic acid structure analysis. Among various potential sites for modification with selenium, ribose 2′-methylseleno groups have attracted most attention so far. Since the pioneering work by Egli, Huang and coworkers, which led to successful multi-wavelength anomalous dispersion (MAD)-phasing of an A-form DNA duplex via 2′-methylseleno uridine (1–3), our laboratory elaborated advanced procedures for the preparation of 2′-methylseleno-modified RNA, site-specifically labeled at any of the four standard nucleosides, adenosine, cytidine, guanosine and uridine (Figure 1) (4–7). Thereby, the method we used for oligonucleotide synthesis relied on nucleoside phosphoramidites protected with the 2′-*O*-[(triisopropylsilyl)oxy]methyl (TOM) protecting group (TOM chemistry) (8–13). Importantly, the application of *threo*-1,4-dimercapto-2,3-butanediol (DTT) during all steps of RNA preparation, including the solid-phase synthesis cycle, was a major breakthrough for the high performance of the *Se*-approach (5). This resulted in the preparation of highly pure 2′-methylseleno modified RNAs with up to a hundred nucleotides, exemplified by the aptamer domain of the adenine riboswitch (5,14). Successful applications of the *Se*-derivatized RNAs in X-ray structure determination refer to the group I intron (15), to the Diels–Alder ribozyme (16), to a short RNA duplex that has been studied in context with the impact of 2′-methylseleno groups on crystallization behavior and crystal packing (7), and very recently, to HIV-1 genomic RNA dimerization initiation site (DIS) constructs bound to aminoglycoside antibiotics (17,18).

In the present work, we report on preparation of oligoribonucleotides with site-specific 2′-methylseleno groups based on the 2′-*O*-*bis*(acetoxyethoxy)methyl (ACE) RNA solid-phase synthesis method. Nucleoside phosphoramidites providing a fluorine-labile silyl protecting group at the ribose 5′-OH and the acid-labile orthoester protecting group at the ribose 2′-OH were introduced for chemical RNA synthesis in the late nineties (19). Within a very short time, this innovative strategy turned out to be highly competitive to commonly used RNA synthesis methods based on 5′-*O* 4,4′-dimethoxytritylated (DMT) nucleoside building blocks and laid the basis for one of the largest custom RNA synthesis services today. In particular, the very good quality of ACE oligoribonucleotides that are commercially available contributed to the high reputation of the method. In research laboratories, usage of the ACE RNA approach has been limited (20–22) and is generally considered complex

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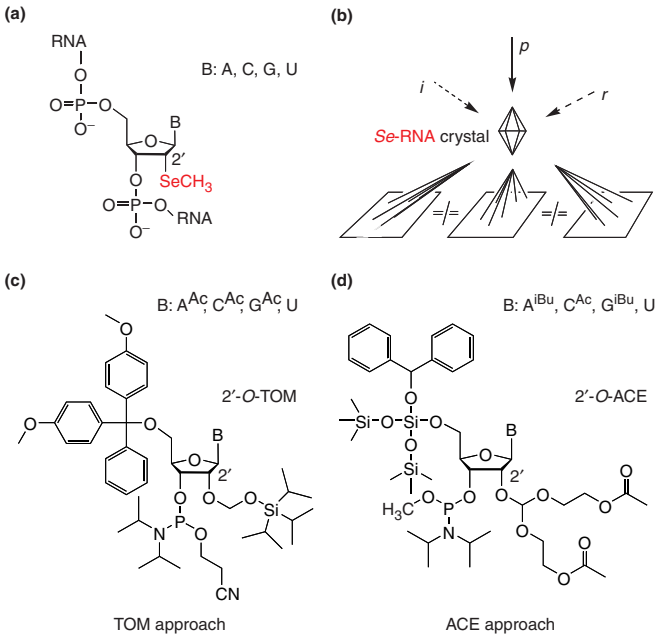


Figure 1. *Se*-modified RNA for X-ray crystallography. (**a**) 2′-Methylseleno-modified RNA represents a highly requested derivative for RNA crystallography. (**b**) A single crystal with an anomalous scattering center such as selenium is required during X-ray structure determination using advanced techniques for phase determination, such as MAD, SAD (single-wavelength anomalous diffraction) or SIRAS (single isomorphous replacement with anomalous scattering). (**c**) Solid-phase synthesis of 2′-methylseleno RNA has been developed based on 5′-*O*-(4,4′-dimethoxytrityl) (DMT)-2′-*O*-silyl protected nucleoside building blocks. (**d**) Goal of the present study is the synthesis of 2′-methylseleno RNA by 2′-*O*-*bis*(acetoxyethoxy)methyl (ACE) RNA solid-phase synthesis. For this, novel building blocks and adaptation of the established ACE solid-phase synthesis cycle are required.

because of non-standard instrumentation, a long optimization period, and not least because of high costs when compared to the 5′-*O*-DMT methods. Under the aspect that our previously established concept of selenium-modified RNA for X-ray structure analysis would strongly benefit from compatibility with high-quality ACE RNA synthesis, we put great efforts into the adaptation of this approach for the preparation of 2′-methylseleno containing oligoribonucleotides. We show here that such derivatives are readily available via the novel nucleoside phosphoramidite building blocks and ACE RNA solid-phase synthesis procedures outlined subsequently.

MATERIALS AND METHODS

Synthesis of 2′-methylseleno modified nucleosides for ACE RNA synthesis

General. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker DRX 300 MHz, or Varian Unity 500 MHz instrument. The chemical shifts are reported relative to TMS and referenced to the residual proton signal of the deuterated solvents: $CDCl_3$ (7.26 p.p.m.), d_6-DMSO (2.49 p.p.m.) for ^{1}H-NMR spectra; $CDCl_3$ (77.0 p.p.m.) or d_6-DMSO (39.5 p.p.m.) for ^{13}C-NMR spectra. ^{31}P-shifts are relative to external 85% phosphoric acid. ^{1}H- and ^{13}C-assignments were based on COSY and HSQC experiments. UV-spectra were recorded on a Varian Cary 100 spectrophotometer. Analytical thin-layer chromatography (TLC) was carried out on silica 60F-254 plates. Flash column chromatography was carried out on silica gel 60 (230–400 mesh or 70–230 mesh). All reactions were carried out under argon atmosphere. Chemical reagents and solvents were purchased from commercial suppliers and used without further purification. Benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (BzHCl) was obtained from Dharmacon. Organic solvents for reactions were dried overnight over freshly activated molecular sieves (4 Å).

Synthesis of 2′-methylseleno adenosine phosphoramidite (A10)

*N^6-Acetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(trimethylsilyl)adenosine (**A2**).* To a suspension of adenosine **A1** (1.0 g; 3.742 mmol) in DMF (12 ml) and pyridine (12 ml), 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.3 g; 4.116 mmol) was added dropwise. The mixture was stirred for 2 h at room temperature, during which time it turned into a clear solution. Then, chlorotrimethylsilane (947 µl; 7.484 mmol) was added and stirring was continued for 2 h. The resulting white suspension was treated with acetyl chloride (292 µl; 4.116 mmol) and stirred for 1.5 h with occasional shaking. After completion of the reaction, the yellow solution was quenched by addition of 5% aqueous $NaHCO_3$ and extracted with dichloromethane. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 99.8/0.2 – 99/1 v/v). Yield: 2.127 g of **A2** as white foam (91%). TLC (CH_2Cl_2/CH_3OH, 94/6): R_f = 0.49; ^{1}H-NMR (500 MHz, DMSO): δ 0.15 (s, 9H, $((CH_3)_3)Si$); 1.03 (m, 28H, 2× $((CH_3)_2CH)_2Si$); 2.26 (s, 3H, $COCH_3$); 3.95 (dd, J = 1.5, 8.0 Hz, 1H, H1-C(5′)); 4.07 (m, 1H, H-C(4′)); 4.13 (dd, J = 1.5, 8.0 Hz, 1H, H2-C(5′)); 4.72 (dd, J = 2.7, 5.4 Hz, 1H, H-C(3′)); 4.77 (d, J = 2.7 Hz, 1H, H-C(2′)); 5.97 (s, 1H, H-C(1′)); 8.47 (s, 1H, H-C(8)); 8.59 (s, 1H, H-C(2)); 10.72 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, DMSO): δ 0.65 ($(CH_3)_3Si$); 12.76, 12.83, 13.17, 17.23, 17.34, 17.40, 17.52, 17.61, 17.76 (2× $((CH_3)_2CH)_2Si$); 24.79 ($COCH_3$); 60.70 (C(5′)); 69.80 (C(3′)); 75.57 (C(2′)); 81.19 (C(4′)); 90.23 (C(1′)); 124.21; 142.38 (C(8)); 150.07, 151.36; 152.00 (C(2)); 169.25 ($COCH_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 270 (16 000) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{27}H_{49}N_5O_6Si_3$, 624.96; found 624.19.

*N^6-Acetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)adenosine (**A3**).* A mixture of *p*-toluenesulfonic acid monohydrate (671 mg; 3.526 mmol), dioxane (20 ml) and molecular sieves (1.5 g) was stirred for 2.5 h at room temperature. A solution of **A2** (2.0 g; 3.205 mmol) in dioxane (10 ml) was added and stirring was continued for 1.5 h. The reaction mixture was then quenched by the addition of triethylamine (4.5 ml), evaporated and coevaporated with dichloromethane. The crude product was

purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 99.5/0.5 – 97/3 v/v). Yield: 1.384 g of **A3** as white foam (78%). TLC (CH_2Cl_2/CH_3OH, 94/6): R_f = 0.46; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.09 (m, 28H, 2× $((CH_3)_2CH)_2Si$); 2.62 (s, 3H, $COCH_3$); 3.41 (s, br, 1H, HO-C(2′)); 4.02–4.14 (m, 3H, H_2-C(5′), H-C(4′)); 4.60 (m, 1H, H-C(2′)); 5.09 (m, 1H, H-C(3′)); 6.02 (d, J = 0.9 Hz, 1H, H-C(1′)); 8.18 (s, 1H, H-C(8)); 8.62 (s, 1H, H-C(2)); 9.09 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.63, 12.75, 13.02, 13.26, 16.88, 16.94, 16.96, 17.08, 17.23, 17.30, 17.39 (2× $((CH_3)_2CH)_2Si$); 25.64 ($COCH_3$); 61.69 (C(5′)); 70.79 (C(3′)); 75.06 (C(2′)); 82.26 (C(4′)); 89.79 (C(1′)); 122.43; 141.96 (C(8)); 149.38, 150.47; 152.31 (C(2)); 170.58 ($COCH_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 271 (11 700) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{24}H_{41}N_5O_6Si_2$, 552.78; found 552.16.

*N^6-Acetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(trifluoromethanesulfonyl)adenosine (**A4**).* To a solution of compound **A3** (380 mg; 0.689 mmol) in CH_2Cl_2 (13 ml), 4-dimethylaminopyridine (253 mg; 2.067 mmol) was added at 0°C. The mixture was treated with trifluoromethanesulfonyl chloride (109 µl; 1.034 mmol) and stirred for 15 min at 0°C. The reaction mixture was then diluted with CH_2Cl_2, washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4 and evaporated. The crude product can be used for the next step without further purification. For analysis, the product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 100/0 – 99.3/0.7 v/v). Yield: 259 mg of **A4** as white foam (55%). TLC (CH_2Cl_2/CH_3OH, 94/6): R_f = 0.51; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.08 (m, 28H, 2× $((CH_3)_2CH)_2Si$); 2.63 (s, 3H, $COCH_3$); 4.05 (dd, J = 2.6, 13.4 Hz, 1H, H1-C(5′)); 4.12 (m, 1H, H-C(4′)); 4.20 (m, 1H, H2-C(5′)); 5.23 (dd, J = 4.8, 9.0 Hz, 1H, H-C(3′)); 5.77 (d, J = 4.5 Hz, 1H, H-C(2′)); 6.17 (s, 1H, H-C(1′)); 8.20 (s, 1H, H-C(8)); 8.61 (s, 1H, H-C(2)); 8.89 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.75, 12.80, 12.88, 13.22, 16.66, 16.73, 17.17, 17.21, 17.32 (2× $((CH_3)_2CH)_2Si$); 25.70 ($COCH_3$), 59.53 (C(5′)); 68.16 (C(3′)); 77.19 (CF_3); 81.63 (C(4′)); 87.05 (C(1′)); 87.99 (C(2′)); 122.29; 141.48 (C(8)); 149.54, 150.20; 152.67 (C(2)); 170.55 ($COCH_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 270 (16 100) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{25}H_{40}F_3N_5O_8SSi_2$, 684.85; found 684.20.

*N^6-Acetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)(β-D-arabinofuranosyl)adenine (**A5**).* To a solution of crude **A4** (prepared from 744 mg of **A3**; 1.348 mmol) in toluene (36 ml), potassium trifluoroacetate (1.025 g; 6.740 mmol), 18-crown-6 (713 mg; 2.696 mmol) and *N*-ethyldiisopropylamine (346 µl; 2.022 mmol) were added. The mixture was stirred for 16 h at 80°C. After completion of the reaction, the mixture was filtrated over celite and the solvent was evaporated. The residue was then suspended in dichloromethane, and again filtrated over celite. The filtrate was washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 100/0 – 97.5/2.5 v/v). Yield: 498 mg of **A5** as slightly yellow foam (67% over two steps). TLC (CH_2Cl_2/CH_3OH, 92/8): R_f = 0.48; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.05 (m, 28H, 2× $((CH_3)_2CH)_2Si$); 2.50 (s, 3H, $COCH_3$); 3.88 (m, 1H, H-C(4′)); 4.07 (m, 2H, H_2-C(5′)); 4.26 (s, br, 1H, HO-C(2′)); 4.61 (t, J = 7.8 Hz, 1H, H-C(3′)); 4.67 (m, 1H, H-C(2′)); 6.28 (d, J = 6.0 Hz, 1H, H-C(1′)); 8.30 (s, 1H, H-C(8)); 8.53 (s, 1H, H-C(2)); 9.07 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.49, 12.94, 13.07, 13.48, 16.90, 16.94, 17.02, 17.30, 17.35, 17.46 (2× $((CH_3)_2CH)_2Si$); 25.52 ($COCH_3$), 61.62 (C(5′)); 75.06 (C(3′)); 77.21 (C(2′)); 81.54 (C(4′)); 83.81 (C(1′)); 121.56; 142.82 (C(8)); 148.94, 150.98; 151.97 (C(2)); 170.69 ($COCH_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 271 (16 400) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{24}H_{41}N_5O_6Si_2$, 552.78; found 552.14.

*N^6-Acetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-O-(trifluoromethanesulfonyl)(β-D-arabinofuranosyl)adenine (**A6**).* To a solution of compound **A5** (49 mg; 0.089 mmol) in CH_2Cl_2 (1.7 ml), 4-dimethylaminopyridine (32 mg; 0.266 mmol) was added at 0°C. The mixture was treated with trifluoromethanesulfonyl chloride (14 µl; 0.133 mmol) and stirred for 15 min at 0°C. The reaction mixture was then diluted with CH_2Cl_2, washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4 and evaporated. The crude product can be used for the next step without further purification. For analysis, the product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 100/0 – 99.3/0.7 v/v). Yield: 31 mg of **A6** as white foam (51%). TLC (CH_2Cl_2/CH_3OH, 94/6): R_f = 0.54; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.05 (m, 28H, 2× $((CH_3)_2CH)_2Si$); 2.64 (s, 3H, $COCH_3$); 3.98 (m, 1H, H-C(4′)); 4.15 (m, 2H, H_2-C(5′)); 5.39 (t, J = 7.2 Hz, 1H, H-C(3′)); 5.49 (t, J = 6.3 Hz, 1H, H-C(2′)); 6.43 (d, J = 6.0 Hz, 1H, H-C(1′)); 8.13 (s, 1H, H-C(8)); 8.66 (s, 1H, H-C(2)); 8.86 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.61-17.38 (2× $((CH_3)_2CH)_2Si$); 25.71 ($COCH_3$); 61.99 (C(5′)); 74.06 (C(3′)); 77.19 (CF_3); 81.00 (C(1′)); 81.23 (C(4′)); 88.27 (C(2′)); 121.84; 141.84 (C(8)); 149.50, 150.78; 152.55 (C(2)); 170.47 ($COCH_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 270 (20 400) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{25}H_{40}F_3N_5O_8SSi_2$, 684.85; found 684.11.

*N^6-Acetyl-3′,5′-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-methylseleno-2′-deoxyadenosine (**A7**).* Sodium borohydride (204 mg; 5.418 mmol) was placed in a sealed 25 ml two-necked round-bottom flask, dried on high vacuum for 15 min to deplete oxygen, kept under argon and suspended in dry THF (7.3 ml). Dimethyldiselenide (174 µl; 1.806 mmol) was slowly injected to this suspension, followed by dropwise addition of anhydrous ethanol; 0.2 ml was required until gas bubbles started to occur in the yellow mixture. The solution was stirred at room temperature for 1.5 h and the almost colorless solution was injected into a solution of crude **A6** (prepared from 498 mg of **A5**; 0.903 mmol) in dry THF (8.6 ml). The reaction mixture was stirred at room temperature for 30 min. Then, aqueous 0.2 M triethylammonium acetate buffer (15 ml, pH 7) was added, and the

solution was reduced to half the volume by evaporation. Dichloromethane was added, and the organic layer was washed twice with 0.2 M triethylammonium acetate buffer and finally with saturated sodium chloride solution. The organic phase was dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 99.8/0.2 – 98/2 v/v). Yield: 335 mg of **A7** as white foam (59% over two steps). TLC (CH_2Cl_2/CH_3OH, 94/6): R_f = 0.47; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.01 (m, 28H, 2× ((CH_3)$_2$CH)$_2$Si); 1.99 (s, 3H, $SeCH_3$); 2.62 (s, 3H, $COCH_3$); 4.09 (m, 3H, H_2-C(5′) + H-C(2′)); 4.19 (m, 1H, H-C(4′)); 4.89 (t, J = 6.8 Hz, 1H, H-C(3′)); 6.31 (d, J = 3.9 Hz, 1H, H-C(1′)); 8.26 (s, 1H, H-C(8)); 8.66 (s, 1H, H-C(2)); 8.89 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 3.40 ($SeCH_3$); 12.70, 12.95, 13.14, 13.47, 16.88, 16.98, 17.13, 17.28, 17.29, 17.34, 17.46 (2× ((CH_3)$_2$CH)$_2$Si); 25.65 (CO*C*H$_3$); 47.12 (C(2′)); 61.73 (C(5′)); 71.47 (C(3′)); 84.68 (C(4′)); 90.14 (C(1′)); 122.36; 141.28 (C(8)); 149.25, 150.57; 152.37 (C(2)); 170.43 (*C*OCH$_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 271 (17 300) nm (mol^{-1} dm^3 cm^{-1}); ESI-MS (m/z): [M + H]$^+$ calcd for $C_{25}H_{43}N_5O_5SeSi_2$, 629.77; found 629.98.

*N^6-Acetyl-2′-methylseleno-2′-deoxyadenosine (**A8**).* Compound **A7** (192 mg; 0.305 mmol) was dissolved in a mixture of 1 M tetrabutylammonium fluoride/0.5 M acetic acid in THF (1.3 ml). The solution was stirred for 2 h at room temperature and the reaction progress was monitored via TLC. Then, the solvent was evaporated and the residue dried under high vacuum. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 100/0 – 97/3 v/v). Yield: 111 mg of **A8** as white foam (94%). TLC (CH_2Cl_2/CH_3OH, 90/10): R_f = 0.42; ^{1}H-NMR (300 MHz, DMSO): δ 1.57 (s, 3H, $SeCH_3$); 2.25 (s, 3H, $COCH_3$); 3.58 (m, 1H, H1-C(5′)); 3.65 (m, 1H, H2-C(5′)); 4.00 (m, 1H, H-C(4′)); 4.18 (dd, J = 3.0, 9.0 Hz, 1H, H-C(2′)); 4.36 (m, 1H, H-C(3′)); 5.10 (t, J = 6.0 Hz, 1H, HO-C(5′)); 5.86 (d, J = 6.0 Hz, 1H, HO-C(3′)); 6.36 (d, J = 9.0 Hz, H-C(1′)); 8.66 (s, 1H, H-C(2)); 8.74 (s, 1H, H-C(8)); 10.68 (s, br, 1H, H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, DMSO): δ 2.87 ($SeCH_3$); 24.81 (CO*C*H$_3$); 46.73 (C(2′)); 62.14 (C(5′)); 73.21 (C(3′)); 87.98 (C(4′)); 89.90 (C(1′)); 124.00; 143.21 (C(8)); 150.17, 152.17; 152.29 (C(2)); 169.30 (*C*OCH$_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 271 (15 000) nm (mol^{-1}dm^{3}cm^{-1}); ESI-MS (m/z): [M + H]$^+$ calcd for $C_{13}H_{17}N_5O_4Se$, 387.27; found 387.84.

*N^6-Acetyl-5′-O-[benzhydryloxy-bis(trimethylsilyloxy) silyl]-2′-methylseleno-2′-deoxyadenosine (**A9**). Solution A:* To a solution of compound **A8** (60 mg; 0.155 mmol) in DMF (0.5 ml), *N,N*-diisopropylamine (22 µl; 0.155 mmol) was added and the mixture was cooled to 0°C. *Solution B: N,N*-diisopropylamine (53 µl; 0.372 mmol) was added dropwise to a solution of benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (132 mg; 0.310 mmol) in dichloromethane (0.3 ml) at 0°C. Solution B was added to solution A at 0°C in three portions (aliquots of 0.5/0.25/0.25 every 30 min) and the reaction progress was monitored by TLC. After 2 h, the reaction mixture was quenched by addition of 5% sodium bicarbonate solution and extracted with dichloromethane. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (hexane/ethyl acetate, 1/1 v/v). Yield: 87 mg of **A9** as colorless oil (72%). TLC (ethyl acetate/hexane, 4/1): R_f = 0.46; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.08, 0.10 (2s, 18 H, 2× (CH_3)$_3$Si); 1.84 (s, 3H, $SeCH_3$); 2.66 (s, 3H, $COCH_3$); 2.73 (m, 1H, HO-C(3′)); 3.82 (m, 2H, H_2-C(5′)); 3.95 (dd, J = 5.1, 8.4 Hz, 1H, H-C(2′)); 4.18 (m, 1H, H-C(4′)); 4.28 (m, 1H, H-C(3′)); 5.94 (s, 1H, OCH(Ph)$_2$); 6.26 (d, J = 9.0 Hz, 1H, H-C(1′)); 7.26–7.36 (m, 10H, H-C(ar)); 8.28 (s, 1H, H-C(8)); 8.66 (s, br, 2H, H-C(2) + H-N^6) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 1.50 (2× (CH_3)$_3$Si); 4.23 ($SeCH_3$); 25.68 (CO*C*H$_3$); 49.68 (C(2′)); 63.14 (C(5′)); 72.75 (C(3′)); 77.05 (OCH(Ph)$_2$); 85.67 (C(4′)); 88.19 (C(1′)); 121.88; 126.31, 126.32, 127.33, 128.30 (C(ar)); 141.49 (C(8)); 143.83, 143.85, 149.13, 151.36; 152.36 (C(2)); 170.62 (*C*OCH$_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 271 (17500) nm (mol^{-1} dm^3 cm^{-1}); HR-ESI-MS (m/z): [M + Na]$^+$ calcd for $C_{32}H_{45}N_5O_7SeSi_3$, 798.1689; found 798.1677.

*N^6-Acetyl-5′-O-[benzhydryloxy-bis(trimethylsilyloxy) silyl]-2′-methylseleno-2′-deoxyadenosine 3′-(methyl-N, N-diisopropyl)phosphoramidite (**A10**).* Compound **A9** (139 mg; 0.179 mmol) was dissolved in a mixture of *N*-ethyldimethylamine (194 µl; 1.794 mmol) in dry dichloromethane (2.3 ml) under argon. After 15 min at room temperature, methyl-*N,N*-diisopropylchlorophosphoramidite (53 mg; 0.269 mmol) was slowly added and the solution was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane, washed with half-saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (ethyl acetate/hexane, 2/3 – 1/1 v/v (+0.5% Et_3N)). Yield: 117 mg of **A10** (mixture of diastereomers) as colorless oil (70%). TLC (ethyl acetate/hexane, 7/3): R_f = 0.47, 0.53; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.07-0.09 (4s, 36 H, 4× (CH_3)$_3$Si); 1.19-1.27 (m, 24H, 2× ((CH_3)$_2$CH)$_2$N); 1.52, 1.57 (2s, 6H, 2× $SeCH_3$); 2.66 (s, 6H, 2× $COCH_3$); 3.35 (d, J = 13.5 Hz, 3H, $POCH_3$); 3.48 (d, J = 13.5 Hz, 3H, $POCH_3$); 3.63 (m, 4H, 2× ((CH_3)$_2$*CH*)$_2$N); 3.80–3.96 (m, 6H, 2× H-C(2′) + 2× H_2-C(5′)); 4.31 (m, 2H, 2× H-C(4′)); 4.57 (dd, J = 5.4, 9.3 Hz, 1H, H-C(3′)); 4.66 (dd, J = 6.2, 11.0 Hz, 1H, H-C(3′)); 5.95, 5.96 (2s, 2H, 2× OCH(Ph)$_2$); 6.45 (2d, J = 8.0, 8.5 Hz, 2H, 2× H-C(1′)); 7.19–7.37 (m, 20H, H-C(ar)); 8.27, 8.28 (2s, 2H, 2× H-C(8)); 8.53 (s, br, 2H, 2× H-N^6); 8.66, 8.68 (2s, 2H, H-C(2)) p.p.m.; ^{31}P-NMR (121 MHz, $CDCl_3$): δ 150.3, 153.0 p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 271 (17 400) nm (mol^{-1} dm^3 cm^{-1}); HR-ESI-MS (m/z): [M + Na]$^+$ calcd for $C_{39}H_{61}N_6O_8PSeSi_3$, 959.2660; found 959.2701.

Synthesis of 2′-methylseleno guanosine phosphoramidite (G3)

*N^2-Acetyl-5′-O-[benzhydryloxy-bis(trimethylsilyloxy) silyl]-2′-methylseleno-2′-deoxyguanosine (**G2**). Solution A:* To a solution of compound **G1** [reference (6)]

(400 mg; 0.994 mmol) in THF (7.0 ml), *N,N*-diisopropylamine (179 µl; 1.99 mmol) was added and the mixture was cooled to 0°C. *Solution B: N,N*-diisopropylamine (179 µl; 1.99 mmol) was added dropwise to a solution of benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (846 mg; 1.99 mmol) in dichloromethane (5.0 ml) at 0°C. Solution B was added to solution A at 0°C in three portions (aliquots of 0.5/0.25/0.25 every 30 min) and the reaction progress was monitored by TLC. After 2 h, the reaction mixture was quenched by addition of 5% sodium bicarbonate solution and extracted with dichloromethane. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/acetone, 95/5 – 85/15 v/v). Yield: 358 mg of **G2** as colorless oil (46%). TLC (CH_2Cl_2/acetone, 7/3): $R_f = 0.71$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.05–0.06 (2s, 18 H, 2× $(CH_3)_3Si$); 1.65 (s, 3H, $SeCH_3$); 2.31 (s, 3H, $COCH_3$); 3.57 (m, 1H, H-C(2′)); 3.67 (m, 1H, HO-C(3′)); 3.77 (m, 2H, H_2-C(5′)); 4.15 (m, 1H, H-C(4′)); 4.31 (m, 1H, H-C(3′)); 5.92 (d, $J = 9$ Hz, 1H, H-C(1′)); 5.93 (s, 1H, $OCH(Ph)_2$); 7.16-7.34 (m, 10H, H-C(ar)); 8.01 (s, 1H, H-C(8)); 10.49 (s, br, 1H, H-N^2); 12.24 (s, br, 1H, H-N(1)) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 1.52 (2× $(CH_3)_3Si$); 3.63 ($SeCH_3$); 24.28 ($COCH_3$); 49.80 (C(2′)); 63.52 (C(5′)); 73.38 (C(3′)); 77.05 ($OCH(Ph)_2$); 85.98 (C(4′)); 87.96 (C(1′)); 120.83, 126.25, 126.32, 127.33, 128.29 (C(ar)); 137.31 (C(8)); 143.72, 143.77, 147.79, 149.13 (C(ar)); 156.16 (C(6)); 172.95 (*C*OCH_3) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 256 (19 300) nm ($mol^{-1}dm^3cm^{-1}$); HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{32}H_{45}N_5O_8SeSi_3$, 815.1656; found 815.1651.

*N^2-Acetyl-5′-O-[benzhydryloxy-*bis*(trimethylsilyloxy)silyl]-2′-methylseleno-2′-deoxyguanosine 3′-(methyl-N,N-diisopropyl)phosphoramidite (**G3**).* Compound **G2** (179 mg; 0.226 mmol) was dissolved in a mixture of *N*-ethyldimethylamine (74 µl; 0.680 mmol) in dry dichloromethane (5.0 ml) under argon. After 15 min at room temperature, methyl-*N,N*-diisopropylchlorophosphoramidite (67 mg; 0.340 mmol) was slowly added and the solution was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane, washed with half-saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/acetone, 100/0 – 95/5 v/v). Yield: 172 mg of **G3** (mixture of diastereomers) as colorless oil (80%). TLC (CH_2Cl_2/acetone, 8/2): $R_f = 0.48$, 0.63; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.07 (m, 36 H, 4× $(CH_3)_3Si$); 1.18-1.27 (m, 24H, 2× $((CH_3)_2CH)_2N$); 1.46, 1.50 (2s, 6H, 2× $SeCH_3$); 2.14, 2.15 (2s, 6H, 2× $COCH_3$); 3.35 (d, $J = 13.3$ Hz, 3H, $POCH_3$); 3.47 (d, $J = 13.2$ Hz, 3H, $POCH_3$); 3.63 (m, 4H, 2× $((CH_3)_2CH)_2N$); 3.76 (m, 4H, 2× H_2-C(5′)); 3.87 (m, 2H, 2× H-C(2′)); 4.25, 4.29 (2m, 2H, 2× H-C(4′)); 4.45, 4.65 (2m, 2H, H-C(3′)); 5.97, 5.98 (2s, 2H, 2× $OCH(Ph)_2$); 6.10, 6.15 (2d, $J = 9.6$, 9.6 Hz, 2H, 2× H-C(1′)); 7.19-7.34 (m, 20H, H-C(ar)); 7.92 (s, 2H, 2× H-C(8)); 8.46 (s, br, 2H, 2× H-N^2); 11.85 (s, br, 2H, 2× H-N(1)) p.p.m.; ^{31}P-NMR (121 MHz, $CDCl_3$): δ 150.5, 152.8 p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 257 (20 800) nm ($mol^{-1}dm^3cm^{-1}$); HR-ESI-MS (m/z): $[M+H]^+$ calcd for $C_{39}H_{61}N_6O_9PSeSi_3$, 953.2790; found 953.2807.

Synthesis of 2′-methylseleno cytidine phosphoramidite (C4)

N^4-Acetyl-2′-methylseleno-2′-deoxycytidine (C2). N^4-Acetyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxycytidine **C1** [reference (4)] (824 mg; 1.055 mmol) was dissolved in a mixture of 1.0 M tetrabutylammonium fluoride/0.5 M acetic acid in THF (3.0 ml). The solution was stirred for 18 h at room temperature, and the reaction progress was monitored via TLC. Then, the solvent was evaporated and the residue partitioned between CH_2Cl_2 and water. The organic layer was concentrated in vacuum and then detritylation was initiated by addition of 4.0 ml of formic acid. The reaction was complete after 2 min. CH_3OH was added followed by evaporation and coevaporation with CH_3OH and toluene for several times. For workup, the crude product was partitioned between water and CH_2Cl_2. The aqueous layer was evaporated and dried under high vacuum. Yield: 305 mg of **C2** as white powder (80%). TLC (CH_2Cl_2/CH_3OH, 90/10): $R_f = 0.38$; ^{1}H-NMR (300 MHz, DMSO): δ 1.89 (s, 3H, $SeCH_3$); 2.10 (s, 3H, $COCH_3$); 3.56 (m, 1H, H-C(2′)); 3.58 (m, 1H, H1-C(5′)); 3.64 (m, 1H, H2-C(5′)); 4.18 (m, 1H, H-C(4′)); 4.22 (m, 1H, H-C(3′)); 5.17 (s, 1H, HO-C(5′)); 5.78 (s, 1H, HO-C(3′)); 6.25 (d, $J = 7.8$ Hz, 1H, H-C(1′)); 7.20 (d, $J = 7.8$ Hz, 1H, H-C(5)); 8.34 (d, $J = 7.8$ Hz, 1H, H-C(6)); 10.92 (s, br, 1H, H-N^4) p.p.m.; ^{13}C-NMR (75 MHz, DMSO): δ 2.33 ($SeCH_3$); 24.25 ($CO*C*H_3$); 47.55 (C(2′)); 60.70 (C(5′)); 71.22 (C(3′)); 86.41 (C(4′)); 89.97 (C(1′)); 95.66 (C(5)); 145.30 (C(6)); 154.61, 162.28; 170.96 (*C*OCH_3) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 262 (12 900) nm ($mol^{-1}dm^3cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{12}H_{17}N_3O_5Se$, 363.14; found 363.03.

*N^4-Acetyl-5′-O-[benzhydryloxy-*bis*(trimethylsilyloxy)silyl]-2′-methylseleno-2′-deoxycytidine (**C3**). Solution A:* To a solution of compound **C2** (80 mg; 0.221 mmol) in DMF (1.0 ml), *N,N*-diisopropylamine (31 µl; 0.221 mmol) was added and the mixture was cooled to 0°C. *Solution B: N,N*-diisopropylamine (70 µl; 0.497 mmol) was added dropwise to a solution of benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (188 mg; 0.442 mmol) in dichloromethane (1.0 ml) at 0°C. Solution B was added to solution A at 0°C in three portions (aliquots of 0.5/0.25/0.25 every 30 min) and the reaction progress was monitored by TLC. After 2 h, the reaction mixture was quenched by addition of 5% sodium bicarbonate solution and extracted with dichloromethane. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/acetone, 1/0 – 1/1 v/v). Yield: 90 mg of **C3** as colorless oil (54%). TLC (CH_2Cl_2/acetone, 3/7): $R_f = 0.65$; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.12–0.24 (2s, 18 H, 2× $(CH_3)_3Si$); 2.14 (s, 3H, $SeCH_3$); 2.22 (s, 3H, $COCH_3$); 2.61 (m, 1H, HO-C(3′)); 3.38 (m, 1H, H-C(2′)); 3.78 (m, 1H, H1-C(5′)); 3.91 (m, 1H, H2-C(5′)); 4.07 (m, 1H, H-C(4′)); 4.10 (m, 1H, H-C(3′)); 5.96 (s, 1H, $OCH(Ph)_2$); 6.35

(d, J = 6.8 Hz, 1H, H-C(1′)); 7.19–7.39 (m, 10H, H-C(ar)); 7.35 (d, J = 7.5 Hz, 1H, H-C(5)); 8.26 (d, J = 7.5 Hz, 1H, H-C(6)); 8.37 (s, br, 1H, H-N^4) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 1.54 (2× $(CH_3)_3Si$); 4.36 ($SeCH_3$); 24.87 ($COCH_3$); 51.29 (C(2′)); 62.55 (C(5′)); 70.90 (C(3′)); 77.09 ($OCH(Ph)_2$); 85.20 (C(4′)); 89.01 (C(1′)); 97.29 (C(5)); 126.21, 127.37, 128.33, 143.72 (C(ar)); 144.38 (C(6)); 155.36, 162.96 (C(ar)); 170.97 ($COCH_3$) p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 263 (12 500) nm ($mol^{-1}\,dm^3\,cm^{-1}$); HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{31}H_{45}N_3O_8SeSi_3$, 775.1595; found 775.1606.

*N^4-Acetyl-5′-O-[benzhydryloxy-bis(trimethylsilyloxy) silyl]-2′-methylseleno-2′-deoxycytidine 3′-(methyl-N,N-diisopropyl)phosphoramidite (**C4**).* Compound **C3** (280 mg; 0.373 mmol) was dissolved in a mixture of *N*-ethyldimethylamine (404 µl; 3.729 mmol) in dry dichloromethane (5.0 ml) under argon. After 15 min at room temperature, methyl-*N,N*-diisopropylchlorophosphoramidite (111 mg; 0.559 mmol) was slowly added and the solution was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane, washed with half-saturated sodium bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/acetone, 99/1–93/7 v/v). Yield: 235 mg of **C4** (mixture of diastereomers) as colorless oil (69%). TLC (CH_2Cl_2/acetone, 7/3): R_f = 0.37, 0.52; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.06-0.09 (4s, 36H, 4× $(CH_3)_3Si$); 1.14–1.27 (m, 24H, 2× $((CH_3)_2CH)_2N$); 1.96, 2.07 (2s, 6H, 2× $SeCH_3$); 2.24 (s, 6H, 2× $COCH_3$); 3.26, 3.49 (2d, 2H, 2× H-C(2′)); 3.31 (d, J = 13.5 Hz, 3H, $POCH_3$); 3.44 (d, J = 13.5 Hz, 3H, $POCH_3$); 3.60 (m, 4H, 2× $((CH_3)_2CH)_2N$); 3.79–3.96 (m, 4H, 2× H_2-C(5′)); 4.23 (m, 2H, 2× H-C(4′)); 4.46 (m, 2H, H-C(3′)); 5.93 (s, 2H, 2× $OCH(Ph)_2$); 6.43, 6.49 (2d, J = 5.5, 7.5 Hz, 2H, 2× H-C(1′)); 7.18–7.35 (m, 22H, H-C(ar), H-C(5)); 8.15, 8.25 (2d, J = 7.8, 7.3 Hz, 2H, 2× H-C(6)); 8.96, 9.05 (2s, br, 2H, 2× H-N^4) p.p.m.; ^{31}P-NMR (121 MHz, $CDCl_3$): δ 150.6, 152.7 p.p.m.; UV/Vis (MeOH): λ_{max} (ε) = 264 (12 800) nm ($mol^{-1}\,dm^3\,cm^{-1}$); HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{38}H_{61}N_4O_9PSeSi_3$, 936.2567; found 936.2587.

Synthesis of 2′-methylseleno uridine phosphoramidite (U4)

*2′-Methylseleno-2′-deoxyuridine (**U2**).* A solution of 5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxyuridine **U1** [reference (4)] (600 mg; 0.962 mmol) in dichloromethane/formic acid (14.4 ml; 5/1 v/v) was stirred at room temperature for 1 h. After addition of methanol (5 ml), the solvents were evaporated at reduced pressure. The residue was coevaporated several times with methanol and toluene until a clear syrup was obtained which was dissolved in dichloromethane/water. Extraction of the product into water, evaporation to dryness and coevaporation with methanol yielded 306 mg of **U2** as white foam (99%). TLC (CH_2Cl_2/MeOH, 88/12): R_f = 0.47; ^{1}H-NMR (300 MHz, DMSO): δ 1.87 (s, 3H, $SeCH_3$); 3.51 (dd, J = 5.6, 8.6 Hz, 1H, H-C(2′)); 3.58 (m, 2H, H_2-C(5′)); 3.88 (m, 1H, H-C(4′)); 4.21 (m, 1H, H-C(3′)); 5.09 (s, br, 1H, HO-C(5′)); 5.71 (d, J = 9.0 Hz, 1H, H-C(5)); 5.75 (m, 1H, HO-C(3′)); 6.20 (d, J = 9.0 Hz, 1H, H-C(1′)); 7.86 (d, J = 9.0 Hz, 1H, H-C(6)); 11.36 (s, br, 1H, H-N(3)) p.p.m.; ^{13}C-NMR (75 MHz, DMSO): δ 2.93 ($SeCH_3$); 46.80 (C(2′)); 61.81 (C(5′)); 72.81 (C(3′)); 87.04 (C(4′)); 89.07 (C(1′)); 102.76 (C(5)); 140.91 (C(6)); 151.12 (C(2)); 163.36 (C(4)) p.p.m.; UV/Vis: λ_{max} (ε) = 260 (8700) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M-H]^-$ calcd for $C_{10}H_{14}N_2O_5Se$, 320.19; found 320.94.

*5′-O-[Benzhydryloxy-bis(trimethylsilyloxy)silyl]-2′-methylseleno-2′-deoxyuridine (**U3**).*

Preparation starting with U2. *Solution A:* To a solution of compound **U2** (299 mg; 0.933 mmol) in DMF (2 ml), *N,N*-diisopropylamine (132 µl; 0.933 mmol) was added and the mixture was cooled to 0°C. *Solution B: N,N*-diisopropylamine (317 µl; 2.237 mmol) was added dropwise to a solution of benzhydryloxy-*bis*(trimethylsilyloxy) chlorosilane (793 mg; 1.866 mmol) in dichloromethane (1.8 ml) at 0°C. Solution B was added to solution A at 0°C in three portions (aliquots of 0.5/0.25/0.25 every 30 min) and the reaction progress was monitored by TLC. After 2 h, the reaction mixture was quenched by addition of 5% sodium bicarbonate solution and extracted with dichloromethane. The combined organic phases were washed with brine, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (hexane/ethyl acetate, 4/1 – 1/1 v/v). Yield: 481 mg of **U3** as white foam (73%).

Preparation starting with U6. Sodium borohydride (18 mg; 0.476 mmol) was placed in a sealed 25 ml two-necked round-bottom flask, dried on high vacuum for 15 min to deplete oxygen, kept under argon, and suspended in dry THF (0.6 ml). Dimethyldiselenide (15 µl; 0.159 mmol) was slowly injected to this suspension, followed by dropwise addition of anhydrous ethanol; 25 µl was required until gas bubbles started to occur in the yellow mixture. The solution was stirred at room temperature for 1 h, and the almost colorless solution was injected into a solution of **U6** (48 mg; 0.078 mmol) in dry THF (0.8 ml). The reaction mixture was stirred at room temperature for 2 h. Then, aqueous 0.1 M triethylammonium acetate buffer (5 ml, pH 7) was added, and the organic solvent was removed by evaporation. Water was added, and the solution extracted with dichloromethane. The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 99/1 – 99/2 v/v). Yield: 33 mg of **U3** as white foam (59%).

TLC (ethyl acetate/hexane, 1/1): R_f = 0.48; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.11, 0.12 (2s, 18 H, 2× $(CH_3)_3Si$); 2.03 (s, 3H, $SeCH_3$); 2.66 (m, 1H, HO-C(3′)); 3.29 (dd, J = 4.8, 8.4 Hz, 1H, H-C(2′)); 3.86 (m, 2H, H_2-C(5′)); 4.15 (m, 2H, H-C(3′), H-C(4′)); 5.59 (d, J = 8.1 Hz, 1H, H-C(5)); 5.95 (s, 1H, $OCH(Ph)_2$); 6.19 (d, J = 8.4 Hz, 1H, H-C(1′)); 7.31 (m, 10H, H-C(ar)); 7.77 (d, J = 8.1 Hz, 1H, H-C(6)); 8.27 (s, br, 1H, H-N(3)) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 1.54 (2× $(CH_3)_3Si$); 4.22 ($SeCH_3$); 50.08 (C(2′)); 63.26 (C(5′)); 71.97 (C(3′/4′)); 77.13 ($OCH(Ph)_2$); 85.11 (C(3′/4′)); 87.15 (C(1′)); 103.01 (C(5));

126.23, 126.28, 127.50, 128.38 (C(ar)); 139.58 (C(6)); 143.64, 143.67 (C(ar)); 150.39 (C(2)); 162.80 (C(4)) p.p.m.; UV/Vis: λ_{max} (ε) = 260 (7800) nm ($mol^{-1}\,dm^3\,cm^{-1}$); HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{29}H_{42}N_2O_8SeSi_3$, 733.1311; found 733.1321.

*5′-O-[Benzhydryloxy-bis(trimethylsilyloxy)silyl]-2′-methylseleno-2′-deoxyuridine 3′-(methyl-N,N-diisopropyl)phosphoramidite (**U4**).* Compound **U3** (106 mg; 0.150 mmol) was dissolved in a mixture of *N*-ethyldimethylamine (162 µl; 1.50 mmol) in dry dichloromethane (2 ml) under argon. After 15 min at room temperature, methyl-*N,N*-diisopropylchlorophosphoramidite (45 mg; 0.225 mmol) was slowly added and the solution was stirred at room temperature for 2 h. The reaction was quenched by the addition of methanol (0.1 ml). The reaction mixture was diluted with dichloromethane, extracted with saturated sodium bicarbonate solution, dried over Na_2SO_4 and the solvent was evaporated. The crude product was purified by column chromatography on SiO_2 (ethyl acetate/hexane, 2/8–3/7 v/v (+0.5% NEt_3)). Yield: 107 mg of **U4** (mixture of diastereomers) as thick, colorless oil (84%). TLC (ethyl acetate/hexane, 3/7): R_f = 0.50; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.07, 0.08, 0.09, 1.10 (4s, 36 H, 4× $(CH_3)_3Si$); 1.17–1.29 (m, 24H, 2× $((CH_3)_2CH)_2N$); 1.92, 1.96 (2s, 6H, 2× $SeCH_3$); 3.17 (m, 1H, H-C(2′)); 3.34 (m, 4H, H-C(2′), $POCH_3$); 3.49 (d, J = 13.1 Hz, 3H, $POCH_3$); 3.66 (m, 4H, 2× $((CH_3)_2CH)_2N$); 3.83 (m, 4H, 2× H_2-C(5′)); 4.23 (m, 2H, 2× H-C(4′)); 4.47, 4.55 (2m, 2H, 2× H-C(3′)); 5.50 (m, 2H, 2× H-C(5)); 5.93 (s, 2H, 2× $OCH(Ph)_2$); 6.38 (2d, J = 9 Hz, 2H, 2× H-C(1′)); 7.22-7.32 (m, 20H, H-C(ar)); 7.70, 7.74 (2d, J = 8.2 Hz, 2H, 2× H-C(6)); 8.61 (s, br, 2H, 2× H-N(3)) p.p.m.; ^{31}P-NMR (121 MHz, $CDCl_3$): δ 150.3, 153.0 p.p.m.; UV/Vis: λ_{max} (ε) = 260 (8400) nm ($mol^{-1}dm^3cm^{-1}$); HR-ESI-MS (m/z): $[M+Na]^+$ calcd for $C_{36}H_{58}N_3O_9PSeSi_3$, 894.2282; found 894.2310.

*5′-O-[Benzhydryloxy-*bis*(trimethylsilyloxy)silyl]-2,2′-anhydro uridine (**U6**). Solution A:* A solution of 2,2′-anhydro uridine **U5** [reference (4)] (25 mg; 0.111 mmol) in pyridine (2.5 ml) was cooled to −15°C. *Solution B:* Benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (118 mg; 0.277 mmol) was added to dichloromethane (1.06 ml) at −15°C. Solution B was added to solution A at −15°C in 5 portions (aliquots of 0.5 over 3.5 h) and the reaction progress was monitored by TLC. After 4 h, the reaction mixture was quenched by addition of 5% citric acid solution and extracted with dichloromethane. The combined organic phases were washed with water and 5% sodium bicarbonate solution, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/CH_3OH, 99/1 – 93/7 v/v). Yield: 32 mg of **U6** as white foam (47%). TLC (CH_2Cl_2/CH_3OH, 92/8): R_f = 0.47; ^{1}H-NMR (300 MHz, $CDCl_3$): δ −0.02, −0.01 (2s, 18H, 2× $(CH_3)_3Si$); 3.42 (m, 2H, H_2-C(5′)); 4.04 (m, 1H, H-C(4′)); 4.36 (m, 1H, H-C(3′)); 4.58 (s, br, 1H, HO-C(3′)); 5.25 (m, 1H, H-C(2′)); 5.84 (s, 1H, $OCH(Ph)_2$); 5.93 (d, J = 7.44 Hz, 1H, H-C(5)); 6.04 (d, J = 5.73 Hz, 1H, H-C(1′)); 7.21 (m, 11H, H-C(6), H-C(ar)) p.p.m.; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 1.57 (2× $(CH_3)_3Si$); 62.40 (C(5′)); 75.58 (C(3′)); 77.01 ($OCH(Ph)_2$); 87.45 (C(4′)); 89.40 (C(2′)); 89.75 (C(1′)); 110.25 (C(5)); 126.59, 127.40, 128.40 (C(ar)); 135.33 (C(6)); 144.07, 144.09 (C(ar)); 159.94 (C(2)); 172.46 (C(4)) p.p.m.; UV/Vis: λ_{260} (ε) = 260 (9800) nm ($mol^{-1}\,dm^3\,cm^{-1}$); ESI-MS (m/z): $[M+H]^+$ calcd for $C_{28}H_{38}N_2O_8Si_3$, 615.88; found 615.29.

ACE RNA solid-phase synthesis of 2′-methylseleno nucleoside containing RNAs

2′-*O*-ACE standard nucleoside phosphoramidites and the corresponding solid-phase supports were obtained from Dharmacon. Oligoribonucleotides containing 2′-methylseleno nucleosides were synthesized on a slightly modified Pharmacia instrumentation (Gene Assembler Plus with a bypassed UV detection unit) following modified DNA/RNA standard methods containing an additional cycle step of treatment with DTT; desilylation (0.55 min): 1.1 M HF/2.9 M Et_3N/DMF; coupling (3.0 min): phosphoramidites/acetonitrile (0.1 M × 130 µl) were activated by benzylthiotetrazole/acetonitrile (0.3 M × 360 µl); capping (3 × 0.4 min): A: Ac_2O/sym-collidine/acetonitrile (20/30/50), B: 4-(dimethylamino)pyridine/acetonitrile (0.5 M), A/B = 1/1; oxidation (1.0 min): I_2 (10 mM) in acetonitrile/sym-collidine/H_2O (10/1/5); DTT treatment (2.0 min): DTT (100 mM) in ethanol/H_2O (2/3). A ready-to-use synthesis method file is provided in the Supplementary Data available online. Solutions of standard amidites, tetrazole solutions and acetonitrile were dried over activated molecular sieves overnight. Solutions of 2′-methylseleno nucleoside phosphoramidites were only dried for 4–6 h over activated molecular sieves before consumption.

Deprotection and purification of 2′-ACE-protected RNAs with 2′-methylseleno modifications

After strand assembly, methyl groups were removed from the phosphate backbone of the RNA attached at the solid support by treatment with disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate in DMF (0.39 M, 2.0 ml) and DTT in H_2O (2 M, 150 µl; final DTT concentration 150 mM) for 20 min at room temperature, followed by filtration of the beads. Then, the beads were removed from the column and additionally treated with DTT in H_2O (150 mM, 200 µl) for 1–3 h at room temperature in a 1.5 ml vial. Cleavage from the solid support and deprotection of acyl groups was performed by $MeNH_2$ in H_2O (40%, 0.74 ml) and DTT in H_2O (2 M, 60 µl; final DTT concentration 150 mM). The mixture was heated to 60°C and held at this temperature for 10 min. Then, the solution was evaporated to dryness, and removal of 2′-*O*-orthoesters was accomplished by treatment with *N,N,N′,N′*-tetramethylethylenediamine (TEMED) acetate buffer (100 mM, 1 mL, pH 3.8) for 30 min at 60°C. The mixture was evaporated to dryness, the residue dissolved in H_2O (0.7 ml), and the solution loaded on a size exclusion column (Amersham HiPrep 26/10 Desalting; 2.6 × 10 cm; Sephadex G25). The crude RNA was eluted with H_2O and dried.

Analysis of crude RNA products after deprotection was performed by anion-exchange chromatography on a Dionex DNAPac100 column (4 × 250 mm) at 80°C. Flow rate: 1 ml/min; eluant A: 25 mM Tris–HCl (pH 8.0), 6 M urea; eluant B: 25 mM Tris–HCl (pH 8.0), 0.5 M $NaClO_4$, 6 M urea; gradient: 0–60% B in A within 45 min; UV-detection at 265 nm. Crude RNA products were purified on a semi-preparative Dionex DNAPac100 column (9 × 250 mm). Flow rate: 2 ml/min; gradient: Δ5–10% B in A within 20 min. Fractions containing RNA were loaded on a C18 SepPak cartridge (Waters/Millipore), washed with 0.1–0.2 M $(Et_3NH)HCO_3$, H_2O, and eluted with H_2O/CH_3CN (6/4). RNA fractions were lyophilized.

Mass spectrometry of 2′-methylseleno group containing RNAs

All experiments were performed on a Finnigan LCQ Advantage MAX ion trap instrumentation connected to an Amersham Ettan micro LC system. RNAs were analyzed in the negative-ion mode with a potential of −4 kV applied to the spray needle. LC: Sample (250 pmol RNA dissolved in 30 µl of 20 mM EDTA solution; average injection volume: 25–30 µl); column (XTerra®MS, C18 2.5 µm; 1.0 × 50 mm) at 21°C; flow rate: 30 µl/min; eluant A: 8.6 mM TEA, 100 mM 1,1,1,3,3,3-hexafluoroisopropanol in H_2O (pH 8.0); eluant B: methanol; gradient: 0–100% B in A within 30 min; UV-detection at 254 nm. Prior to each injection, column equilibration was performed by eluting buffer A for 30 min at a flow rate of 30 µl/min.

RESULTS AND DISCUSSION

2′-*O*-ACE RNA synthesis method

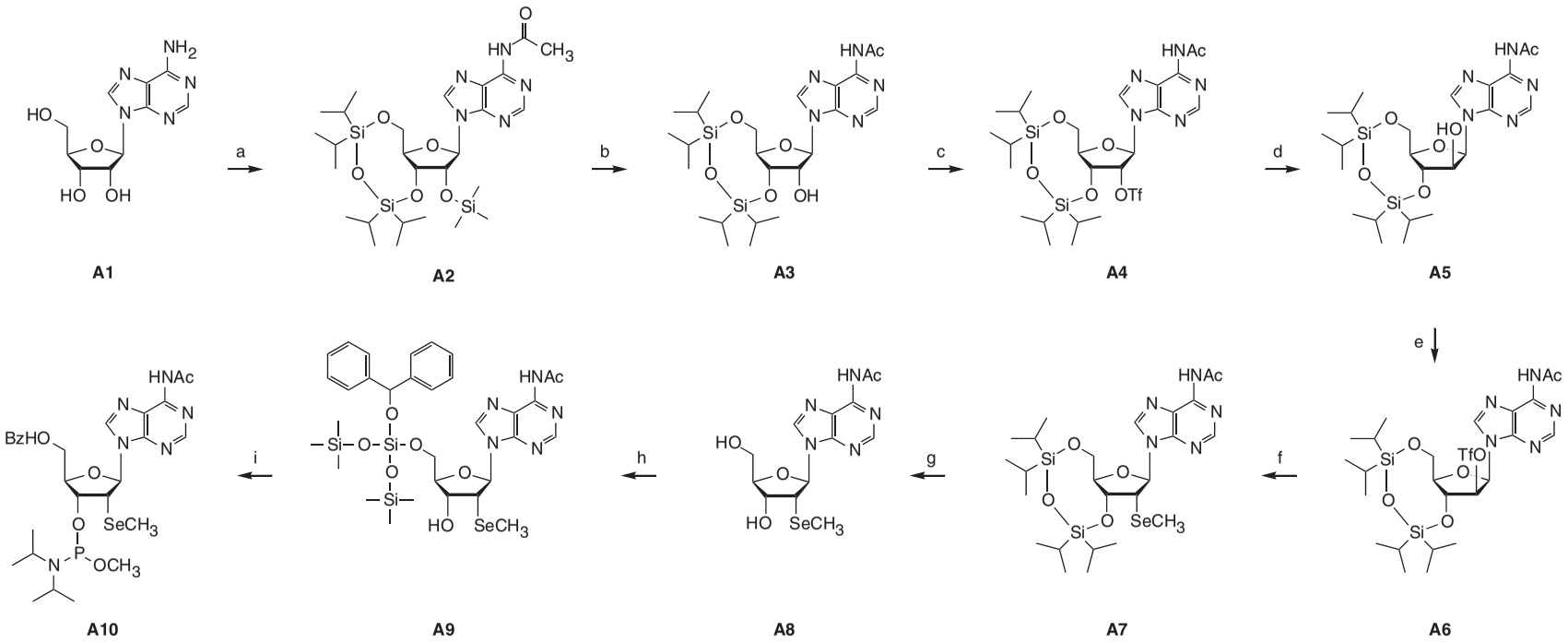
The ACE method for chemical synthesis of RNA was designed under the aspect that mildly acidic aqueous conditions are most desirable for the final 2′-*O* deprotection of the synthesized RNA (19). The loss of orthogonality in combination with the classic 5′-*O*-DMT group was an obstacle to using a mildly acid-labile 2′-*O* protecting group and thus, the concept was achieved based on the fluoride labile 5′-*O*-*bis*(trimethylsilyloxy)cyclododecyloxysilyl ether (DOD), together with the 2′-*O*-*bis*(2-acetoxyethoxy)methyl (ACE) orthoester. The 3′-OH group was derivatized as methyl-*N*,*N*-diisopropylphosphoramidite, since the cyanoethyl group turned out to be unstable with fluoride reagents. After oligonucleotide assembly, the phosphate methyl protecting groups were removed with disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate in DMF. Then, basic conditions (40% aqueous $MeNH_2$) caused oligonucleotide cleavage from the solid support, along with the removal of the acyl protecting groups at the exocyclic amino groups and, importantly, of the acetyl groups at the 2′-orthoesters. The resulting 2′-*O*-*bis*(2-hydroxyethoxy)methyl orthoesters being 10 times more acid labile than prior to the removal of the acetyl groups, therefore required very mild acidic conditions (pH 3.8, 30 min, 60°C) for the final deprotection step (9,19,23–25). For preparation of *Se*-modified RNA oligonucleotides based on the ACE method, we have elaborated the syntheses of appropriate nucleoside phosphoramidites as described subsequently.

Synthesis of 2′-methylseleno adenosine phosphoramidite

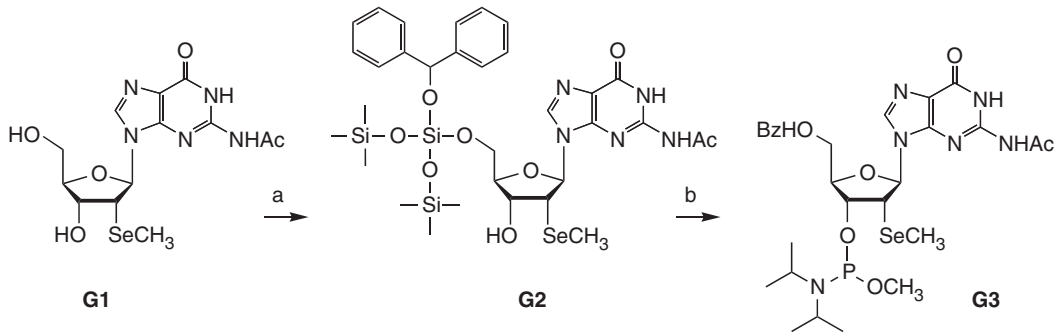
Our route began with the simultaneous protection of the 3′- and 5′-hydroxyl groups of commercially available adenosine **A1** using 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$), followed by protection of the 2′-hydroxyl group as trimethylsilyl ether and reaction with acetyl chloride to furnish the N^6-acetyl adenosine derivative **A2** (Scheme 1). Then, the trimethylsilyl group was cleaved by *p*-toluenesulfonic acid (26). Triflation of the ribose 2′-OH of compound **A3** gave intermediate **A4** which was converted into arabino nucleoside **A5** in diastereoselective manner by treatment with potassium trifluoroacetate and 18-crown-6-ether. After triflation of the arabinose 2′-OH, compound **A6** was reacted with sodium methyl selenide, producing key diastereomer **A7** in high yields. Deprotection of the TIPDS moiety proceeded straightforward using tetrabutylammonium fluoride (TBAF) and acetic acid. Derivative **A8** was transformed regioselectively into the 5′-*O* protected analog **A9** by using benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (BzHCl). Conversion into the corresponding phosphoramidite **A10** was achieved in good yields by reaction with methyl-*N*,*N*-diisopropylchlorophosphoramidite. In principle, intermediate **A8** would be alternatively accessible via N^6-acetyl-5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-{[(triisopropylsilyl)oxy]methyl} (TOM) adenosine which accumulates as regioisomeric by-product during the synthesis of standard 2′-*O*-TOM adenosine phosphoramidite (8). With this precursor, introduction of the selenium moiety proceeds in four steps along the lines described in reference (5), followed by cleavage of the DMT group to provide intermediate **A8**. Such a route, however, would involve ten steps in total starting from adenosine, and overall yields would significantly suffer from the nearly 1:1 ratio of 2′- and 3′-*O*-TOM regioisomers obtained in the third step of synthesis (8). Therefore, we decided to follow the strategy depicted in Scheme 1; our route provides phosphoramidite **A10** in a 13% overall yield in nine steps with seven chromatographic purifications; in total, 0.5 g of **A10** was prepared in the course of this study.

Synthesis of 2′-methylseleno guanosine phosphoramidite

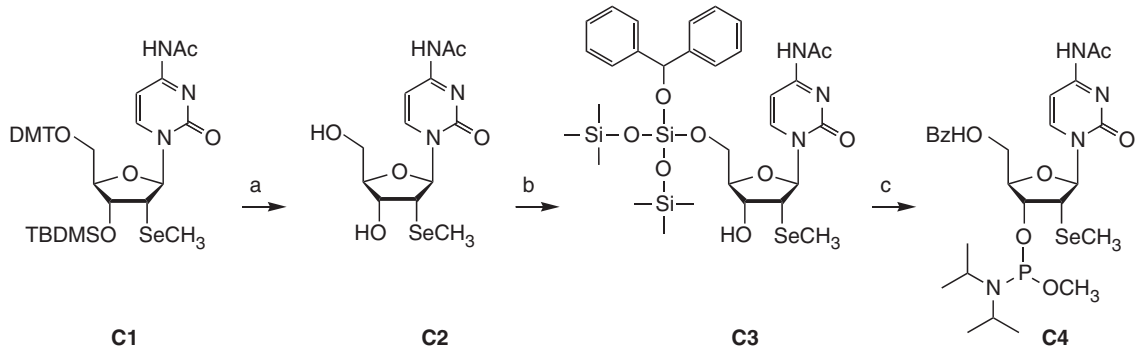
For the guanosine building block, we expanded a previously developed route to obtain N^2-acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxyguanosine phosphoramidite (6). Briefly, commercially available 9-[β-D-arabinofuranosyl]guanine was reacted with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) to protect the 3′- and 5′-hydroxyl groups simultaneously. Then, the 2′-hydroxyl group together with the exocyclic N^2-amino group were acetylated, followed by protection of the guanine lactam moiety with a O^6-(4-nitrophenyl)-ethyl (NPE) group introduced under Mitsunobu conditions (6). Release of the 2′-OH, transformation into the desired 2′-methylseleno moiety, and simultaneous deprotection of the NPE and TIPDS groups yielded derivative



Scheme 1. Synthesis of the 2′-methylseleno adenosine phosphoramidite **A10** (**a**) i. 1.1 eq 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, in DMF/pyridine, room temperature, 2 h; ii. 2 eq chlorotrimethylsilane, room temperature, 2 h; iii. 1.1 eq acetyl chloride, room temperature, 1.5 h, 91%; (**b**) 1.1 eq *p*-toluenesulfonic acid monohydrate, molecular sieves, in dioxane, room temperature, 1.5 h, 78%; (**c**) 1.5 eq trifluoromethanesulfonyl chloride, 3 eq DMAP, in CH_2Cl_2, 0°C, 15 min; (**d**) 5 eq $CF_3COO^-K^+$, 1.5 eq $(iPr)_2NEt$, 2 eq 18-crown-6-ether, in toluene, 80°C, 16 h (67% over (c) and (d)); (**e**) 1.5 eq trifluoromethanesulfonyl chloride, 3 eq DMAP, in CH_2Cl_2, 0°C, 15 min; (**f**) 6 eq $NaBH_4$, 2 eq $CH_3SeSeCH_3$, in THF, room temperature, 30 min (59% over (e) and (f)); (**g**) 1 M TBAF, 0.5 M acetic acid, in THF, room temperature, 2 h, 94%; (**h**) 2 eq benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane, 3.4 eq $(iPr)_2NH$, in CH_2Cl_2/DMF, room temperature, 2 h, 72%; (**i**) 1.5 eq methyl-*N*,*N*-diisopropylchlorophosphoramidite, 10 eq $EtNMe_2$, in CH_2Cl_2, room temperature, 2 h, 70%; (DMAP 4-(dimethylamino)pyridine; TBAF tetrabutylammonium fluoride).



Scheme 2. Synthesis of the 2′-methylseleno guanosine phosphoramidite **G3**; (**a**) 2 eq benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane, 4 eq $(iPr)_2NH$, in CH_2Cl_2/DMF, room temperature, 2 h, 46%; (**b**) 1.5 eq methyl-*N,N*-diisopropylchlorophosphoramidite, 3 eq $EtNMe_2$, in CH_2Cl_2, room temperature, 2 h, 80%.



Scheme 3. Synthesis of the 2′-methylseleno cytidine phosphoramidite **C4**; (**a**) i. 1 M TBAF, 0.5 M acetic acid, in THF, room temperature, 18 h, ii. HCOOH, 2 min, 80%; (**b**) 2 eq benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane, 3.3 eq $(iPr)_2NH$, in CH_2Cl_2/DMF, room temperature, 2.5 h, 54%; (**c**) 1.5 eq methyl-*N,N*-diisopropylchlorophosphoramidite, 10 eq $EtNMe_2$, in CH_2Cl_2, room temperature, 2 h, 69%.

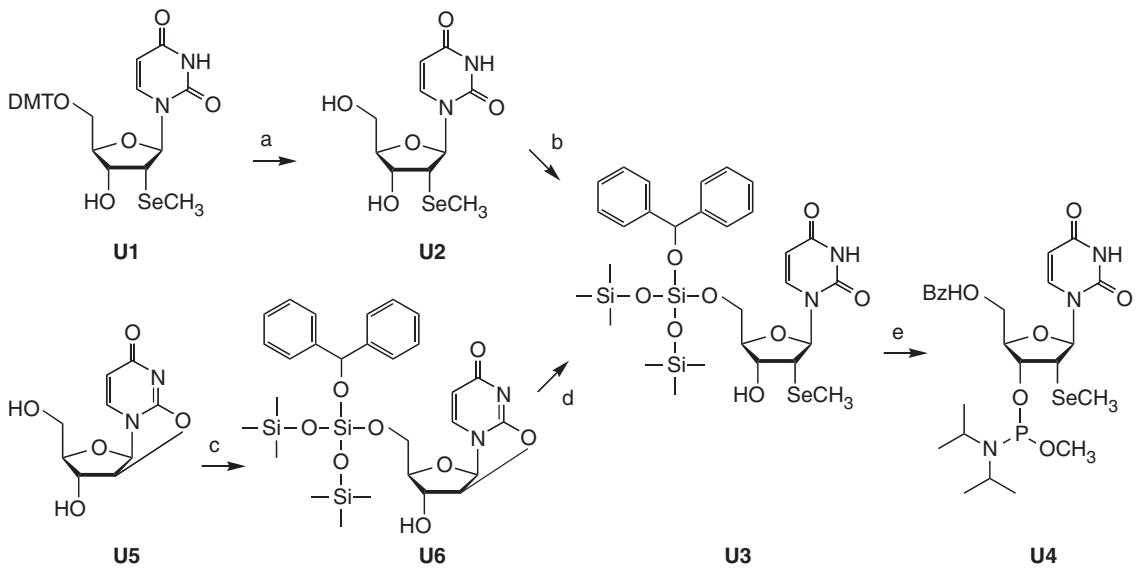
G1 (Scheme 2). This compound was then transformed regioselectively into the 5′-*O* protected analog **G2** by using benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (BzHCl). Conversion into the corresponding phosphoramidite **G3** was achieved in good yields by reaction with methyl-*N*,*N*-diisopropylchlorophosphoramidite. Our route provides phosphoramidite **G3** in a 4% overall yield in nine steps with six chromatographic purifications; in total, 0.2 g of **G3** was prepared in the course of this study.

Synthesis of 2′-methylseleno cytidine phosphoramidite

For synthesis of the *Se*-modified cytidine building block, we relied on a previously developed route to obtain N^4-acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxycytidine phosphoramidite (4). The strategy involved transformation of the nucleobase from the readily available 3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxyuridine (4) into the corresponding cytidine derivative **C1** (Scheme 3). After deprotection, compound **C2** was reacted regioselectively with benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane (BzHCl) to furnish the 5′-*O* protected analog **C3**. Conversion into the corresponding phosphoramidite **C4** was achieved in good yields by reaction with methyl-*N*,*N*-diisopropylchlorophosphoramidite. Our route provides phosphoramidite **C4** in a 10% overall yield in ten steps with eight chromatographic purifications; in total, 0.4 g of **C4** was prepared in the course of this study. We note that an alternative pathway starting from cytidine via the N^4-acetylated, 3′,5′-*O* TIPDS protected analog suffered from very poor yields during triflation reactions that were required for inversion of the configuration at C2′ and introduction of the methylseleno group. This pathway was therefore not further continued.

Synthesis of 2′-methylseleno uridine phosphoramidite

Synthesis of the *Se*-modified uridine building block refers to a previously developed route to obtain 5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxyuridine phosphoramidite (4,27). Thereby, uridine was transformed into 2,2′-anhydro uridine; subsequent protection with DMT at the 5′-OH, followed by introduction of the 2′-methylseleno group with sodium methylselenide gave precursor **U1** (Scheme 4). After release of the DMT group, compound **U2** was transformed regioselectively into the 5′-*O*-benzhydryloxy-*bis*(trimethylsilyloxy)silyl protected analog **U3** by using benzhydryloxy-*bis*(trimethylsilyloxy)-chlorosilane (BzHCl). Conversion into the corresponding phosphoramidite **U4** was achieved in good yields by reaction with methyl-*N*,*N*-diisopropylchlorophosphoramidite. Our route provides phosphoramidite **U4** in a 28% overall yield in six steps with four chromatographic purifications. Alternatively, intermediate **U3** was accessed from 2,2′-anhydro uridine **U5** by direct introduction of the



Scheme 4. Synthesis of the 2′-methylseleno uridine phosphoramidite **U4**; (**a**) HCOOH, in CH_2Cl_2 (1/4 v/v), room temperature, 1 h, 99%; (**b**) 2 eq benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane, 3.4 eq $(iPr)_2NH$, in CH_2Cl_2/DMF, room temperature, 2 h, 73%; (**c**) 2.5 eq benzhydryloxy-*bis*(trimethylsilyloxy)chlorosilane, in pyridine, −15 °C, 4 h; (**d**) 6 eq $NaBH_4$, 2 eq $CH_3SeSeCH_3$, in THF, room temperature, 2 h (28% over (c) and (d)); (**e**) 1.5 eq methyl-*N,N*-diisopropylchlorophosphoramidite, 10 eq $EtNMe_2$, in CH_2Cl_2, room temperature, 2 h, 84%.

BzH group at the 5′-OH to give **U6**, and subsequent introduction of the 2′-methylseleno group. However, the yields over both steps were only about 30%, therefore we preferred the route via the tritylated derivative **U1**; in total, 0.5 g of **U4** was prepared in the course of this study.

Chemical synthesis of *Se*-containing RNA using the ACE method

The preparation of RNA with 2′-methylseleno modified nucleosides relied on the 2′-*O*-ACE RNA synthesis method for strand assembly (9,19,23–25). We used an automated solid-phase synthesizer of the type Pharmacia Gene Assembler Plus where we bypassed the UV detection unit to avoid damage of the flow cell during treatment with HF/TEA solution. The solid-phase synthesis cycle was programmed according to a general description in reference (23). An optimized protocol is provided in the Supplementary Data available online. Therein, the cycle is substantially changed from standard ACE RNA synthesis by an additional step, treating the oligonucleotide chain on the solid support with *threo*-1,4-dimercapto-2, 3-butanediol (DTT) after the capping–oxidation–capping operation. With these protocols, the novel 2′-methylseleno-modified phosphoramidites **A10**, **C4**, **G3** and **U4** were successfully incorporated into oligoribonucleotides (Figure 2, Table 1). The repeated exposure of the growing chain to DTT is a requirement for the reliable synthesis of RNAs (>20 nt) containing multiple *Se*-labels and was previously found to be advantageous for *Se*-containing RNAs prepared by the 2′-*O*-TOM approach (5,6). Deprotection of the methyl groups from the phosphate backbone, cleavage from the solid support and deprotection of acyl groups from 2′-methylseleno-modified RNAs were performed in the presence of DTT as well, added in millimolar amounts to the deprotection solutions of disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate in DMF, of CH_3NH_2 in H_2O and of the *N,N,N′,N′*-tetramethylethylenediamine (TEMED) acetate buffer (pH 3.8). After deprotection, DTT and other low molecular weight components were removed by size exclusion chromatography. RNAs were then purified by anion-exchange chromatography under strong denaturating conditions (6 M urea, 80°C; Figure 2). The molecular weights of the purified RNAs were confirmed by liquid chromatography (LC) electrospray-ionization (ESI) mass spectrometry (MS). Several oligoribonucleotides containing any of the four nucleosides with a 2′-methylseleno moiety were prepared. Their sequences **S1**–**S11** are listed in Table 1. Sequences **S7** – **S10** represent self-complementary 16 nt RNAs, that contain two isolated G–A mispairs. Crystal structures of the non-modified duplex and of a G_{Se} containing analog have been recently solved in cooperation with A. Serganov to rationalize the influence of 2′-methylseleno groups on crystallization and crystal packing (6). Sequence **S6** represents the minimal binding motif of the tandem zinc finger domain of protein TIS11d (28). This protein binds to the class II AU-rich element in the 3′-untranslated region of target mRNAs and promotes their deadenylation and degradation.

Se-RNA preparation with 2′-*O*-TOM- versus 2′-*O*-ACE RNA chemistry

For reason of comparison, we synthesized the non-modified oligoribonucleotide 5′-rGCA GAG UUA AAU CUG U-3′ and the 2′-methylseleno-modified analog 5′-rGCA_{Se} GAG UUA AAU CUG U-3′ with both, the TOM and the ACE solid-phase synthesis methods. From the HPLC profiles of the crude deprotected oligos (Figure 3), it is obvious that the novel access for 2′-methylseleno-containing RNA is of very high quality

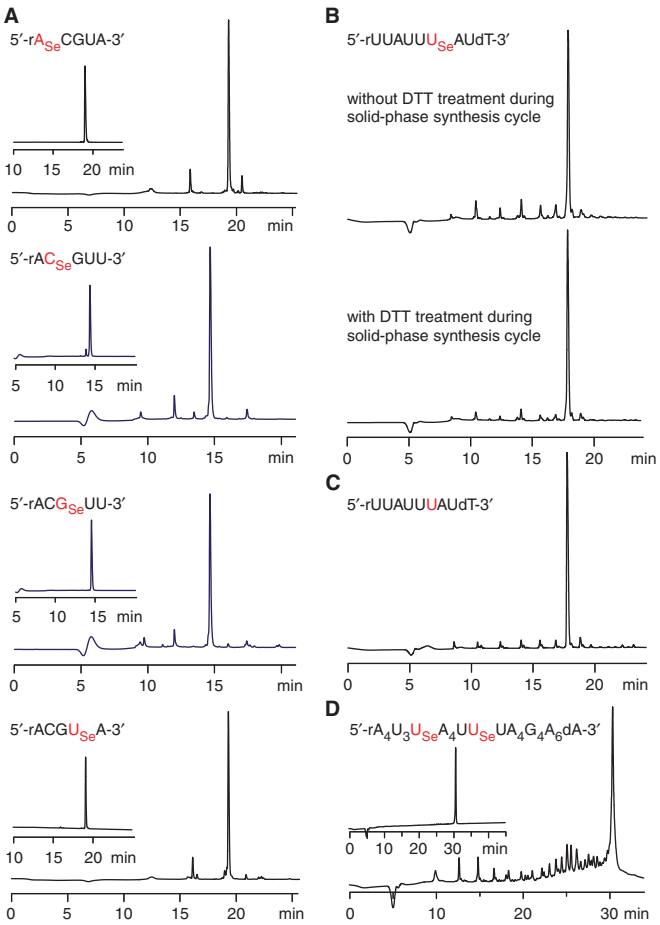


Figure 2. HPLC-traces of deprotected 2′-methylseleno-modified RNA (anion exchange HPLC: Dionex DNAPac (4 × 250 mm), 80°C, 1 ml/min, 0–60% B in 45 min; A: 25 mM Tris–HCl, 6 M urea, pH 8.0; B: same as A + 0.5 M $NaClO_4$); (**a**) Crude and purified (inset) pentamers with 2′-methylseleno modifications at either A, C, G or U. (**b**) 2′-Methylseleno uridine-modified A/U-rich nonamer synthesized without (top) and with (bottom) *threo*-1,4-dimercapto-2,3-butanediol (DTT) treatment during solid-phase synthesis. Both nonamers were deprotected in the presence of DTT. (**c**) Non-modified nonamer as in (b) synthesized for reason of comparison. (**d**) Crude 30 nt RNA with two U_{Se} labels. Deprotection procedure includes four steps: 1. 0.39 M disodium 2-carbamoyl-2-cyanoethylene-1,1-dithiolate, 150 mM DTT, DMF/H_2O (13/1), 20 min, room temperature; 2. 150 mM DTT in H_2O, 1–3 h, room temperature; 3. 40% CH_3NH_2 in H_2O, 150 mM DTT, 10 min, 60°C; 4. 0.1 M TEMED acetate buffer (pH 3.8), 150 mM DTT, 30 min, 60°C.

and can compete with the established *Se*-TOM approach. We note that for the *Se*-ACE method, complete deprotection of acyl groups and of 2′-*O* protecting groups is much faster (10 min plus 30 min) when compared to the corresponding deprotection procedures (5 h plus overnight) required for *Se*-TOM chemistry. Taken together, our goal to transfer the *Se*-approach to the well-reputed ACE RNA synthesis has been satisfactorily achieved.

RNA crystallography

For X-ray structure analysis, we consider RNA with covalent 2′-methylseleno groups best applicable for sizes up to about 80 nt. *Se*-RNA of this dimension can be

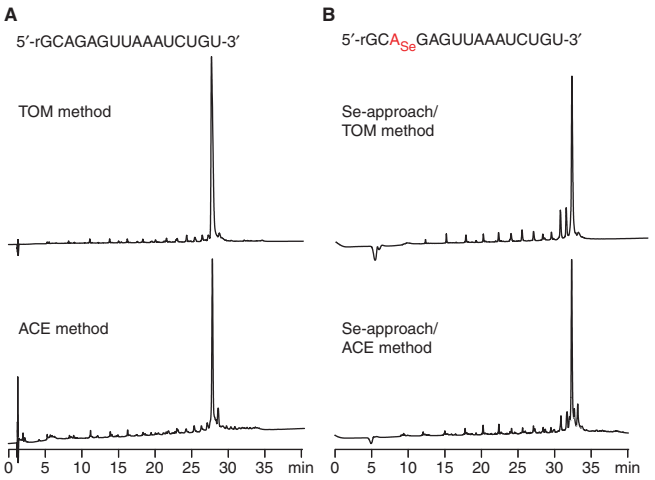


Figure 3. Comparison of TOM versus ACE chemistry for the synthesis of 2′-methylseleno containing oligoribonucleotides, exemplified by the sequence rGCAGAGUUAAAUCUGU. HPLC-traces of crude, deprotected non-modified RNA (**a**) and of A_{Se}-modified RNA (**b**); anion exchange HPLC: Dionex DNAPac (4 × 250 mm), 80°C, 1 ml/min, 0-60% B in 45 min; A: 25 mM Tris–HCl, 6 M urea, pH 8.0; B: same as A + 0.5 M $NaClO_4$.

readily obtained by solid-phase synthesis in combination with enzymatic ligation procedures (4,29,30). For RNAs up to about 35 nt, the *Se*-approach is in competition with 5-iodo and 5-bromo pyrimidine derivatization (31–37). We render the *Se*-approach superior since all four 2′-methylseleno nucleoside phosphoramidites are available, and therefore a great flexibility for adequate positioning within the RNA target is attained. This is important since the *Se*-labels should always be placed in double helical regions of a complex fold to minimize structural perturbance (5). In addition, 5-halogen pyrimidine derivatives are highly photo-reactive species (38–40). Inherent radiation damage of 5-halogen-modified nucleic acids during MAD data collection has been reported as a limitation (35). For medium-size RNA (up to 100 nt), the *Se*-approach competes with heavy metal ion derivatization (41–44). Search for a suitable heavy atom is a time-consuming process which requires soaking of the RNA crystals with dozens of compounds at various concentrations, therefore demanding many reasonably good crystals. This can be a serious obstacle, as had been encountered for the Diels–Alder ribozyme where the *Se*-approach finally delivered the key derivative to enable structure determination (16). Moreover, we have recently shown that 2′-methylseleno-modified model duplexes gave crystals in many more buffer conditions compared to their unmodified counterparts, and thus *Se*-modifications hold promise to actively support the crystallization process (6).

CONCLUSION

In the present study, we have shown that highly requested, 2′-methylseleno-functionalized RNA that represents a key derivative for RNA crystallography, is readily accessible by the ACE synthesis protocols elaborated here.

Table 1. RNAs with 2′-methylseleno modifications prepared by ACE RNA solid-phase synthesis[a]

No.	Sequence[a]	Length	Scale	Isolated yield		Molecular weight	
		(nt)	(μmol)	OD $_{260\,nm}$	(nmol)	(Calcd amu)	Found[b] (amu)
S1	5′–A_{Se}CGUA–3′	5	1	10	164	1630.0	1630.3
S2	5′–AC_{Se}GUU–3′	5	1	19	356	1607.0	1607.4
S3	5′–ACG_{Se}UU–3′	5	1	26	472	1607.0	1607.4
S4	5′–ACGU_{Se}A–3′	5	1	9	143	1630.0	1630.3
S5	5′–GCGUGU_{Se}CCG–3′	9	1	13	147	2923.7	2923.5
S6	5′–UUAUUU_{Se}AUdT–3′	9	2	54	547	2814.6	2815.1
S7	5′–GCA_{Se}GAGUUAAAUCUGU–3′	16	1	12	65	5183.1	5181.7
S8	5′–GCAGAGUUAAAUC_{Se}UGC–3′	16	1	64	345	5181.1	5181.7
S9	5′–GCAG_{Se}AGUUAAAUCUGC–3′	16	1	69	372	5181.1	5182.6
S10	5′–GCAGAGUU_{Se}AAAUCUGU–3′	16	1	8	43	5183.1	5183.7
S11	5′–$A_4U_3U_{Se}A_4UU_{Se}UA_4G_4A_6$dA–3′	30	1	49	147	9855.0	9854.4

[a]A_{Se}, 2′-methylseleno adenosine; C_{Se}, 2′-methylseleno cytosine; G_{Se}, 2′-methylseleno guanosine; U_{Se}, 2′-methylseleno uridine.
[b]LC-ESI MS.

The study further shows that the methodological transfer to ACE-based synthesis is not only feasible but highly satisfying since the quality of the modified *Se*-RNAs can well compete with the quality of TOM-made *Se*-RNA. We are convinced that this new access to 2′-methylseleno RNA will contribute to a fast dissemination of the *Se*-approach for RNA X-ray structure analysis.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Conflict of interest statement. None declared.

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