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Chemical Synthesis of Selenium-Modified Oligoribonucleotides and Their Enzymatic Ligation Leading to an U6 SnRNA Stem−Loop Segment

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Abstract: The derivatization of nucleic acids with selenium is highly promising to facilitate nucleic acids structure determination by X-ray crystallography using the multiwavelength anomalous dispersion (MAD) technique. The foundation for such an approach has been laid by Huang, Egli, and co-workers and was exemplified on small DNA duplexes. Here, we present a comprehensive study on the preparation of RNAs containing 2′-*Se*-methylpyrimidine nucleoside labels. This includes the synthesis of a novel 2′-*Se*-methylcytidine phosphoramidite **11** and its incorporation into oligoribonucleotides by solid-phase synthesis. Deprotection of the oligonucleotides is achieved in the presence of millimolar amounts of *threo*-1,4-dimercapto-2,3-butandiol (DTT). With this additive, oxidation products and follow-up side-products are suppressed and acceptable HPLC traces of the crude material are obtained, so far tested for sequences of up to 22-mers. Moreover, an extensive investigation on the enzymatic ligation of the selenium-containing oligoribonucleotides demonstrates the high flexibility of the selenium approach. Our target sequence, an U6 snRNA stem−loop motif comprising all naturally occurring nucleoside modifications beside the *Se*-label is achieved by ligation using T4 RNA ligase.

Introduction

High-resolution structure analysis of biomolecules is of tremendous importance to gain insight into fundamental biological processes. X-ray crystallography using the multiwavelength anomalous dispersion (MAD) technique has been widely used in recent years and accounts for the majority of all new protein crystal structures.[2] This approach implicates the use of selenomethionyl-modified proteins. The selenomethionine replaces natural methionine residues as a result of overexpression of the target protein in the presence of selenomethionine.[3,4] For MAD experiments selenium serves as an effective anomalous scattering center and greatly facilitates phase determination of protein crystals. The first steps to apply this successful concept in nucleic acid structure analysis have been made recently. Huang, Egli, and co-workers reported a 2′-*Se*-methyl-uridine (U_{Se}) phosphoramidite **4** that allowed for the incorporation into small oligonucleotides by solid-phase synthesis.[5] The crystal structure of the A-form DNA duplex $[d(GCGTA)U_{Se}d(ACGC)]_2$ was obtained proving the principle of the concept.[6] Therein, the 2′-*Se*-methylribofuranoses display C3′-endo puckers, consistent with A-form geometry. Moreover, Egli and co-workers documented the preparation and crystal structure of a Z-form DNA duplex with a phosphoroselenoate modified backbone, $[d(C_{PSe}GCGCG)]_2$.[7] The phosphoroselenoate modification was accomplished during solid-phase oligonucleotide synthesis by replacing the standard oxidation agent with potassium selenocyanide. The resulting diastereomeric mixture of phosphoroselenoates had to be separated by anion exchange HPLC. Only very short sequences are therefore readily accessible. For DNA sequences, this limitation might be overcome if nonenzymatic template-directed ligation of oligonucleotide 3′-phosphoroselenoate anions to oligonucleotide 5′-iodides is considered, according to a procedure published by Kool.[8] Diastereomerically pure products would be directly obtained.

The rapidly growing knowledge about the significance of small RNAs in many biological processes is also accompanied by a great demand for RNA structure analysis by X-ray crystallography. In this context, selenium-modified RNA would be immensely valuable in order to facilitate phasing of diffraction data. Unfortunately, little data are available with respect to chemical preparation.[9−11] Only the synthesis of a single hexamer and a short RNA−DNA hybrid have been documented without providing experimental details.[5] Therefore, we have

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(1) The following abbreviations are used: MAD, multiwavelength anomalous dispersion; DMT, dimethoxytrityl; TBDMS, *tert*-butyldimethylsilyl; TOM, (triisopropylsilyl)oxymethyl; Pac, phenoxyacetyl; TBAF, tetrabutylammonium fluoride; DMAP, 4-(dimethylamino)-pyridine; DTT, *threo*-1,4-dimercapto-2,3-butandiol; ATP, adenosine triphosphate; EDTA, ethylendiaminetetraacetate; Tris, 2-amino-2-hydroxymethyl-1,3-propandiol; TMS, tetramethylsilane; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; TLC, thin-layer chromatography; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight; FT ICR ESI-MS, Fourier transformation ion cyclotron resonance electrospray ionization mass spectrometry.

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focused on the development of methodologies for the preparation of selenium-modified RNAs as their availability still represents the bottleneck for broad systematic crystallization screenings. Here, we present a robust preparation protocol for such RNAs. Our approach relies on the incorporation of 2′-*Se*-methyl modified uridine and cytidine nucleosides, **4** and **11**. To the best of our knowledge, this is the first study that demonstrates comprehensively the synthetic feasibility of high-purity selenium-labeled RNA with a length of up to thirty nucleotides and in amounts sufficient for crystallization screenings.

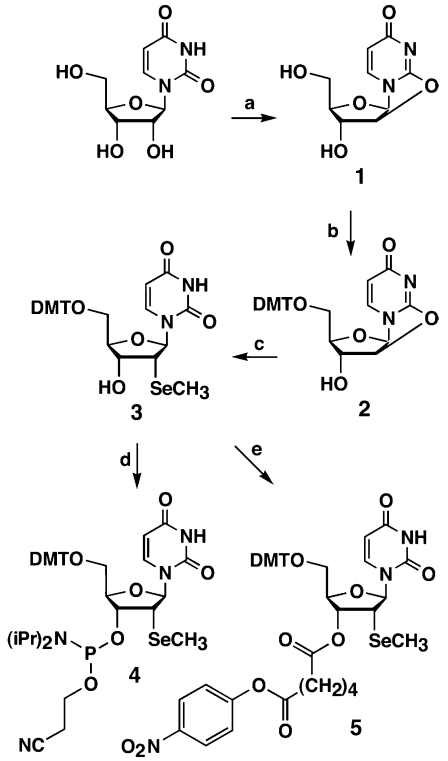
Results and Discussion

We started our studies on selenium-modified RNA based on the knowledge reported on 2′-*Se*-methyl modified DNA.[5,6] Ribose 2′-*Se*-methyl moieties are in principle comparable with the naturally occurring ribose 2′-*O*-methyl modifications and can therefore be considered as an appropriate replacement. These modifications support the C3′-endo type ribose puckering beneficial for A-form geometry. From the crystal structure of the A-form DNA duplex $[d(GCGTA)U_{Se}d(ACGC)]_2$, it is also known that the methylseleno moieties are directed toward the minor groove as generally observed for 2′-*O*-alkylated nucleosides.[6] The van der Waals radius of selenium is 2.00 Å as compared to 1.40 Å for oxygen. This may cause structural perturbations and effect crystallization efforts. However, these perturbations have to be considered less than those observed for 5-bromo- or 5-iodopyrimidine oligonucleotides, which represent the majority of probes with covalent derivatization in nucleic acid crystallography.[12−15] 5-Halogen modifications can cause disruption or severe alterations of nucleobase stacking; they are light-sensitive and may cause decomposition of the oligonucleotide during long-time exposure to X-ray sources.

Syntheses of 2′-*Se*-Methyl Pyrimidine Nucleoside Phosphoramidites. As a first step we intended to improve the accessibility of 2′-*Se*-methyluridine phosphoramidite **4** (Scheme 1). Therefore, uridine was directly transformed into 2,2′-anhydrouridine **1** using diphenyl carbonate in DMF.[16] The 5′-OH was then protected as dimethoxytrityl (DMT) ether to form compound **2**. Subsequent treatment with sodium methylselenide in THF was used for ring-opening to furnish the 2′-*Se*-methyl derivative **3**.[5] Conversion into the corresponding phosphoramidite **4** was achieved in good yields by reaction with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite. Compared to the procedure by Huang, key intermediate **2** was obtained from uridine in two steps instead of five via the 5′-*O*-DMT−3′-*O*-TBDMS functionalized intermediate.[5]

Initial attempts to synthesize the corresponding 2′-*Se*-methylcytidine phosphoramidite **11** by an analogous route starting from commercially available 2,2′-anhydrocytidine failed. We therefore decided on a strategy that involves transformation of the nucleobase. For this, the 3′-OH of derivative **3** was protected applying TBDMS chloride and imidazole in DMF to obtain compound **6** (Scheme 2). Then, reaction of **6** with 2,4,6-triisopropylbenzenesulfonyl chloride in the presence of triethylamine and DMAP in dichloromethane resulted in regioselective O^4-trisylation. After workup, the trisylated derivative **7** was used without further purification and directly converted into **8** upon treatment with aqueous ammonium hydroxide in THF in 79% yield over the two steps. Noteably, treatment of **8** with 7 M NH_3 in anhydrous methanol resulted in the O^4-methyluridine derivative. Acetylation of the amino function was then achieved with acetic anhydride in pyridine to provide **9**, followed by cleavage of the 3′-*O*-TBDMS group with 1 M TBAF and 0.5 M acetic acid in THF. Finally, conversion of **10** into the 2′-*Se*-methylcytidine phosphoramidite **11** proceeded by reaction with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in the presence of ethyldimethylamine in 82% yield.

Scheme 1. Synthesis of 2′-*Se*-methyl Uridine Phosphoramidite[a]



[a] (a) 1.1 equiv of diphenyl carbonate, 3.6% sodium hydrogencarbonate in DMF, 120 °C, 4 h, 75%.[16] (b) 1.1 equiv of DMT-Cl in pyridine, room temperature, 6 h, 67%.[16] (c) 2 equiv of dimethyldiselenide, 6 equiv of sodium borohydride in THF, room temperature, 2 h, 92%.[5] (d) 1.5 equiv of (2-cyanoethyl) *N*,*N*-diisopropylchlorophosphoramidite, 10 equiv of ethyldimethylamine, in CH_2Cl_2, room temperature, 2 h, 93%.[5] (e) 1 equiv of DMAP, 4.5 equiv of bis-(4-nitrophenyl)hexandioate in pyridine/DMF, room temperature, 24 h, 85%.

Oligoribonucleotide Synthesis and Purification. All oligoribonucleotides were synthesized using nucleobase-acetylated and 2′-*O*-TOM protected nucleoside phosphoramidites (Table 1).[17,18] The incorporation of 2′-*Se*-methyluridine and cytidine phosphoramidites was performed with standard DNA/RNA solid-phase synthesis protocols with coupling yields higher than 98%

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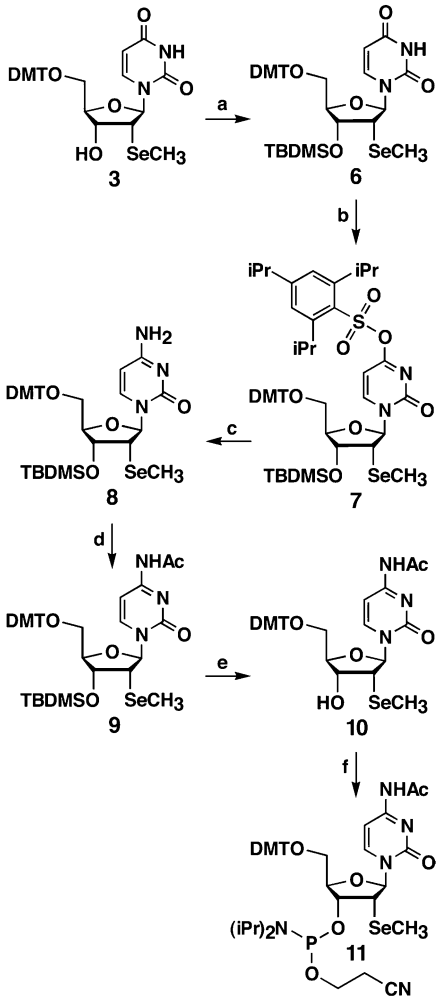
Scheme 2. Synthesis of 2′-*Se*-methyl Cytidine Phosphoramidite[a]



[a] (a) 2 equiv of TBDMS−Cl, 4 equiv of imidazole in DMF, room temperature, 18 h, 95%. (b) 1.5 equiv of 2,4,6-triisopropylbenzenesulfonyl chloride, 10 equiv of NEt_3, 0.12 equiv of DMAP in CH_2Cl_2, room temperature, 1 h. (c) aqueous ammoniumhydroxide in THF, room temperature, 12 h, 79% (over (b) and (c)). (d) 2.5 equiv of acetic anhydride in pyridine, room temperature, 30 min, 94%. (e) 1 M TBAF, 0.5 M acetic acid in THF, room temperature, 2.5 h, 87%. (f) 1.5 equiv of (2-cyanoethyl) *N*,*N*-diisopropylchlorophosphoramidite, 10 equiv of ethyldimethylamine, in CH_2Cl_2, room temperature, 2 h, 82%.

according to the trityl assay (supporting information). For phosphite oxidation, a 10 mM solution of I_2 in acetonitrile/2,4,6-collidine/water was applied for 1 min after each coupling. Even though the 2′-*Se*-methyl nucleosides were placed at the 3′-end of a sequence and therefore repeatedly exposed to oxidative conditions, the coupling yields throughout the various syntheses were comparable to those of non-selenium sequences. This suggested that the selenium moieties are sufficiently stable during strand assembly although oxidation products and follow-up elimination products could not be completely excluded at this level.

After strand assembly, we originally encountered severe difficulties with respect to deprotection of the selenium-containing oligoribonucleotides. Standard conditions, such as CH_3NH_2 in ethanol/water for the deprotection of acetyl and cyanoethyl groups and cleavage from the solid support followed by treatment with 1 M TBAF in THF to cleave the silyl ethers, resulted for the majority of the crude products in HPLC traces showing multiple peaks. Variation of the conditions using CH_3NH_2 in ethanol, NH_3 in methanol, or aqueous ammonia

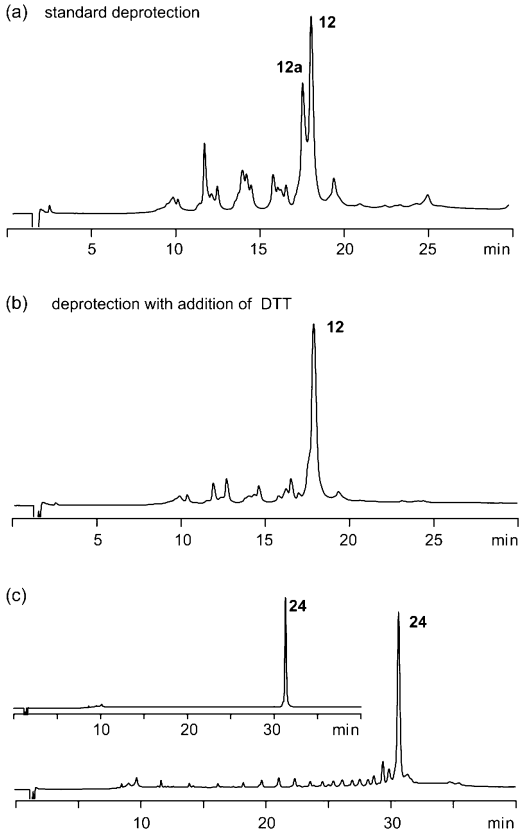


Figure 1. Optimization of the deprotection of *Se*-derivatized oligoribonucleotides. (a) HPLC trace of crude $CGCGU_{Se}GG$ **12** after standard deprotection conditions for 2′-*O*-TOM RNAs (1, $MeNH_2$ in $EtOH/H_2O$, 6 h, room temperature; 2, 1 M TBAF in THF, 12 h, room temperature). Major products were isolated and characterized individually by MALDI-TOF MS: **12** (*m*/*z* calcd $[M + H]^+$ 2313.4, found 2312.9±2); oxidation product **12a** ($[M + H]^+$, found 2328.9±2); for details see supporting information. (b) HPLC trace of crude $CGCGU_{Se}GG$ **12** obtained by addition of 10 mM DTT during treatment with the above-mentioned deprotection solutions. (c) HPLC trace of crude and purified (inset) $AAGC_{Se}ACACAAACC(dA)$-(dG)(dA)CGGCC **24** (50 mM DTT). Anion exchange HPLC: Dionex DNAPac (4 × 250 mm), 80 °C, 1 mL/min, 0−40% B in 30 min (0−60 B in 45 min for (c)); A, 25 mM Tris·HCl, 6 M urea, pH 8.0; B, same as A + 0.5 M $NaClO_4$.

combined with varying conditions for TOM deprotection with fluoride at different temperatures and with different reaction times were not successful. However, we finally found that addition of millimolar amounts of *threo*-1,4-dimercapto-2,3-butandiol (DTT) to the solutions of CH_3NH_2 in ethanol/water drastically improved the quality of the crude materials and also improved reproducibility.[19] This is illustrated for sequence **12**, 5′-$CGCGU_{Se}GG$ in Figure 1. Concerning this sequence, we also purified the major byproduct **12a** that was observed when deprotection conditions without DTT had been applied. By MALDI-TOF mass spectrometry a $[M + H]^+$ signal was obtained with 16 mass units higher than expected for **12**, 5′-$CGCGU_{Se}GG$, indicating that the selenium moiety was most likely oxidized (supporting information). From the above, it is obvious that DTT reduces the amount of oxidation products. Oxidation of the Se moieties most likely occurs during strand assembly when after each coupling step the phosphite triester is transformed into the corresponding phosphate triester by treatment with I_2. To understand how DTT acts on the oxidized

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Table 1. Solid Phase Synthesis of RNA Oligonucleotides Containing 2′-*Se*-Methyl Nucleosides[a]

			isolated yield		molecular weight	
no.	sequence[a]	scale, μmol	$OD^{260\ nm}$	nmol	calcd amu	found[b] amu
12	5′-CGCGU$_{Se}$GG-3′	0.5	8.3	116	2312.4	2311.9
13	5′-CGCGU$_{Se}$GGC-3′	0.7	10.4	131	2617.6	2618.6
14	5′-pU$_{Se}$GACACGCUp-3′	0.3	8.3	86	3051.7	3054.5
15	5′-pUGACACGCU$_{Se}$p-3′	0.3	4.4	46	3051.7	3053.3
16	5′-pUGACAC$_{Se}$GCUp-3′	0.3	2.3	24	3051.7	3054.4
17	5′-pUGACmACGCU$_{Se}$p-3′	0.3	2.9	30	3065.7	3064.1
18	5′-pUGACmAC$_{Se}$GCUp-3′	0.3	2.1	22	3065.7	3067.3
19	5′-GC$_{Se}$GGCGGCGGC-3′	0.3	10.3	93	3652.2	3655.2
20	5′-GCGGCGGCGGU$_{Se}$-3′	3	115	1000	3653.2	3655.7
21	5′-GC$_{Se}$GGCGGC-3′	2	22.0	270	2656.6	2657.8
22	5′-U$_{Se}$GCGGCGGCGGC-3′	1	16.9	140	3958.3	3959.1
23	5′-anthracene(heg)-GGAGCU$_{Se}$CGCC$_{Se}$C-3′	2	126		4168.6	4168.2
24	5′-AAGC$_{Se}$CACACAAACC(dA)(dG)(dA)CGGCC-3′	2	67.8	270	7057.3	7059.7
25	5′-AAGC$_{Se}$CACACAAACC(dA)(dG)(dA)C$_{Se}$GGCC-3′	0.3	2.5	10	7134.3	7136.6

[a] U$_{Se}$, 2′-*Se*-methyluridine; C$_{Se}$, 2′-*Se*-methylcytidine; Cm, 2′-*O*-methylcytidine; dA, 2′-*O*-deoxyadenosine; dG, 2′-*O*-deoxyguanosine; heg, hexaethyleneglycol. [b] MALDI-TOF MS; molecular weights detected as $[M + H]^+$; $[M + Na]^+$ for sequence **23**.

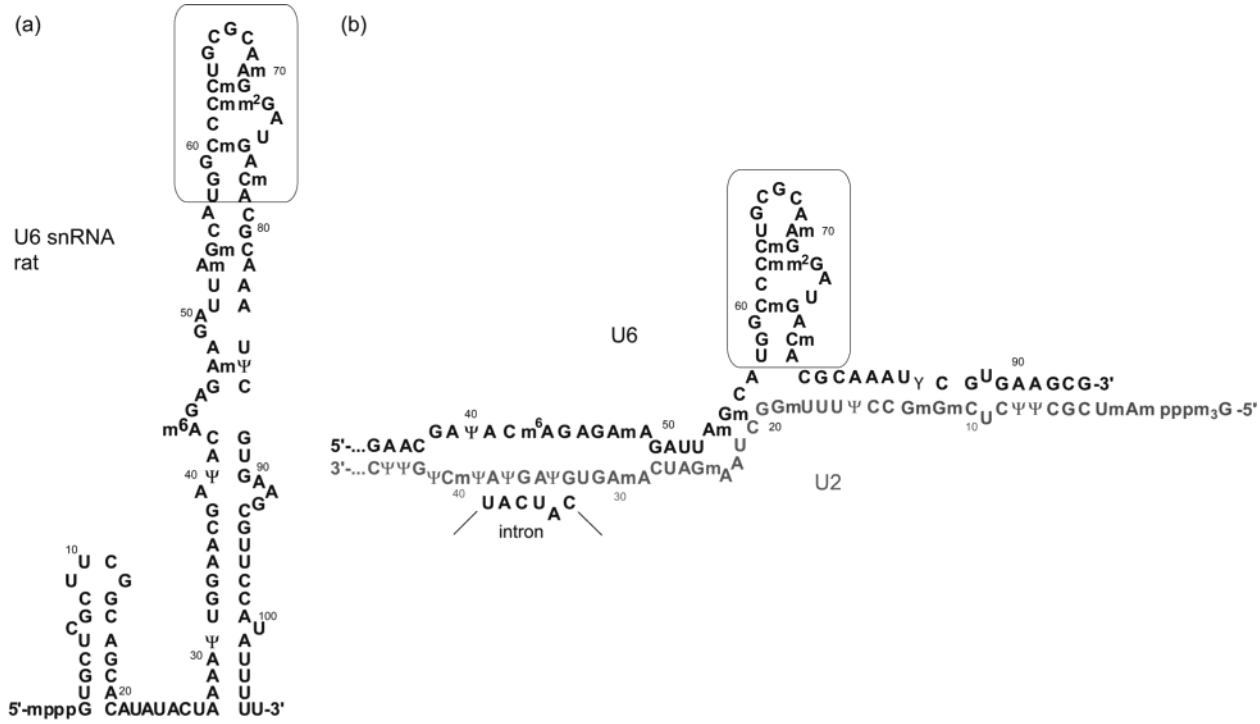

Figure 2. (a) Secondary structure of rat spliceosomal U6 snRNA.[21] (b) U2/U6 snRNA interaction at the catalytic center of the spliceosome, maintaining the boxed stem−loop segment of nucleotides 57−78. Am, 2′-*O*-methyladenosine; Cm, 2′-*O*-methylcytidine; Gm, 2′-*O*-methylguanosine; Um, 2′-*O*-methyluridine; m^{6}A, N^6-methyladenosine; m^{2}G, N^2-methylguanosine; **Ψ**, pseudouridine.

products, such as **12a**, is issue of our ongoing work. In this context, we note that the use of a 1.1 M solution of *tert*-butylhydroperoxide in THF instead of treatment with I_2 was tested and yielded products of comparable quality.

We further note that after evaporation of the basic deprotection solution, the nonvolatile DTT was not separated from the crude product but directly mixed with the TBAF/THF solutions to cleave the TOM groups. DTT was removed after the two-step deprotection procedure together with salts by size exclusion chromatography on a Sephadex column.

Purification of the crude deprotected oligoribonucleotides was then accomplished by anion exchange chromatography on semipreparative columns under strong denaturating conditions (6 M urea, 80 °C). Table 1 shows a series of oligoribonucleotides with up to 22 nucleotides and containing up to two methylseleno nucleosides. The molecular weights of the purified sequences have been confirmed by MALDI-TOF mass spectrometry (supporting information). Sequences **19**−**24** have been prepared in larger amounts to enable crystallization screenings that are currently in progress in the laboratory of our collaborators.

Enzymatic Ligation of Oligoribonucleotides Containing 2′-*Se*-Methyl Nucleosides. We intended to prove the accessibility also of larger seleno-RNAs by a comprehensive enzymatic ligation study using T4 RNA ligase. T4 RNA ligase possesses the ability to join the 3′-hydroxyl and the 5′-phosphate termini of individual ribonucleic acid strands.[20] As target sequence we have chosen a rat spliceosomal U6 snRNA stem−loop motif (Figure 2, box).[21] This motif comprising the

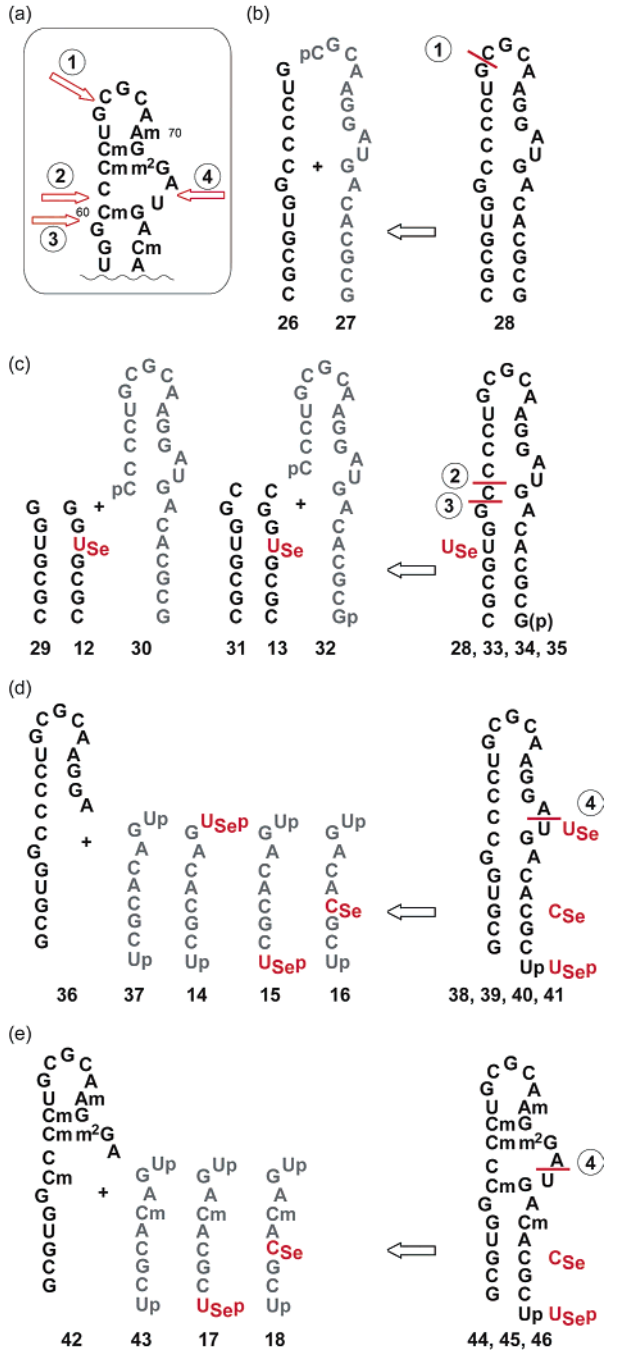

Figure 3. (a) Target stem−loop motif of rat spliceosomal U6 snRNA. Arrows indicate ligation sites. (b−e) Conception of the different ligation experiments with T4 RNA ligase. Details are given in the text. U_{Se}, 2′-*Se*-methyluridine; C_{Se}, 2′-*Se*-methylcytidine.

nucleotides 57−78 should be stabilized by additional cytidine-guanosine base pairs and should contain all the naturally occurring nucleoside modifications beside the selenium label (Figure 3). As indicated by arrows, we considered four potential sites for the enzymatic ligation of such an oligoribonucleotide (Figure 3a). First experience was collected from the ligation of nonmodified sequences to optimize the general reaction and buffer conditions. Strand concentrations of 40−120 μM and 0.4 U/μL T4 RNA ligase in Tris buffer (pH 7.8) in the presence of $MgCl_2$, DTT, and ATP at 37 °C turned out to be appropriate. Good overall ligation yields were, however, only obtained if the donor substrate (providing the 5′-phosphate terminus) was represented by a 5′,3′-bisphosphate oligoribonucleotide. When 5′-phosphate oligoribonucleotides with 3′-OH termini were used as donor substrates, cyclization of the donor and byproducts from repeated ligation of the donor with already formed ligation products occurred significantly (Figure 3b,c and supporting information; **28**, **33**−**35**). The latter could only be avoided when the 5′-phosphate oligoribonucleotide and the partner strand to be ligated formed a highly stable duplex prior to the treatment with ligase and when the ligation position was situated in a loop or bulge region of the resulting product (Figure 3b; **28**).

Among donors, 5′-terminal pyrimidines are known to be slightly preferred over purines. Among acceptors, 3′-terminal adenosines are the best acceptors, cytidines and guanosines show intermediate reactivity, and terminal uridine residues are poor substrates.[20] With respect to ligation site 4 of our target sequence, optimal requirements are therefore fulfilled (Figure 3d-e; Table 2). We also found that 2′-*Se*-methyl nucleosides in the 5′-terminal position of the donor (**14**) can be ligated as efficiently as standard nucleosides (Figure 3d and supporting information; **39**). T4 RNA ligase recognizes only the first nucleobase and first two phosphates of the donor. With respect to the acceptor substrates the requirements are more stringent. The recognition site for the acceptor encompasses three nucleosides and their bridging phosphates.[20] Thus, a 2′-*Se*-methyl nucleoside in the third position from the 3′-OH terminus did hamper ligation almost completely compared to the unmodified counterpart (Figure 3c and Supporting Information; **12** versus **29**), whereas a 2′-*Se*-methyl nucleoside positioned four nucleosides apart from the 3′-OH terminus did not (Figure 3c; **13** versus **31**). Remarkably, the modified nucleoside N^2-methylguanosine in the second position from the 3′-OH terminus of the acceptor **42** did also not reduce ligation rate or yield (Figure 3e; Table 2, **45**, **46**). Figure 4 illustrates two typical sets of HPLC traces for the ligation of sequences comprising the naturally occurring methylations, one referring to the 2′-*Se*-methyluridine target sequence **45**, the other referring to the 2′-*Se*-methylcytidine target **46**.

The identities of the ligation products without selenium were confirmed by co-injection with the identical sequences obtained by solid-phase synthesis (**28**, **34**, **38**, **44**; supporting information). For the subset of ligations listed in Table 2 we have isolated the product compounds by anion exchange HPLC and characterization was done by MALDI-TOF mass spectrometry.

The set of experiments presented above has shown that the selenium-modified RNAs are stable throughout enzymatic ligation conditions. Our U6 snRNA target sequences are only about 30 nucleotides in length. We have chosen this length to enable a broad study with respect to the optimization of various ligation parameters and thereby permitting a fast and reliable characterization of the full-length products. The ligation approach is amenable for longer selenium-containing RNAs and also for the isolation of larger amounts. This is currently in progress in our laboratory for different target sequences of larger size and with multiple Se labels.

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Table 2. Selected Ligation Experiments of an U6 snRNA Stem−Loop Motif with T4 RNA Ligase[a]

					isolated yield			molecular weight	
no	ligation product[a]	scale nmol	ligation conditions[b]	calcd yield, %[c]	$OD^{260\ nm}$	nmol	%	calcd amu	found[d] amu
38	5′-GCGUGGCCCCUGCGCAAGG AUGACACGCUp-3′	20	100 μM, 0.20 U/μL, 37 °C, 4 h	48	2.3	7.5	37	9392.7	9399.7
40	5′-GCGUGGCCCCUGCGCAAGG AUGACACGCU$_{Se}$p-3′	20	120 μM, 0.30 U/μL, 22 °C, 12 h	52	2.7	8.8	44	9469.6	9475.8
41	5′-GCGUGGCCCCUGCGCAAGG AUGACAC$_{Se}$GCUp-3′	8	40 μM, 0.21 U/μL, 37 °C, 4 h	44	1.0	3.3	41	9469.6	9474.6
44	5′-GCGUGGCmCCmCmUGCGCA AmGm^{2}GAUGACmACGCUp-3′	10	80 μM, 0.20 U/μL, 37 °C, 4 h	45	1.1	3.5	35	9476.8	9482.8
45	5′-GCGUGGCmCCmCmUGCGCA AmGm^{2}GAUGACmACGCU$_{Se}$p-3′	13	100 μM, 0.30 U/μL, 22 °C, 15 h	60	2.0	6.4	49	9553.8	9559.9
46	5′-GCGUGGCmCCmCmUGCGCA AmGm^{2}GAUGACmAC$_{Se}$GCUp-3′	17	140 μM, 0.40 U/μL, 22 °C, 12 h	44	2.2	7.1	41	9553.8	9561.8

[a] U_{Se}, 2′-*Se*-methyluridine; C_{Se}, 2′-*Se*-methylcytidine; Cm, 2′-*O*-methylcytidine; Am, 2′-*O*-methyladenosine; m^2G, N^2-methylguanosine. [b] Concentrations of each ligation fragment and of T4 RNA ligase in ligation buffer (50 mM Tris.HCl, pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP), reaction temperature, and reaction time. [c] Yield calculated from HPLC traces taking into account different extinction coefficients of fragments and ligation products. [d] MALDI-TOF MS; molecular weights detected as $[M + H]^+$.

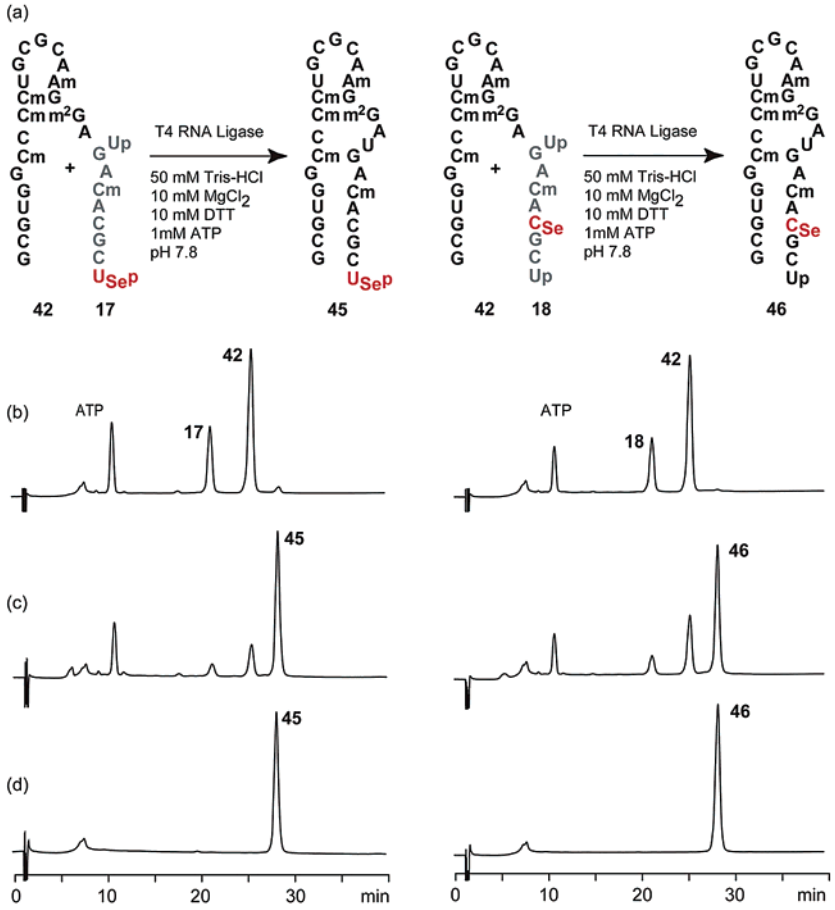

Figure 4. (a) Enzymatic ligation of 2′-*Se*-methyluridine and 2′-*Se*-methylcytidine derivatized oligoribonucleotides with T4 RNA ligase ($c_{(42,17,18)} = 120\ \mu$M each; 0.40 U/$\mu$L ligase). HPLC traces of reaction mixtures at ligation start (b), after 12 h at 22 °C (c), and of the isolated products (d). 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 $NaClO_4$; see also Table 2.

Conclusion

With the present work we have documented a robust protocol for the synthesis of 2′-*Se*-methyluridine and -cytidine phosphoramidites and their incorporation into oligoribonucleotides. By solid-phase synthesis sequences with up to 25 nucleotides and including up to two Se labels are readily accessible. Key feature for the successful and reproducible preparation is the use of millimolar amounts of *threo*-1,4-dimercapto-2,3-butandiol (DTT) during the deprotection procedure. Moreover, various Se-labeled sequences of an U6 snRNA stem−loop motif of about 30 nucleotides in size have been achieved by enzymatic ligation with T4 RNA ligase. This basic ligation study involving the chemically synthesized Se-labeled oligoribonucleotides should demonstrate the flexibility of the selenium approach and the potential for an extension to even larger target sequences. This may attract the interest of structural biologists dealing with RNA X-ray crystallography.

Experimental Section

Materials. ^{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 ppm) or DMSO-d_6 (2.49 ppm) for ^{1}H NMR spectra; $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.5 ppm) for ^{13}C NMR spectra. ^{31}P-shifts are relative to external 85% phosphoric acid. UV spectra were recorded on a Varian Cary 100. Analytical thin-layer chromatography (TLC) was carried out on silica 60F-254 plates. Compounds on the plates were visualized by dipping into a solution of ethanol (180 mL), anisaldehyde (10 mL), concentrated H_2SO_4 (10 mL), and acetic acid (2 mL) and subsequent heating with a heat gun. Flash column chromatography was carried out with silica gel 60 (230−400 mesh). Packing of silica gel columns was performed with 1% Et_3N added to the corresponding starting eluent. All reactions were carried out under Ar atmosphere. Chemical reagents and solvents were purchased from commercial suppliers and used without further purification. Organic solvents for reactions were dried overnight over freshly activated molecular sieves (4 Å).

Phosphoramidite Synthesis. We note that selenium is highly toxic and that handling of the selenium-derivatized compounds requires wearing of protective clothing and a good fume hood.

2,2′-*O*-Anhydro(*β*-D-arabinofuranosyl)uracil (1) was prepared with slight modifications according to ref 16. To a stirred suspension of diphenyl carbonate (1.92 g; 9.0 mmol) in *N*,*N*-dimethylformamide (2.2 mL), uridine (2.0 g; 8.2 mmol) was added. The slurry was heated to 80 °C at which point sodium bicarbonate (25 mg; 0.3 mmol) was added and the reaction mixture was heated to 120 °C. Gas evolution was observable, a clear solution resulted which soon deposited the solid product. Once the evolution of gas subsided (after 3−4 h), the suspension was cooled to room temperature, the precipitate was isolated by filtration, and the product was washed with methanol. Yield: 1.4 g of **1** as white powder (75%). TLC (CH_2Cl_2/MeOH 85/15): R_f 0.2. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.17−3.28 (m, 2 H, H_2–C(5′)); 4.06 (m, 1 H, H−C(4′)); 4.37 (m, 1 H, H−C(3′)); 4.95 (triplettoid, 1 H, HO−C(5′)); 5.18 (d, J = 6.2 Hz, 1 H, H−C(2′)); 5.84 (m, 2 H, H−C(5) + HO−C(3′)); 6.29 (d, J = 6.2 Hz, 1 H, H−C(1′)); 7.82 (d, J = 7.4 Hz, 1 H, H−C(6)) ppm.

2,2′-*O*-Anhydro-5′-O-(4,4′-dimethoxytrityl)(*β*-D-arabinofuranosyl)uracil (2) was prepared with slight modifications according to ref 16. To a stirred suspension of **1** (500 mg; 2.2 mmol) in dry pyridine (5 mL) at room temperature, 4,4′-dimethoxytrityl chloride (820 mg, 2.4 mmol) was added in two portions over a period of 2 h. The suspension turned into a clear solution during 1−3 h. The resulting orange solution was stirred for another 4−6 h and the reaction was monitored by TLC (CH_2Cl_2/MeOH = 9/1); when the reaction was complete, methanol (0.5 mL) was added and the solution was stirred for 30 min. The solvents were evaporated; the residue was dissolved in dichloromethane and extracted with 5% citric acid, water, and sodium bicarbonate solution. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH = 98/2 to 90/10 (+1% NEt_3)). Yield: 775 mg of **2** as colorless foam (67%). TLC (CH_2Cl_2/MeOH 9/1): R_f 0.6. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.80 (dd, J = 10.0, 7.5 Hz, 1 H, H−C(5′)); 2.93 (dd, J = 10.0, 4.0 Hz, 1 H, H−C(5′)); 3.72 (s, 6 H, 2 OCH_3); 4.20 (m, 1 H, H−C(4′)); 4.29 (m, 1 H, H−C(3′)); 5.19 (d, J = 6.0 Hz, 1 H, H−C(2′)); 5.86 (d, J = 7.5 Hz, 1 H, H−C(5)); 5.94 (s, br, 1 H, HO−C(3′)); 6.31 (d, J = 6.0 Hz, 1 H, H−C(1′)); 6.82 (m, 4 H, H−C(ar)); 7.12−7.28 (m, 9 H, H−C(ar)); 7.93 (d, J = 7.5 Hz, 1 H, H−C(6)) ppm.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxy-2′-*Se*-methyluridine (3) was prepared with slight modifications according to ref 5. Sodium borohydride (215 mg, 5.67 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 mL). Dimethyl diselenide (180 μL; 1.89 mmol) was slowly injected to this suspension, followed by dropwise addition of anhydrous ethanol; 0.5 mL were required till 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 **2** (500 mg; 0.945 mmol) in dry THF (10 mL). The reaction mixture was stirred at room temperature for 2 h and monitored by TLC (CH_2Cl_2/MeOH = 92/8). When the reaction was complete, water (2 mL) was added and the pH of the mixture was adjusted to 7 by the dropwise addition of 20% acetic acid. The solvents were evaporated under reduced pressure at 40 °C, water was added, and the product was repeatedly extracted into ethyl acetate. The combined ethyl acetate extracts were washed with saturated sodium chloride solution. The organic layer 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 + 1−2% MeOH). Yield: 540 mg of **3** as colorless foam (92%). TLC (CH_2Cl_2/MeOH 92/8): R_f 0.75. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 3 H, $SeCH_3$); 2.62 (d, J = 3.0 Hz, 1 H, HO−C(3′)); 3.51−3.61 (m, 3 H, H_2−C(5′), H−C(2′)); 3.81 (s, 6 H, 2 OCH_3); 4.19 (m, 1 H, H−C(4′)); 4.39 (m, 1 H, H−C(3′)); 5.38 (d, J = 8.3 Hz, 1 H, H−C(5)); 6.18 (d, J = 7.5 Hz, 1 H, H−C(1′)); 6.85 (m, 4 H, H−C(ar)); 7.25−7.38 (m, 9 H, H−C(ar)); 7.78 (d, J = 8.3 Hz, 1 H, H−C(6)); 8.11 (s, br, 1 H, NH) ppm. FT ICR ESI-MS m/z calcd for $C_{31}H_{32}N_2O_7Se$ $[M + Na]^+$ 647.127 56; found 647.127 56.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxy-2′-*Se*-methyluridine 3′-(2-Cyanoethyl)diisopropylphosphoramidite (4) was prepared with slight modifications according to ref 5. Compound **3** (300 mg, 0.48 mmol) was dissolved in a mixture of ethyldimethylamine (520 μL; 4.8 mmol) in dry dichloromethane (5 mL) under argon. After 15 min at room temperature, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (170 mg; 0.72 mmol) was slowly added and the solution was stirred at room temperature for 2 h. The reaction was quenched by the addition of MeOH (0.2 mL). The reaction mixture was diluted with dichloromethane, extracted with saturated sodium bicarbonate solution, and dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by column chromatography on SiO_2 (ethyl acetate/hexane 1/1 to 7/3 (+1% NEt_3)) and isolated as a 1:1 mixture of diastereoisomers. Yield: 368 mg of **4** as colorless foam (93%). TLC (ethyl acetate/hexane 7/3): R_f 0.6. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05−1.28 (m, 24 H, $((CH_3)_2CH)_2N$); 2.04, 2.08 (2s, 6 H, $SeCH_3$); 2.37, 2.62 (2m, 4 H, CH_2-CN); 3.41−3.69 (m, 10 H, $((CH_3)_2CH)_2N$, $POCH_2$, H_2−C(5′)); 3.79 (2s, 12 H, OCH_3); 3.90, 3.93 (2m, 4 H, $POCH_2$); 4.24, 4.30 (2m, 2 H, H−C(4′)); 4.63, 4.68 (2m, 2 H, H−C(3′)); 5.25, 5.31 (2d, 2 H, J = 8.0 Hz, H−C(5)); 6.33 (d, 2 H, H−C(1′)); 6.84 (m, 8 H, H−C(ar)); 7.24−7.37 (m, 18 H, H−C(ar)); 7.80 (d, 2 H, J = 8.0 Hz, H−C(6)); 8.36 (s, br, 2 H, NH) ppm. ^{31}P NMR (121 MHz, $CDCl_3$): 151.14, 151.88. FT ICR ESI-MS m/z calcd for $C_{40}H_{49}N_4O_8PSe$ $[M + Na]^+$ 847.235 60, found 847.232 85; calcd $[M + K]^+$ 863.209 54, found 863.208 37.

3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-2′-*Se*-methyluridine (6). Compound **3** (500 mg, 0.80 mmol) was dissolved in DMF (4 mL) and mixed with imidazole (218 mg; 3.20 mmol) and *tert*-butyldimethylsilyl chloride (242 mg; 1.60 mmol). The colorless solution was kept under argon and stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure. The product was precipitated from the resulting oil by the addition of water, isolated by filtration, washed with water and hexanes, and dried under high vacuum. The crude product was used without further purification. An analytical sample was prepared by column chromatography on silica gel (CH_2Cl_2 + 0−3% MeOH). Yield: 560 mg of **6** as white solid (95%). TLC (CH_2Cl_2/MeOH 98/2): R_f 0.65. UV (MeOH): $\lambda(\epsilon)$ 260 (11 600) nm (L mol^{-1} cm^{-1}). ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.02 (s, 3 H, $SiCH_3$); 0.12 (s, 3 H, $SiCH_3$); 0.86 (s, 9 H, $SiC(CH_3)_3$); 2.04 (s, 3 H, $SeCH_3$); 3.38 (dd, J = 11.0, 2.3 Hz, 1 H, H1−C(5′)); 3.47 (m, 1 H, H−C(2′)); 3.56 (dd, J = 11.0, 3.0 Hz, 1 H, H2−C(5′)); 3.81 (s, 6 H, 2 OCH_3)); 4.09 (m, 1 H, H−C(4′)); 4.48 (m, 1 H, H−C(3′)); 5.33 (d, J = 8.3 Hz, 1 H, H−C(5)); 6.34 (d, J = 7.1 Hz, 1 H, H−C(1′)); 6.85 (d, J = 8.7 Hz, 4 H, H−C(ar)); 7.25−7.38 (m, 9H, H−C(ar)); 7.88 (d, J = 8.3 Hz, 1 H, H−C(6)); 7.95 (s, br, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.80 ($SiCH_3$); −4.73 ($SiCH_3$); 3.35

($SeCH_3$); 18.07 (Si*C*(CH_3)$_3$); 25.66 (SiC(*C*H_3)$_3$); 48.61 (C(2′)); 55.24 (OCH_3); 62.38 (C(5′)); 73.50 (C(3′)); 85.57 (C(4′)); 87.27; 90.01 (C(1′)); 102.50 (C(5)); 113.28 (C(ar)); 127.24, 127.97, 128.18, 130.12 (C(ar)); 134.94, 135.08; 140.18 (C(6)); 144.05, 150.31, 158.80, 163.12 ppm. FT ICR ESI-MS *m*/*z* calcd for $C_{37}H_{46}N_2O_7SeSi$ $[M + Na]^+$ 761.214 21, found 761.214 96.

3′-*O*-*tert*-Butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-2′-*Se*-methylcytidine (8). A solution of **6** (500 mg; 0.68 mmol) and DMAP (10 mg; 0.08 mmol) in dry dichloromethane (5 mL) was treated under argon with triethylamine (950 μL; 6.8 mmol). Subsequently, 2,4,6-triisopropylbenzenesulfonyl chloride (310 mg; 1.02 mmol) was slowly added. The solution was stirred for 1 h at room temperature The reaction mixture was diluted with dichloromethane, extracted with saturated sodium bicarbonate solution, dried over Na_2SO_4, and evaporated. The intermediate **7** (yellow foam) was dried under vacuum for 0.5 h, then dissolved in THF (10 mL), treated with aqueous ammonia (15 mL; 32%), and stirred at room temperature overnight. The solvents were evaporated and the crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH = 98/2 to 92/8). Yield: 395 mg of **8** as slightly yellow foam (79%). TLC (CH_2Cl_2/MeOH 95/5): R_f 0.45. UV (MeOH): $\lambda(\epsilon)$ 260 (10 300) nm (L mol^{-1} cm^{-1}). ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.08 (s, 3 H, $SiCH_3$); 0.08 (s, 3 H, $SiCH_3$); 0.81 (s, 9 H, SiC(CH_3)$_3$); 2.13 (s, 3 H, $SeCH_3$); 3.30 (dd, J = 2.3, 10.5 Hz, 1 H, H1−C(5′)); 3.58 (m, 1 H, H−C(2′)); 3.63 (dd, J = 3.0, 10.5 Hz, 1 H, H2−C(5′)); 3.80 (s, 6 H, 2 OCH_3)); 4.14 (m, 1 H, H−C(4′)); 4.46 (m, 1 H, H−C(3′)); 5.30 (d, J = 7.5 Hz, 1 H, H−C(5)); 6.38 (d, J = 3.9 Hz, 1 H, H−C(1′)); 6.84 (d, J = 7.6 Hz, 4 H, H−C(ar)); 7.26−7.40 (m, 9 H, H−C(ar)); 8.12 (d, J = 7.5 Hz, 1 H, H−C(6)) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.90 ($SiCH_3$); −4.69 ($SiCH_3$); 3.08 ($SeCH_3$); 18.07 (Si*C*(CH_3)$_3$); 25.68 (SiC(*C*H_3)$_3$); 49.12 (C(2′)); 55.27 (OCH_3); 61.95 (C(5′)); 72.10 (C(3′)); 84.65 (C(4′)); 87.00; 90.71 (C(1′)); 94.90 (C(5)); 113.24, 113.27 (C(ar)); 127.13, 127.92, 128.34, 130.25 (C(ar)); 135.27, 135.35; 141.23 (C(6)); 144.27, 158.78, 165.45 ppm. FT ICR ESI-MS *m*/*z* calcd for $C_{37}H_{47}N_3O_6SeSi$ $[M + Na]^+$ 760.230 18, found 760.228 80.

***N*^{4}-Acetyl-3′-*O*-*tert*-butyldimethylsilyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-2′-*Se*-methylcytidine (9).** A solution of **8** (200 mg; 0.27 mmol) in dry pyridine (0.5 mL) was cooled to 0 °C under argon, treated with acetic anhydride (65 μL; 0.68 mmol), allowed to warm to room temperature, and stirred for 30 min. The reaction was quenched by the addition of methanol (0.3 mL). The solvents were evaporated; the oily residue was dissolved in dichloromethane and extracted with 5% citric acid, water, and saturated sodium bicarbonate solution. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH = 99/1 to 95/5). Yield: 197 mg of **9** as colorless foam (94%). TLC (CH_2Cl_2/MeOH 97/3): R_f 0.65. UV (MeOH): $\lambda(\epsilon)$ 272 (6000), 300 (5800) nm (L mol^{-1} cm^{-1}). ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.06 (s, 3 H, $SiCH_3$); 0.08 (s, 3 H, $SiCH_3$); 0.82 (s, 9 H, SiC(CH_3)$_3$); 2.15 (s, 3 H, $SeCH_3$); 2.27 (s, 3 H, $COCH_3$); 3.38 (dd, J = 2, 10.5 Hz, 1 H, H1−C(5′)); 3.58 (m, 1 H, H−C(2′)); 3.67 (dd, J = 2, 10.5 Hz, 1 H, H2−C(5′)); 3.82 (s, 6 H, 2 OCH_3)); 4.20 (m, 1 H, H−C(4′)); 4.44 (m, 1 H, H−C(3′)); 6.39 (d, J = 3.9 Hz, 1 H, H−C(1′)); 6.86 (d, J = 8.0 Hz, 4 H, H−C(ar)); 7.08 (d, J = 7.5 Hz, 1 H, H−C(5)); 7.28−7.40 (m, 9 H, H−C(ar)); 8.47 (d, J = 7.5 Hz, 1 H, H−C(6)); 10.37 (s, br, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.00 ($SiCH_3$); −4.74 ($SiCH_3$); 3.18 ($SeCH_3$); 18.03 (Si*C*(CH_3)$_3$); 24.78 (CO*C*H_3); 25.62 (SiC(*C*H_3)$_3$); 49.26 (C(2′)); 55.22 (OCH_3); 61.55 (C(5′)); 71.68 (C(3′)); 85.04 (C(4′)); 87.24; 91.15 (C(1′)); 96.99 (C(5)); 113.30 (C(ar)); 127.26, 127.96, 128.34, 130.19 (C(ar)); 135.02, 135.13, 143.88; 144.69 (C(6)); 155.06, 158.83, 163.11, 170.97 ppm. FT ICR ESI-MS *m*/*z* calcd for $C_{39}H_{49}N_3O_7SeSi$ $[M + Na]^+$ 802.240 80, found 802.241 21.

***N*^{4}-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-2′-*Se*-methylcytidine (10).** Compound **9** (200 mg; 0.26 mmol) was treated with 0.5 mL of 1 M TBAF/0.5 M AcOH in THF. The solution was stirred at room temperature for 2.5 h. The solvent was evaporated and the product was isolated by column chromatography on SiO_2 (CH_2Cl_2/MeOH = 98/2 to 94/6). Yield: 150 mg of **10** as colorless foam (87%). TLC (CH_2Cl_2/MeOH 97/3): R_f 0.40. UV (MeOH): $\lambda(\epsilon)$ 272 (7200), 300 (7400) nm (L mol^{-1} cm^{-1}). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.17 (s, 3 H, $SeCH_3$); 2.19 (s, 3 H, $COCH_3$); 3.00 (s, br, 1 H, HO−C(3′)); 3.54 (2dd, J = 3.0, 11.3 Hz, 2 H, H_2−C(5′)); 3.66 (m, 1 H, H−C(2′)); 3.81 (s, 6 H, 2 OCH_3)); 4.15 (m, 1 H, H−C(4′)); 4.39 (m, 1 H, H−C(3′)); 6.30 (d, J = 4.5 Hz, 1 H, H−C(1′)); 6.86 (d, J = 9.2 Hz, 4 H, H−C(ar)); 7.14 (d, J = 7.5 Hz, 1 H, H−C(5)); 7.26−7.42 (m, 9 H, H−C(ar)); 8.30 (d, J = 7.5 Hz, 1 H, H−C(6)); 9.48 (s, br, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 4.65 ($SeCH_3$); 24.75 (CO*C*H_3); 51.46 (C(2′)); 55.23 (OCH_3); 62.25 (C(5′)); 70.01 (C(3′)); 84.43 (C(4′)); 87.20; 90.22 (C(1′)); 97.00 (C(5)); 113.37 (C(ar)); 127.17, 128.03, 128.15, 130.06 (C(ar)); 135.21, 135.42, 144.23; 144.49 (C(6)); 155.35, 158.76, 162.88, 170.80 ppm. FT ICR ESI-MS *m*/*z* calcd for $C_{33}H_{35}N_3O_7Se$ $[M + H]^+$ 666.172 17, found 666.172 08, calcd $[M + Na]^+$ 688.154 14; found 688.153 44.

***N*^{4}-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-2′-*Se*-methylcytidine 3′-(2-Cyanoethyl)diisopropylphosphoramidite (11).** Compound **10** (110 mg; 0.165 mmol) was dissolved in a mixture of ethyldimethylamine (180 μL, 1.6 mmol) in dry dichloromethane (5 mL) under argon. After 15 min at room temperature, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (60 mg; 0.25 mmol) was slowly added and the solution was stirred at room temperature for 2 h. The reaction was quenched by the addition of methanol (0.2 mL). The reaction mixture was diluted with dichloromethane, extracted with saturated sodium bicarbonate solution, and dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by column chromatography on SiO_2 (ethyl acetate/hexane 7/3 (+1% NEt_3)) and isolated as a 1:1 mixture of diastereoisomers. Yield: 118 mg of **11** as colorless foam (82%). TLC (CH_2Cl_2/ MeOH 96/4): R_f 0.55. UV (MeOH): $\lambda(\epsilon)$ = 274 (7830), 300 (7950) nm (L mol^{-1} cm^{-1}). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.98−1.25 (m, 24 H, ((CH_3)$_2$CH)$_2$N); 2.11 (s, 6 H, $COCH_3$); 2.15, 2.17 (2s, 6 H, $SeCH_3$); 2.33, 2.59 (2m, 4 H, CH_2CN); 3.41−3.70 (m, 12 H, ((CH_3)$_2$C*H*)$_2$N, $POCH_2$, H_2−C(5′), H−C(2′)); 3.78 (2s, 12 H, OCH_3); 3.80−3.93 (m, 2 H, $POCH_2$); 4.29 (m, 2 H, H−C(4′)); 4.55, 4.61 (2m, 2 H, H−C(3′)); 6.36 (m, 2 H, H−C(1′)); 6.81 (m, 8 H, H−C(ar)); 6.89, 6.95 (2d, br, 2 H, H−C(5)); 7.22−7.37 (m, 18 H, H−C(ar)); 8.30 (m, 2 H, H−C(6)); 8.78 (s, br, 2 H, NH) ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ 151.23, 151.49 ppm. FT ICR ESI-MS *m*/*z* calcd for $C_{42}H_{52}N_5O_8PSe$ $[M + H]^+$ 866.280 22, found 866.278 66.

Oligoribonucleotide Synthesis. 5′-*O*-DMT-2′-*O*-TOM-protected nucleoside phosphoramidites (A, C, G, U), the corresponding 2′-*O*-TOM-standard nucleoside-CPG supports (500 Å), phosphoramidites of *N*^{4}-acetyl-5′-*O*-DMT-2′-*O*-methylcytidine, *N*^{6}-benzoyl-5′-*O*-DMT-2′-*O*-methyladenosine, *N*^{6}-Pac-5′-*O*-DMT-2′-deoxyadenosine, and *N*^{2}-*i*Pr−Pac-5′-*O*-DMT-2′-deoxyguanosine were obtained from GlenResearch, Sterling, VA, or Xeragon AG, Switzerland. The phosphoramidite of 5′-*O*-DMT-*N*^{2}-methyl-*O*^{6}-(4-nitrophenyl-2-ethyl)-2′-*O*-TOM-guanosine was synthesized as described in ref 22. Anthracene hexaethylene glycol phosphoramidite was synthesized as described in ref 23. CPG support **47** for the synthesis of 3′-phosphate oligoribonucleotides was prepared according to ref 24; phosphoramidite **48** for 5′-phosphate labeling was prepared according to ref 25 (Chart 1). 2′-*Se*-methyluridine CPG support **49** was prepared via compound **5**.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxy-2′-*Se*-methyluridine 3′-(4-nitrophenyl hexandioate) (5). Compound **3** (165 mg; 0.26 mmol) was dissolved in pyridine (2 mL) and DMF (2 mL). The mixture was treated with DMAP (33 mg; 0.26 mmol) and bis-(4-nitrophenyl)hexandioate (475 mg; 1.23 mmol). The reaction solution was stirred at room temperature for 24 h. The solvents were evaporated the residue dissolved

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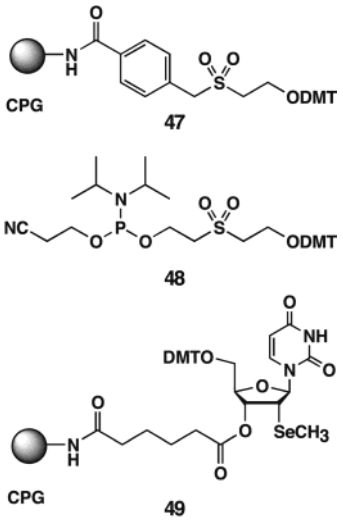
Chart 1. Solid-Phase Supports and Phosphorylation Reagent



in dichloromethane, extracted with half-saturated sodium bicarbonate solution, and dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/acetone 8/1 to 7/1). Yield: 195 mg of **4** as colorless foam (85%). TLC (CH_2Cl_2/acetone 7/1): R_f 0.7. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.79−1.83 (m, 4 H, 2 CH_2); 2.09 (s, 3 H, $SeCH_3$); 2.47 (m, 2 H, CH_2); 2.66 (m, 2 H, CH_2); 3.54 (2 dd, J = 2.5, 8.9 Hz, 2 H, H_2−C(5′)); 3.64 (dd, J = 6.3, 9.0 Hz, 1 H, H−C(2′)); 3.80 (s, 6 H, 2 OCH_3); 4.18 (m, 1 H, H−C(4′)); 5.34 (d, J = 9.0 Hz, 1 H, H−C(5)); 5.53 (dd, J = 2.7, 6.3 Hz, 1 H, H−C(3′)); 6.32 (d, J = 9.0 Hz, 1 H, H−C(1′)); 6.85 (m, 4 H, H−C(ar)); 7.25−7.34 (m, 11 H, H−C(ar)); 7.76 (d, J = 9.0 Hz, 1 H, H−C(6)); 8.27 (d, J = 10.0 Hz, 2 H, H−C(ar)) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 4.92 ($SeCH_3$); 24.01 ($COCH_3$); 33.57 (CH_2); 33.84 (CH_2); 45.21 (C(2′)); 55.26 (OCH_3); 63.05 (C(5′)); 74.98 (C(3′)); 83.29 (C(4′)); 87.58; 88.67 (C(1′)); 102.89 (C(5)); 113.36 (C(ar)); 122.40; 125.20; 127.36; 128.09; 130.06, 130.13 (C(ar)); 134.75; 134.90; 139.82 (C(6)); 144.02; 145.29; 150.46, 155.32; 158.83; 163.03; 170.72; 172.22 ppm.

Preparation of Solid Support 49. To a solution of the active ester **5** (120 mg, 0.14 mmol) in 2 mL of DMF was added long-chain alkylamino 500 Å CPG (0.5 g) followed by ethyldiisopropylamine (200 μL, 1.17 mmol) and pyridine (20 μL, 0.25 mmol). The mixture was agitated for 24 h at room temperature. After filtration, the solids were washed with DMF, methanol, and dichloromethane, dried, suspended in a mixture of 5 mL capping solution A/B (1/1; see below), and shaken for 20 min at room temperature. After filtration, the solids were washed with DMF, methanol, and dichloromethane and dried under high vacuum. Loading of the support was 35 μmol/g.

All oligoribonucleotides were synthesized on a Pharmacia Gene Assembler Plus following DNA/RNA standard methods: detritylation, dichloroacetic acid/1,2-dichloroethane (4/96, 2 min); coupling, phosphoramidites/acetonitrile (0.1 M × 120 μL, 2.5 min); activation by benzyl thiotetrazole/acetonitrile (0.35 M × 360 μL); capping, A, Ac_2O/sym-collidine/acetonitrile (20/30/50), B, DMAP/acetonitrile (0.5 M), A/B = 1/1 (1 min); alternatively, when support **47** was used, capping, A, Ac_2O/sym-collidine/THF (1/1/8), B, *N*-methylimidazole in THF (16/84); oxidation, I_2 (10 mM) in acetonitrile/sym-collidine/H_2O (10/1/5), (1 min). Amidite solutions, tetrazole solutions, and acetonitrile were dried over activated molecular sieves overnight. All sequences were synthesized trityl-off.

Oligoribonucleotide Deprotection and Purification. Deprotection of the selenium-containing oligoribonucleotides and cleavage from the solid support were achieved with $MeNH_2$ in EtOH (8 M, 0.75 mL) and $MeNH_2$ in water (40%, 0.75 mL) containing 10−150 mM DTT for 5−6 h at room temperature; the solution was then evaporated to dryness. Removal of the 2′-*O*-silyl ethers was achieved by treatment with TBAF·3H_2O in THF (1 M, 0.90 mL) for at least 12 h at room temperature. The reaction was quenched by the addition of Tris·HCl (1 M, pH 7.4, 0.95 mL). The volume of the solution was reduced to 1 mL and directly applied on a Sephadex G 10 column (30 × 1.5 cm) controlled by UV detection at 270 nm. The product was eluted with water and evaporated to dryness.

All oligoribonucleotides were purified by anion-exchange chromatography on a semipreparative Dionex DNAPac column (9 × 250 mm) at 80 °C. Flow rate, 2 mL/min; eluant A, 25 mM Tris·HCl, 6 M urea, in H_2O (pH 8.0); eluant B, 25 mM Tris·HCl, 0.5 M $NaOCl_4$, 6 M urea, in H_2O (pH 8.0); detection at 265 nm, Δ5−10% B in A within 20 min. Fractions containing the purified oligonucleotide were desalted by loading onto a C18 SepPak cartridge (Waters/Millipore), followed by elution with 0.1−0.2 M (Et_3NH)HCO_3, water, and then H_2O/CH_3CN (6/4). Combined fractions containing the oligonucleotide were lyophilized to dryness.

Enzymatic Ligations Using T4 RNA Ligase. General procedure for the ligations of **28**, **33**−**35**, and **39**: 2 nmol containing aliquots from aqueous stock solutions of the oligonucleotides to be ligated were mixed, 5 μL of ligation buffer (500 mM Tris·HCl, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP, pH 7.8 at 25 °C) were added and the volume was adjusted to 50 μL by the addition of water to give a final oligonucleotide concentration of 40 μM for each oligonucleotide fragment and a final ligation buffer concentration of 50 mM Tris·HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, pH 7.8 at 25 °C. The reaction solution was heated to 90 °C for 4 min and allowed to cool to 25 °C with a cooling rate of 1 °C/min. Then, 0.5 μL (10 U) of T4 RNA ligase in storage solution (New England Biolabs, 20000 U/mL in 50 mM KCl, 10 mM Tris·HCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol) were added to give a final T4 RNA ligase concentration of 0.2 U/μL. The reaction solution was incubated at 37 °C. After 4 h aliquots (5 μL) of the reaction solution were inactivated by heat (95 °C; 3 min) and analyzed by anion-exchange HPLC (Dionex DNAPac column, 4 × 250 mm, 80 °C, 0−60% B in 45 min; A, 25 mM Tris·HCl, 6 M urea, pH 8.0; B, 25 mM Tris·HCl, 6 M urea, 0.5 M sodium perchlorate, pH 8.0) and denaturating polyacrylamide gel electrophoresis (15% polyacrylamide, 7 M urea). Ligations of **38**, **40**, **41**, and **44**−**46**: ligation experiments were performed as described above; scale, 5−20 nmol; concentration of each ligation fragment, 40−140 μM; concentration of T4 RNA ligase, 0.2−0.4 U/μL; ligation temperature, reaction time, 37 °C, 4 h or 22 °C, 12−15 h (see Table 2). The T4 RNA ligase was heat inactivated at 95 °C for 3 min and the ligation product isolated by anion-exchange HPLC (Dionex DNAPac column, 4 × 250 mm) as described for synthetic products in the preceding section. The identities of the isolated ligation products were confirmed by MALDI-TOF mass spectrometry (Table 2).

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Supporting Information Available: HPLC traces concerning the deprotection optimization of Se-labeled oligoribonucleotides (**12**, **23**, and **24**); HPLC traces and PAGE of selected ligation experiments with T4 RNA ligase; trityl assays for strand assembly; MALDI-TOF and FT-ICR-MS spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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