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An Unconventional Acid-Labile Nucleobase Protection Concept for Guanosine Phosphoramidites in RNA Solid-Phase Synthesis

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Abstract

We present an innovative *O*⁶-*tert*-butyl/*N*²-*tert*-butyloxycarbonyl protection concept for guanosine (G) phosphoramidites. This concept is advantageous for 2'-modified G building blocks because of very efficient synthetic access when compared with existing routes that usually employ *O*⁶-(4-nitrophenyl)ethyl/*N*²-acyl protection or that start from 2-aminoadenosine involving enzymatic transformation into guanosine later on in the synthetic path. The new phosphoramidites are fully compatible with 2'-*O*-*t*BDMS or TOM phosphoramidites in standard RNA solid-phase synthesis and deprotection, and provide excellent quality of tailored RNAs for the growing range of applications in RNA biophysics, biochemistry, and biology.

Keywords

nucleosides; oligonucleotides; protecting groups; RNA; solid-phase synthesis

Introduction

Naturally occurring nucleic acid modifications such as methylation or pseudouridylation have attracted much attention lately because of their unquestionable albeit rather poorly understood roles in epigenetics.[1–3] Likewise, artificial modifications have become increasingly important for the manipulation of nucleic acid properties and to create chemical diversity that is needed for applications in the life sciences.[4] Examples are manifold and include nucleobase-, ribose-, and backbone modifications that, for instance, can improve pharmacokinetic properties of therapeutic oligonucleotides.[5, 6] Other examples include isotope- and dye-labeled RNAs for structural and functional analysis using NMR and fluorescence spectroscopy.[7–9]

Solid-phase synthesis using nucleoside phosphoramidites is the method of choice to introduce a modification of interest in a site-specific manner into RNA. Continuously optimized over the last decades, RNAs of up to 50 to 70 nucleotides are readily accessible. [10–12] The nucleoside building blocks require suitable protection of nucleobase amino and ribose 2'-hydroxy groups. Whereas the former are usually acyl protected, silyl protection (*t*BDMS or TOM)[13, 14] of the latter are most widely applied, and orthoesters (ACE),[15]

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2-cyanoethoxymethyl (CEM),[16] or thiocarbamates (TC)[17] also represent powerful alternatives. For all five approaches, the syntheses of the standard nucleoside phosphoramidites (A, C, G, U) have been very well optimized and are robust. With respect to modified nucleosides, the situation can be quite different.[18, 19] For instance, although ribose 2'-modified pyrimidine nucleoside phosphoramidites are well accessible, the corresponding purine counterparts—in particular guanosine derivatives—are much more challenging and time-consuming in terms of synthesis. This is true for simple 2'-*O*-aminoalkyl modified building blocks, and even more for 2'-SeCH₃, 2'-SCF₃ or 2'-N₃ modifications, to name a few.

To resolve this unsatisfactory situation, we devised a new protecting group pattern for guanosine phosphoramidites that employs *O*⁶-*tert*-butyl, *N*²(bis-[*tert*-butyloxycarbonyl]) (*O*⁶-*t*Bu, *N*²-Boc₂) protection of the guanine nucleobase (Figure 1). At first sight, this seems disadvantageous because orthogonality to the 5'-*O*-DMT group during iterative detritylation during automated strand assembly gets lost. However, our hypothesis was that this pattern should nevertheless work efficiently in solid-phase synthesis for the following reasons (Figure 1): After phosphoramidite coupling, deprotection of *t*Bu/Boc from guanine bases as a concomitant result of the detritylation step of the subsequent coupling cycle is not expected to cause interference, referring to an early study by Hayakawa and co-workers on protection-group-free DNA synthesis. These authors investigated the use of 2'-deoxynucleoside phosphoramidites without base protection and demonstrated that, in contrast to NH₂-free 2'-deoxycytidine or 2'-deoxyadenosine amidites, the corresponding 2'-deoxyguanosine amidites were efficiently coupled without byproducts, yielding DNA oligomers in high quality.[20] However, the major problem with their approach was the reduced solubility and aggregation of the NH₂-free 2'-deoxyguanosine building block.

Encouraged by the assumption that *O*⁶-*t*Bu-*N*²(Boc)₂ guanosine phosphoramidites should be very well soluble, and, importantly, under the foreseeable aspect that such a protection pattern would shorten and/or make synthetic routes towards 2'-modified guanosine phosphoramidites more convenient, we set out to realize this concept.

Results and Discussion

First, we conceived the synthesis of the unmodified guanosine building blocks **2** and **4** (Scheme 1) with either 2'-*O*-*t*BDMS or 2'-*O*-TOM protection. These should then be tested and evaluated with respect to their compatibility in standard RNA solid-phase synthesis, before moving on to 2'-modified guanosines. Access to the starting material, namely *O*⁶-*tert*-butyl-*N*²(Boc)₂ guanosine **1** in large quantities, was straightforward by using the procedure originally introduced by Montesarchio and co-workers,[21] involving two steps from commercially available 2',3',5'-*O*-triacetyl guanosine[22] with one chromatographic purification in 74% overall yield. Tritylation of compound **1** was achieved under standard conditions in 76% yield. Subsequently, compound **1a** was *t*BDMS-protected according to Ogilvie[23] with silver nitrate in rather low regioselectivity (ca. 3:2). However, the overall yield for **1b** was increased to 55% by using base-induced 2',3'-*O*-*t*BDMS equilibration. Finally, phosphitylation with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite under basic conditions in tetrahydrofuran (THF) gave building block **2** in 86% yield.

Because 2',3'-*O*-BDMS equilibration is time consuming, we demonstrated that faster access to large amounts of building block **2** can be achieved directly from guanosine by masking the 3'- and 5'-OH groups with the di-*tert*-butylsilyl clamp,[24] followed by BDMS protection of the 2'-OH group to give compound **5** (Scheme 2). Then, introduction of the *O*⁶-*t*Bu *N*²(Boc)₂ pattern was conducted with di-*tert*-butyldicarbonate in triethylamine to provide **6** after chromatographic purification (64%). Selective cleavage of the 3',5'-*O*-di-*tert*-butylsilyl moiety using HF in pyridine gave the corresponding *O*⁶-*t*Bu, *N*²(Boc)₂ and 2'-*O*-BDMS-protected guanosine **7** in 93% yield. Finally, tritylation and phosphitylation by following standard procedures furnished building block **2**. Starting from guanosine, our route yields **2** in 29% overall yield in five steps with four chromatographic purifications; in total 1.2 g of **2** was synthesized in the course of this study.

Concerning the corresponding guanosine building blocks for the 2'-*O*-{[(triisopropylsilyl)oxy]methyl}(TOM) RNA approach,[14] we noted that the original paper reported high regioselectivity of the tomylation reaction (with a ratio of 12:1 in favor of the 2'-*O* over 3'-*O* isomer)[14] when the exocyclic *N*² amino group of the guanine moiety was protected with a single acyl group only. In analogy, we therefore decided to first cleave one of the two *N*² Boc groups of compound **1**. This was achieved in a robust manner by using aqueous NaOH solution in methanol (Scheme 1). Compound **3** was obtained in 88% yield and subsequent tritylation under standard conditions gave **3a** in 78% yield. Then, tomylation of the 2'-hydroxyl group was conducted via the corresponding cyclic 2',3'-*O*-di-*tert*-butylstannylidene derivative formed in situ in the presence of ethyldiisopropylamine and *t*Bu₂SnCl₂ in 1,2-dichloroethane at 50°C. Treatment with 1.3 equivalents of TOM-Cl[14] gave a mixture of 2'-*O*- and 3'-*O*-alkylated regioisomers in a favorable ratio of 4:1. The desired 2'-*O*-alkylated derivative **3b** was easily isolated in pure form by chromatography on silica gel as the first-eluting isomer in 62% yield. For comparison, direct tomylation of the *N*²-Boc₂ derivative **1a** gave only 32% yield of 2'-*O*-TOM isomer, whereas 40% of 3'-*O*-TOM isomer was isolated. Starting with compound **1**, our route provides **4** in 35% overall yield in four steps with four chromatographic purifications; in total, 1.8 g of **4** was obtained in the course of this study.

To assess the new *t*Bu/Boc protection concept in standard RNA solid-phase synthesis, we prepared a series of RNAs of different length, containing varying numbers of guanosines in the sequence (Figure 2, Table 1). Building blocks **2** and **4** were applied in combination with standard *N*-acylated 2'-*O*-TOM and/or 2'-*O*-BDMS adenosine, cytidine and uridine phosphoramidites. The oligomers were assembled on standard polystyrene or controlled pore glass (CPG) supports, by using the standard implementations of solid-phase synthesis cycles on commercial DNA/RNA synthesizers. Coupling yields of building blocks **2** and **4** were higher than 98% according to the trityl assay.

Cleavage from the solid support and deprotection of the RNA was performed under standard conditions with methylamine in ethanol/water, or, alternatively, in methylamine/ammonia in water followed by treatment with tetrabutylammonium fluoride (TBAF) in THF; importantly, no additional (acidic) deprotection step was applied. Salts were removed by size-exclusion chromatography on a Sephadex G25 column. In general, the crude products gave a major product peak in anion-exchange (AE) HPLC analysis (Figure 2). RNA

oligomers were purified by AE chromatography under strong denaturing conditions (6 M urea, 80°C). The molecular weights of the purified RNAs were confirmed by liquid chromatography-electrospray ionization (LC-ESI) mass spectrometry (MS) (Figure 2). This result supports our hypothesis that the *t*Bu/Boc groups at the guanine bases are concomitantly cleaved during cleavage of the trityl groups (dichloroacetic acid in dichloroethane) when the RNA strand is assembled on solid phase, and that the deprotected guanine nucleobases do not interfere further with successive phosphoramidite coupling.

Our in-depth product analysis included minor peaks that eluted slightly slower or faster than the desired major RNA peak. We assigned these minor products to molecular weights that were either 54 or 56 mass units higher than the desired RNA. The former corresponds to the commonly occurring cyanoethyl-RNA adducts (plus 54 amu)[25] whereas the latter likely refers to *tert*-butyl RNA adducts (plus 56 amu). To support this hypothesis, we changed the solvent of the detritylation solution from dichloroethane to acetonitrile, which we expected to act as carbocation scavenger,[40] and, indeed, the plus 56 amu byproduct either diminished below the detection limit, or decreased significantly (see the Supporting Information, Figure S1).

A major driving force to develop the *t*Bu/Boc guanine approach was the foreseeable advantage of generating short and efficient synthetic routes for 2'-modified guanosine building blocks of RNA solid-phase synthesis. For instance, thus far, 2'-SeCH₃ or 2'-N₃ guanosine building block synthesis[26, 27] relied on *N*²-acetyl-*O*-(4-nitrophenyl)ethyl (NPE) or *N*²-dimethylform-amidine-*O*-NPE functionalization to protect the guanine lactam moiety against electrophilic reagents. Introduction of the *O*-NPE group was accomplished under Mitsunobu conditions.[28] Although optimized over the years in our laboratory, we experienced unsatisfactory yields for this transformation. Additionally, extensive purification protocols (to remove triphenylphosphine oxide) were required, rendering such a pathway time consuming and not very attractive.

We decided to test the *O*-*t*Bu-*N*²(Boc)₂ pattern first for a 2'-SCF₃ guanosine building block. Indeed, the *O*-*t*Bu-*N*²(Boc)₂-protected guanosine derivative **8**[29] can be readily transformed into the phosphoramidite building block **10**, and directly used in RNA solid-phase synthesis (Scheme 3, Table 1, see the Supporting Information). Starting from guanosine, the overall yield was 26%. The present route is therefore two steps shorter compared with the original synthesis (14% overall yield)[29] in which we cleaved the *O*-*t*Bu/*N*²Boc₂ pattern and replaced it with *N*²-[(dimethylamino)methylene] protection for the final building block.[29]

In addition, we demonstrate the convenience of the *t*Bu/Boc pattern for the synthesis of a 2'-*O*-([3-phthalimidopropyl]oxymethyl) guanosine building block (**13**; Scheme 4, Table 1, see the Supporting Information). Starting from compound **3a**, alkylation of the 2'-OH via the cyclic 2',3'-*O*-di-*tert*-butylstannylidene derivative formed in situ, followed by treatment with ([3-phthalimidopropyl]oxymethyl)chloride (**11**) gave the 2'-*O*-([3-phthalimidopropyl]oxymethyl) guanosine derivative **12** after chromatographic separation. Phosphitylation under standard conditions furnished building block **13** in four steps from compound **1** in 29% overall yield. Our path is therefore significantly more efficient

compared with a 10-step route that required eight chromatographic purifications and gave appropriately protected 2'-*O*-(*N*-trifluoroacetyl-2-aminoethyl) guanosine phosphoramidite in 6% overall yield.[30] It is also more convenient compared with a five-step route to 2'-*O*-(3-phthalimidopropyl) guanosine phosphoramidite.[31] Both these routes start with 2-aminoadenosine and require enzymatic transformation using adenosine deaminase (ADA) from calf intestine. We conducted the five-step route several times; in our hands, the overall yield of 1% was very unsatisfactory. Additionally, the availability of ADA is limited and back orders of several months are not unusual, representing an additional handicap. We also consider our path to be superior to a more recent route that was described by Herdewijn and co-workers for 2'-*O*-aminoethoxy(and propoxy)methyl guanosine phosphoramidites because of ease-of-handling.[32]

We point out that we have a constant need for amino-modified building blocks, such as derivative **13**. They are required to synthesize prefunctionalized RNA (containing NH₂-, N₃- and alkyne tethers) for subsequent selective labeling with fluorophores (Cy3, Cy5, Cy7, using NHS and click chemistry)[33, 34] to generate high-performance biotinylated RNA probes for 2- and 3-color single-molecule FRET experiments to study RNA folding and dynamics.[35–37]

As a final example to demonstrate the convenience of the new protection pattern for 2'-modified G building blocks, we synthesized the 2'-deoxy-2'-azidoguanosine phosphodiester derivative **18** (Scheme 5, see the Supporting Information). This derivative can be integrated into a combined phosphotriester/phosphoramidite approach to synthesize 2'-azido RNA, which is a valuable tool for bioconjugation and siRNA technologies.[27]

Conclusion

We have introduced an unconventional, acid-labile protection concept for G building blocks in RNA solid-phase synthesis. The major strength of the *O*⁶-*t*BuN²(Boc)₂ and *O*⁶-*t*BuN²HBoc pattern is that synthetic routes towards 2'-modified G building blocks become advantageous over existing concepts through handling of well soluble derivatives and reducing the number of synthetic steps. The new *t*Bu/Boc building blocks are fully compatible with standard RNA synthesis and deprotection methods, making RNAs with 2'-modified guanosine residues easily available. New initiatives in RNA solid-phase synthesis—such as the one described here—are much needed to generate multiply labeled RNA for biophysical applications and for diagnostic and biotechnological approaches.

Experimental Section

General

All chemicals were purchased from commercial suppliers and used without further purification. All reactions were carried out under argon atmosphere. Marchery–Nagel Polygram SILG/UV₂₅₄ pre-coated polyester sheets (0.2 mm silica gel with fluorescent indicator) were used for thin-layer chromatography; the compounds were visualized at 254 nm. Flash column chromatography was carried out on silica gel purchased from Sigma–Aldrich (pore size 60 Å, 70–230 mesh, 63–200 μm). Triethylamine (1%) was added to the

solvents when packing silica gel columns. All mixtures of liquids in this document are understood as v/v mixtures. Mass spectrometry experiments were performed with a Finnigan LCQ Advantage MAX ion trap instrument. ^1H , ^{13}C , ^{31}P NMR spectra were recorded with a Bruker DRX 300 MHz spectrometer; ^{19}F NMR spectra were measured with a Bruker Avance II + 600 MHz spectrometer equipped with a TCI Prodigy Cryo-Probe. The chemical shifts are referenced to the residual proton signal of the deuterated solvents: CDCl_3 (^1H : $\delta = 7.26$ ppm, ^{13}C : $\delta = 77.1$ ppm), $[\text{D}_6]\text{DMSO}$ (^1H : $\delta = 2.5$ ppm, ^{13}C : $\delta = 39.5$ ppm), ^{31}P shifts are relative to external 85% phosphoric acid, ^{19}F shifts are relative to external CCl_3F . ^1H - and ^{13}C -assignments were based on COSY and HSQC experiments.

O⁶-*tert*-Butyl-*N*-(*tert*-butyloxycarbonyl)guanosine (3)

Compound **1**[21] (1.45 g, 2.69 mmol) was dissolved in methanol (75 mL) before an aqueous sodium hydroxide solution (1.0 mL, 10 M) was added. The reaction mixture was stirred overnight, evaporated to dryness and further dried under high vacuum. The crude product was purified by column chromatographic purification on SiO_2 (6% CH_3OH in dichloromethane) to give **3** (1.05 g, 88%) as a colorless foam. TLC (6% CH_3OH in dichloromethane): $R_f = 0.24$; ^1H NMR (300 MHz, $[\text{D}_6]\text{DMSO}$): $\delta = 1.48$ (s, 9H; Boc), 1.67 (s, 9H; C(6)-*O*-C(CH_3)₃), 3.50–3.68 (m, 2H; H1, H2-C(5')), 3.93 (dd, $J = 7.06, 3.78$ Hz, 1H; H-C(4')), 4.17 (dd, $J = 7.94, 4.52$ Hz, 1H; H-C(3')), 4.58 (dd, $J = 11.17, 5.70$ Hz, 1H; H-C(2')), 4.92 (t, $J = 6.00$ Hz, 1H; HO-C(5')), 5.14 (d, $J = 4.62$ Hz, 1H; HO-C(3')), 5.44 (d, $J = 5.91$ Hz, 1H; HO-C(5')), 5.86 (d, $J = 6.00$ Hz, 1H; H-C(1')), 8.32 (s, 1H; H-C(8)), 9.85 ppm (s, 1H; NH); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 28.02, 28.15$ (2 $\times$ OC(CH_3)₃), 61.48 (C(5')), 70.53 (C(3')), 73.49 (C(2')), 85.67 (C(4')), 87.02 (C(1')), 140.40 ppm (C(8)); ESI-MS: m/z calcd for $\text{C}_{19}\text{H}_{30}\text{N}_5\text{O}_7$: 440.22 [$M+\text{H}$]⁺; found: 440.10.

O⁶-*tert*-Butyl-*N*-(*tert*-butyloxycarbonyl)-5'-*O*-(4,4'-dimethoxytrityl)guanosine (3a)

Compound **3** (1.0 g, 2.28 mmol) was coevaporated with pyridine (6 mL) and subsequently dissolved in anhydrous pyridine (7 mL). 4-(Dimethylamino)pyridine (29 mg, 0.237 mmol) and *N,N*-diisopropyl ethylamine (0.5 mL, 2.87 mmol) was added, followed by 4,4'-dimethoxytriphenylmethyl chloride (880 mg, 2.62 mmol) in two portions over a period of 1 h. The solution was stirred for 13 h before more 4,4'-dimethoxytriphenylmethyl chloride (100 mg, 0.295 mmol) was added and stirring was continued for 3 h. The reaction was ended by the addition of methanol (0.5 mL), and the mixture was evaporated, and subjected to column chromatographic purification on SiO_2 (1–6%, CH_3OH /dichloromethane + 1% NEt_3) to give **3a** (1.32 g, 78%) as a colorless foam. TLC (6%, CH_3OH /dichloromethane + 1% NEt_3): $R_f = 0.74$. ^1H NMR (300 MHz, CDCl_3): $\delta = 1.66$ (s, 9H; boc), 1.83 (s, 9H; C(6)-*O*-C(CH_3)₃), 3.29–3.33 (m, 2H; H1, H2-C(5')), 3.86 (s, 6H; 2 $\times$ OCH₃), 4.51 (d, $J = 4.98$ Hz, 1H; H-C(3')), 4.61 (triplettoid, 1H; H-C(4')), 5.03 (dd, $J = 5.72, 8.12$ Hz, 1H; H-C(2')), 6.07 (d, $J = 6.40$ Hz, 1H; H-C(1')), 6.83–7.40 (m, 13 H; HC(Ar)), 7.72 (s, 1H; NH), 8.19 ppm (s, 1H; HC(8)); ^{13}C NMR (75 MHz, CDCl_3): $\delta = 28.23, 28.46$ (2 $\times$ O-C(CH_3)₃), 55.08 (2 $\times$ OCH₃), 63.81 (C(5')), 73.82 (C(3')), 76.57 (C(2')), 86.75 (C(4')), 91.79 (C(1')), 113.07, 126.69–129.94 (C(Ar)), 138.82 ppm (C(8)); ESI-MS: m/z calcd for $\text{C}_{40}\text{H}_{48}\text{N}_5\text{O}_9$: 742.35 [$M+\text{H}$]⁺; found: 742.07.

***O*⁶-*tert*-Butyl-*N,N*-bis(*tert*-butoxycarbonyl)-3',5'-*O*-di-*tert*-butylsilyl-2'-*O*-*tert*-butyldimethylsilyl guanosine (6)**

Compound **5** [24, 38, 39] (1.56 g, 2.90 mmol) was dissolved in acetonitrile (10 mL) and treated with triethylamine (3.60 mL, 26.1 mmol), di-*tert*-butyldicarbonate (5.06 g, 23.2 mmol) and 4-(dimethylamino)pyridine (177 mg, 1.45 mmol) for 2 days. The reaction was quenched with methanol and the volatiles were removed under reduced pressure. The crude product was subjected to column chromatographic purification on SiO₂ (1–3%, CH₃OH/dichloromethane) to give **6** (1.46 g, 64%) as a yellow foam. TLC (6%, CH₃OH/dichloromethane): *R*_f = 0.82; ¹H NMR (300 MHz, [D₆]DMSO): δ = 0.07, 0.08 (2×s, 6H; Si(CH₃)₂), 0.87, 1.00, 1.06 (3×s, 27 H; 3×*t*bu), 1.35 (s, 18 H; C(2)-N(Boc)₂), 1.65 (s, 9H; C(6)-*O*-C(CH₃)₃), 3.93–4.01 (m, 2H; H-C(4'), H-C(5')), 4.38 (dd, *J* = 7.76, 3.98 Hz, 1H; H-C(5')), 4.63 (d, *J* = 4.98 Hz, 1 H, H-C(2')), (dd, *J* = 8.91, 5.04 Hz, 1H; H-C(3')), 5.99 (s, 1H; H-C(1')), 8.58 ppm (s, 1H; H-C(8)); ¹³C NMR (75 MHz, CDCl₃): δ = −4.22, −4.87 (Si(CH₃)₂), 26.02–28.40 (6×C(CH₃)₃), 68.06 (C(5')), 75.01 (C(3')), 75.70 (C(2')), 84.24 (C(4')), 92.86 (C(1')), 141.00 ppm (C(8)); ESI-MS: *m/z* calcd for C₃₈H₆₈N₅O₉Si₂: 794.46 [*M*+H]⁺; found: 794.42.

***O*⁶-*tert*-Butyl-*N,N*-bis(*tert*-butoxycarbonyl)-2'-*O*-*tert*-butyldimethylsilyl guanosine (7)**

Compound **6** (246 mg, 0.309 mmol) was dissolved in dichloromethane (1.7 mL) in a plastic vessel, cooled in an ice bath, and treated dropwise with HF/pyridine [prepared prior by diluting 47 μL (70% HF, 30% pyridine) with 280 μL pyridine in an ice-cooled plastic vessel]. The reaction was allowed to proceed at 0 °C for 2 h before it was ended and poured into a large excess of half-saturated sodium hydrogencarbonate solution. The mixture was extracted with dichloromethane, dried over sodium sulfate, and evaporated under reduced pressure to give **7** (189 mg, 93%) as a white foam, which was used without further purification. TLC (6%, CH₃OH/dichloromethane): *R*_f = 0.57; ¹H NMR (300 MHz, [D₆]DMSO): δ = −0.17, 0.76 (2×s, 6H; 2×Si-(CH₃)₂), 0.76 (s, 9H; Si-C(CH₃)₃), 1.38 (s, 18 H; C(2)-N(Boc)₂), 1.65 (s, 9H; C(6)-*O*-C(CH₃)₃), 3.57, 3.71 (m, 2H; H-1-, H-2-C(5')), 3.99 (m, 1 H, H-C(4')), 4.14 (dd, *J* = 4.92, 8.82 Hz, 1H; H-C(3')), 4.70 (triplettoid, 1H; H-C(2')), 5.02 (m, 2H; HO-C(3'), HO-C(5')), 5.92 (d, *J* = 5.34 Hz, 1H; H-C(1')), 8.63 ppm (s, 1H; H-C(8)); ¹³C NMR (75 MHz, [D₆]DMSO): δ = −5.29, −5.06 (Si(CH₃)₂), 25.59 (SiC(CH₃)₃), 27.37 (N((CO)OC(CH₃)₃)₂), 27.94 (*O*-C(CH₃)₃), 61.03 (C(5')), 70.10 (C(3')), 75.33 (C(2')), 85.84 (C(4')), 88.07 (C(1')), 142.71 ppm (C(8)); ESI-MS: *m/z* calcd for C₃₀H₅₂N₅O₉Si: 654.35 [*M*+H]⁺; found: 654.27.

***O*⁶-*tert*-Butyl-*N,N*-bis(*tert*-butoxycarbonyl)-2'-*O*-*tert*-butyl dimethyl silyl-5'-*O*-(4,4'-dimethoxy triphenylmethyl) guanosine (7a)**

Identical to **1b**, see the Supporting Information). Compound **7** (1.0 g, 1.53 mmol) was coevaporated with anhydrous pyridine and dissolved in anhydrous pyridine (5 mL). 4-(Dimethylamino)pyridine (19 mg, 0.153 mmol) was added, and the reaction mixture was stirred for 30 min at ambient temperature before 4,4'-dimethoxytriphenylmethyl chloride (570 mg, 1.68 mmol) was added in two portions over a period of 1 h. Stirring was continued overnight. After that time, methanol (700 μL) was added to end the reaction, immediately thereafter the solvents were evaporated (note that extended reaction times would cause

isomerization of 2'/3'-*O*-tBDMS group!) and once coevaporated with toluene. The crude product was subjected to column chromatographic purification on SiO₂ (0–2%, CH₃OH/dichloromethane, 1% NEt₃) to give **7a** (1.13 g, 78%) (identical to **1b**, the Supporting Information) as a yellow foam. TLC (3%, CH₃OH/dichloromethane + 1% NEt₃) *R*_f = 0.72; ¹H NMR (300 MHz, CDCl₃): δ = 0.00, 0.03 (m, 6H; 2×C(2')-*O*-Si-(CH₃)₂), 0.88 (s, 9H; C(2')-*O*-Si-C(CH₃)₃), 1.38 (s, 18 H; C(2)-N(Boc)₂), 1.71 (s, 9H; C(6)-*O*-C(CH₃)₃), 2.63 (d, *J* = 5.70 Hz, 1H; HO-C(3')), 3.43, 3.48 (2×m, 2H; H1, H2-C(5')), 3.75 (s, 6H; 2×OCH₃), 4.18 (m, 1H; H-C(4')), 4.28 (m, 1H; H-C(3')), 4.73 (triplettoid, 1H; H-C(2')), 6.05 (d, *J* = 4.14 Hz, 1H; H-C(1')), 6.80–7.44 (3×m, 13 H; H-C(Ar)), 8.14 ppm (s, 1H; H-C(8)); ¹³C NMR (75 MHz, CDCl₃): δ = −5.3, −4.57 (Si(CH₃)₂), 25.71 (SiC(CH₃)₃), 27.91 (N((CO)OC(CH₃)₃)₂), 28.33 (O-C(CH₃)₃), 63.44 (C(5')), 71.28 (C(3')), 76.40 (C(2')), 83.93 (C(4')), 88.24 (C(1')), 113.28, 127.00–130.06 (C(Ar)), 140.42 ppm (C(8)); ESI-MS: *m/z* calcd for C₅₁H₇₀N₅O₁₁Si: 956.48 [*M*+H]⁺; found: 956.29.

O⁶-tert-Butyl-*N,N*-bis(tert-butyloxycarbonyl)-2'-*O*-tert-butyl dimethylsilyl-5'-*O*-(4,4'-dimethoxytrityl)guanosine 3'-(2-cyanoethyl diisopropylphosphoramidite) (2**)**

Compound **7a** (or **1b**, see SI) (500 mg, 0.523 mmol) was dissolved in absolute tetrahydrofuran (2.5 mL), then 2,4,6-trimethylpyridine (694 mL, 5.23 mmol) and *N*-methylimidazole (29 μL, 0.366 mmol) were added quickly. Immediately thereafter, the resulting mixture was treated with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (372 mg, 1.57 mmol) for 1.5 h until TLC showed full conversion. The reaction was ended by the addition of methanol (100 μL) and stirring was continued for 5 min. Within that time a thick white precipitate formed, which dissolved when it was partitioned between ethyl acetate and half-saturated sodium hydrogencarbonate solution. The organic layer was dried over sodium sulfate and gave a yellow oil after evaporation. The crude product was subjected to column chromatographic purification on SiO₂ (7:3 to 5:5, hexane/ethyl acetate + 1% NEt₃) to give **2** (521 mg, 86%) as a colorless foam. TLC (3%, CH₃OH/dichloromethane + 1% NEt₃): *R*_f = 0.89; ¹H NMR (300 MHz, CDCl₃): δ = 0.00, 0.07 (m, 6H; 2×C(2')-*O*-Si-(CH₃)₂), 0.84, 0.86 (m, 12 H; N(CH(CH₃)₂)₂), 0.91 (s, 9H; C(2')-*O*-Si-C(CH₃)₃), 1.44, 1.45 (2×s, 18 H; 2×C(2)-N(Boc)₂), 1.78 (s, 9H; C(6)-*O*-C(CH₃)₃), 2.54 (2×m, 2×2H; OCH₂CH₂CN), 3.35–3.67 (m, 4H; H1-, H2-C(5'), N(CH(CH₃)₂)₂), 3.83 (s, 6H; 2×OCH₃), 4.05, 4.16 (2×m, 2H; OCH₂CH₂CN), 4.33–4.46 (m, 2H; H-C(3'), H-C(4')), 4.76, 4.85 (2×triplettoid, 1H; H-C(2')), 6.11, 6.14 (2×d, *J* = 4.47, 5.10 Hz, 1H; H-C(1')), 6.88, −6.90, 7.20–7.43, 7.51 (3×m, 13 H; H-C(Ar)), 8.24, 8.28 ppm (2×s, 1H; H-C(8)); ¹³C NMR (75 MHz, CDCl₃): δ = −4.61 (2×C(2')-*O*-Si-(CH₃)₂), 18.20, 20.22 (2×OCH₂CH₂CN), 25.81 (C(2')-*O*-Si-C(CH₃)₃), 25.93 (N(CH(CH₃)₂)₂), 28.00 (N((CO)OC(CH₃)₃)₂), 28.45 (O-C(CH₃)₃), 49.90, 50.03 (2×C(5')), 55.25, 55.31 (2×OCH₃), 56.64, 56.75 (OCH₂CH₂CN), 62.79, 63.11 (N(CH(CH₃)₂)₂), 72.40, 72.57 (C(3')), 75.42, 75.46 (C(2')), 82.81 (C(4')), 87.31, 88.40 (C(1')), 113.43, 121.46–135.59 (C(Ar)), 140.71 ppm (C8); ³¹P NMR (121 MHz, CDCl₃): δ = 149.92, 151.17 ppm; ESI-MS: *m/z* calcd for C₆₀H₈₇N₇O₁₂PSi: 1157.45 [*M*+H]⁺; found: 1157.38.

***O*⁶-*tert*-Butyl-*N*-(*tert*-butyloxycarbonyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[(3-phthalimidopropoxy)methyl]guanosine (**12**)**

Compound **3a** (410 mg, 0.553 mmol) was dissolved in 1,2-dichloroethane (4 mL). *N,N*-Diisopropylethylamine (320 μ L, 1.84 mmol) and di-*tert*-butyltin dichloride (176 mg, 0.579 mmol) were added, and the reaction mixture was stirred for 2 h at ambient temperature. After that time, **11** (164 mg, 0.646 mmol) was added along with more *N,N*-diisopropylethylamine (500 μ L, 2.87 mmol). The reaction was allowed to proceed for 1 h at ambient temperature before it was heated to 50°C for 6 h. The reaction was allowed to cool to ambient temperature, quenched by the addition of methanol, diluted with dichloromethane, and washed with aqueous half-saturated sodium hydrogencarbonate solution. The combined organic layers were dried over sodium sulfate, filtered, and evaporated under reduced pressure to yield, under high vacuum, the crude product as a red foam, which was subjected to column chromatographic purification on SiO₂ (toluene/hexane/ethyl acetate, 3:3:4 + 1% NEt₃) to give pure **12** as a colorless foam, and a fraction containing a mixture of **12** and the corresponding 3'-*O* isomer. This fraction was again subjected to column chromatographic purification on SiO₂ (2%, CH₃OH/chloroform + 1% NEt₃) to increase the total yield of **12** (270 mg, 51%). TLC (3%, CH₃OH/dichloromethane): *R*_f = 0.46; ¹H NMR (300 MHz, CDCl₃): δ = 1.49 (s, 18 H; C(2)-N(Boc)₂), 1.74 (s, 9H; C(6)-*O*-C(CH₃)₃), 1.92 (m, 2H; CH₂CH₂CH₂), 2.91 (d, *J* = 5.76 Hz, 1H; HO-C(3')), 3.25–3.65 (m, 6H; CH₂CH₂CH₂, H1-, H2-C(5')), 3.76 (s, 6H; 2×OCH₃), 4.19 (m, 1H; H-C(4')), 4.53 (dd, *J* = 5.41, 10.84 Hz, 1H; H-C(3')), 4.80 (m, 1H; H-C(2')), 4.86 (dd, *J* = 6.60 Hz, 2H; OCH₂O), 6.09 (d, *J* = 3.69 Hz, 1H; H-C(1')), 6.77–7.42 (m, 13 H; CH(Ar)), 7.66, 7.78 (2×m, 4H; CH(Ar)), 7.92 ppm (s, 1H; H-C(8)); ¹³C NMR (75 MHz, CDCl₃): δ = 28.40, 28.67 (O-C(CH₃)₃, N((CO)OC(CH₃)₃)₂), 28.84 (CH₂CH₂CH₂), 35.49 (CH₂CH₂CH₂), 55.29 (2×OCH₃), 63.91 (C(5')), 65.87 (CH₂CH₂CH₂), 76.00 (C(2')), 78.04 (C(3')), 84.87 (C(4')), 91.17 (C(1')), 96.01 (OCH₂O), 123.28 127.87, 128.15, 130.09, 130.15 (C(Ar)), 133.99 ppm (C(8)); ESI-MS: *m/z* calcd for C₅₂H₅₉N₆O₁₂: 959.42 [*M*+H]⁺; found: 959.06.

***O*⁶-*tert*-Butyl-*N*-(*tert*-butyloxycarbonyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-[(3-phthalimidopropoxy)methyl]guanosine 3'-(2-cyanoethyl diisopropylphosphoramidite) (**13**)**

Compound **12** (159 mg, 0.166 mmol) was dissolved in dichloromethane (3.5 mL). *N,N*-Diisopropylethylamine (258 μ L, 1.48 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (118 mg, 0.498 mmol) were added and the reaction was allowed to proceed at ambient temperature for 4 h. After that time, the reaction was quenched by the addition of methanol and volatiles were evaporated. The crude product was dissolved in a mixture of hexane/ethyl acetate (6:4) and filtered through a silica plug. The filtrate was evaporated and the crude product was subjected to column chromatographic purification on SiO₂ (ethyl acetate/hexane, 1:1 to 7:3) to give **13** (162 mg, 84%) as a colorless foam.

Alternative preparation—A mixture of **12** and the corresponding 2'-*O* isomer (80 mg, 1:1 ratio according to NMR analysis) was dissolved in dichloromethane (2 mL) and treated with *N,N*-diisopropylethylamine (130 μ L) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (90 mg, 0.380 mmol) for 3 h at ambient temperature. The reaction was ended by the addition of methanol (300 μ L) and stirring was continued for 5

min, before the reaction mixture was diluted with dichloromethane and partitioned with half-saturated sodium hydrogencarbonate solution. The combined organic layers were dried over sodium sulfate, filtered, and evaporated. The crude product was subjected to column chromatographic purification on SiO₂ (ethyl acetate/hexane, 1:1 to 6:4), which yielded two well-separated fractions. The faster migrating fraction (TLC (ethyl acetate/hexane, 6:4) R_f = 0.78) contained the two diastereomers of compound **13** (41 mg, 85%) as a colorless foam, as confirmed by ¹H, ¹³C, and ³¹P NMR analysis. The second fraction (TLC (ethyl acetate/hexane, 6:4) R_f = 0.70) contained the two diastereoisomers of *O*⁶-*tert*-butyl-*N,N*-bis(*tert*-butyloxycarbonyl)-5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-[(3-phthalimidopropoxy)methyl]-guanosine 2'-(2-cyanoethyl diisopropylphosphoramidite). TLC (ethyl acetate/hexane, 6:4): R_f = 0.78. ¹H NMR (300 MHz, CDCl₃): δ = 1.15, 1.17 (2×s, 12 H; N(CH(CH₃)₂)₂), 1.48 (s, 9H; C(2)-NH(boc)), 1.74 (s, 9H; C(6)-*O*-C(CH₃)₃), 1.86 (m, 2H; CH₂CH₂CH₂), 2.36, 2.60 (2×t, J = 6.06, 6.03 Hz, 2H; OCH₂CH₂CN), 3.40–3.67 (m, 10 H; H1-, H2-C(5'), OCH₂CH₂CN, N(CH(CH₃)₂)₂, 2×CH₂CH₂CH₂), 3.73, 3.76 (2×s, 6H; 2×OCH₃), 3.85 (m, 2H; OCH₂CH₂CN), 4.11 (m, 2H; N(CH(CH₃)₂)₂), 4.25, 4.34 (2×m, 1H; 2×H-C(4')), 4.55 (m, 1H; H-C(3')), 4.67–4.74 (m, 3H; H-C(2'), OCH₂O), 5.01, 5.10 (m, 2H; H-C(2')), 6.02, 6.07 (2×d, J = 5.88, 4.77 Hz, 1H; H-C(1')), 6.76–7.31 (m, 13 H; H-C(Ar_{DMT})), 7.67, 7.79 (2×m, 4H; H-C(Ar_{phthal})), 7.92, 7.94 ppm (2×s, 1H; H-C(8)); ³¹P NMR (121 MHz, CDCl₃): δ = 151.21, 151.61 ppm; ESI-MS: m/z calcd for C₆₁H₇₆N₈O₁₃P: 1159.53 [*M*-H]⁺; found: 1159.26.

Solid-phase synthesis of RNA using *t*Bu/Boc-protected guanosine phosphoramidites

Standard phosphoramidite chemistry was applied for RNA solid-phase synthesis using the new *O*⁶-*tert*-butyl-*N,N*-bis(*tert*-butyloxycarbonyl) or *O*⁶-*tert*-butyl-*N*-(*tert*-butyloxycarbonyl)-protected guanosine phosphoramidites (**2**, **4**, **10**, and **13**) in combination with 2'-*O*-*t*BDS or 2'-*O*-TOM nucleoside phosphoramidite building blocks (ChemGenes) and polystyrene support (GE Healthcare, Custom Primer Support™, 80 μmolg⁻¹; PS 200). All oligonucleotides were synthesized with a ABI 392 Nucleic Acid Synthesizer by following standard methods: detritylation (80 s) with dichloroacetic acid/1,2-dichloroethane (4:96); coupling (2.0 min) with phosphoramidites/acetonitrile (0.1 M×130 μL) and benzylthiotetrazole/acetonitrile (0.3 M×360 μL); capping (3×0.4 min, Cap A/Cap B=1:1) with Cap A: 4-(dimethylamino)pyridine in acetonitrile (0.5 M) and Cap B: Ac₂O/sym-collidine/acetonitrile (2:3:5); oxidation (1.0 min) with I₂ (20 mM) in tetrahydrofuran (THF)/pyridine/H₂O (35:10 :5). The solutions of amidites and tetrazole, and acetonitrile were dried over activated molecular sieves (4 Å) overnight.

Deprotection of RNA

The solid support was treated each with MeNH₂ in EtOH (33%, 0.5 mL) and MeNH₂ in water (40%, 0.5 mL) for 4–7 h at RT. The supernatant was removed and the solid support was washed three times with ethanol/water (1:1, v/v) or alternatively tetrahydrofuran/water (1:1, v/v). The supernatant and the washings were combined and the whole mixture was evaporated to dryness. To remove the 2'-silyl protecting groups, the resulting residue was treated with tetrabutylammonium fluoride trihydrate (TBAF·3 H₂O) in THF (1 M, 1 mL) at 37°C overnight. The reaction was quenched by the addition of triethylammonium acetate buffer (1 M, pH 7.4, 1 mL). The volume of the solution was reduced and the solution was

desalted with a size exclusion column (GE Healthcare, H₂Prep 26/10 Desalting; 2.6×10 cm; Sephadex G25) eluting with H₂O, the collected fraction was evaporated to dryness and dissolved in H₂O (1 mL). Analysis of the crude RNA after deprotection was performed by anion-exchange chromatography on a Dionex DNAPac PA-100 column (4×250 mm) at 80°C. Flow rate: 1 mL min⁻¹, eluant A: 25 mM Tris *x* HCl (pH 8.0), 6 M urea; eluant B: 25 mM Tris-HCl (pH 8.0), 0.5 M NaClO₄, 6 M urea; gradient: 0–60% B in A within 45 min or 0–40% B in 30 min for short sequences up to 15 nucleotides, ultraviolet detection at 260 nm. For oligoribonucleotides containing 2'-SCF₃ guanosine, the deprotection solutions additionally contained 150 mM of *threo*-1,4-dimercapto-2,3-butandiol (DTT) as described in ref. [29].

Purification of RNA

Crude RNA products were purified on a semi-preparative Dionex DNAPac PA-100 column (9×250 mm) at 80°C with flow rate 2 mL min⁻¹. Fractions containing RNA were loaded on a C18 SepPak Plus cartridge (Waters/Millipore), washed with 0.1–0.15 M (Et₃NH)⁺ HCO₃⁻, H₂O and eluted with H₂O/CH₃CN (1:1). RNA containing fractions were lyophilized. Analysis of the quality of purified RNA was performed by anion-exchange chromatography with the same conditions used for crude RNA; the molecular weight was confirmed by liquid chromatography-electrospray ionization (LC-ESI) mass spectrometry. Yields were determined by ultraviolet photometrical analysis of oligonucleotide solutions.

Mass spectrometry of RNA

All experiments were performed with a Finnigan LCQ Advantage MAX ion trap instrumentation connected to an Amersham Ettan micro LC system. RNA sequences were analyzed in the negative-ion mode with a potential of -4 kV applied to the spray needle. LC: Sample (200 pmol RNA dissolved in 30 µL of 20 mM EDTA solution; average injection volume: 30 µL); column (Waters XTerraMS, C18 2.5 µm; 1.0×50 mm) at 21°C; flow rate: 30 µL min⁻¹; eluant A: 8.6 mM triethylamine, 100 mM 1,1,1,3,3,3-hexafluoroisopropanol in H₂O (pH 8.0); eluant B: methanol; gradient: 0–100% B in A within 30 min; ultraviolet detection at 254 nm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A new protection pattern (*t*Bu/Boc) for guanosine phosphoramidites (top left) that is compatible with standard solid-phase RNA synthesis, is introduced in this work. After strand assembly on solid phase, the RNA carries all protecting groups except the *t*Bu/Boc groups, which were cleaved during iterative detritylation (top right). Subsequently, standard deprotection provides the free RNA (bottom). The approach is advantageous over existing methods for the preparation of RNA with site-specific, 2'-modified guanosines.

Scheme 1.

Synthesis of guanosine phosphoramidites with *t*Bu and Boc nucleobase protection. Reagents and conditions: a) DMT-Cl (1.1 equiv), 4-(dimethylamino)pyridine (0.1 equiv), EtN(*i*Pr)₂ (0.5 equiv), pyridine, 4.5 h, RT, 76% of **1a**; b) AgNO₃ (1.4 equiv), *tert*-butyldimethylsilyl chloride (tBDMS-Cl) (2.5 equiv), pyridine (2.3 equiv), THF, RT, 6 h, 55% of **1b** (after one round of 2'-*O*,3'-*O* tBDMS equilibration in methanol/triethylamine, 9:1; 10 min; RT; starting from the pure, isolated 3'-*O* tBDMS isomer; for a detailed protocol see ref. [7]); c) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (3 equiv), 2,4,6-trimethylpyridine (10 equiv), *N*-methylimidazole (0.7 equiv), THF, 1.5 h, RT, 86% of **2**; d) 0.13 M NaOH, in CH₃OH/H₂O (75:1), RT, overnight, 88% of **3**; e) DMT-Cl (1.3 equiv), 4-(dimethylamino)pyridine (0.1 equiv), EtN(*i*Pr)₂ (1.3 equiv), pyridine, 16 h, RT, 78% of **3a**; f) *t*Bu₂SnCl₂ (1.1 equiv), ethyldiisopropylamine (3.5 equiv), ClCH₂CH₂Cl, RT, 2 h, then TOM-Cl (1.3 equiv), ethyldiisopropylamine (3.4 equiv), 50°C, 6 h, separation of 2' isomer by chromatography (2' isomer: 62% of **3b**, 3' isomer: 15%); g) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (3 equiv), EtN(*i*Pr)₂ (7.6 equiv), CH₂Cl₂, 3.5 h, RT, 83% of **4**.

Scheme 2.

Alternative route to *t*Bu/*Boc*₂-protected guanosine phosphoramidite **2** for RNA solid-phase synthesis. Reagents and conditions: a) di-*tert*-butyl silylditriplate (1.1 equiv), 1*H*-imidazole (5.0 equiv), 0°C, 30 min, then *tert*-butyldimethyl chlorosilane (4.8 equiv), 60°C, 2 h, 72% of **5**; b) di-*tert*-butyldicarbonate (8 equiv), triethylamine (9 equiv), 4-(dimethylamino)pyridine (0.5 equiv), RT, 2 d, 64% of **6**; c) 0.9 M HF in pyridine/CH₂Cl₂, plastic vessel, 0 °C, 2 h, 93% of **7**; d) DMT-Cl (1.1 equiv), 4-(dimethylamino)pyridine (0.1 equiv), pyridine, overnight, RT, 78% of **7a** (identical to **1b**); e) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (3 equiv), 2,4,6-trimethylpyridine (10 equiv), *N*-methylimidazole (0.7 equiv), THF, 1.5 h, RT, 86% of **2**.

Figure 2.

Selection of RNAs that were synthesized by using *t*Bu/Boc-protected amidites **2** or **4** (indicated in *italic style*). Anion exchange HPLC traces (top) of a) 6 nt single-stranded RNA, b) 6 nt G-quadruplex-forming RNA, c) 21 nt RNA, and d) 8 nt RNA, and corresponding LC-ESI mass spectra (bottom) HPLC conditions: Dionex DNAPac column (4 × 250 mm), 80°C, 1 mLmin⁻¹, 0–60% buffer B in 45 min; buffer A: Tris-HCl (25 mM), urea (6 M), pH 8.0; buffer B: Tris-HCl (25 mM), urea (6 M), NaClO₄ (0.5 M), pH 8.0. For LC-ESI MS conditions, see the Supporting Information. The RNA 3'-terminal guanosines originated from solid supports and carried standard acyl protection patterns (see the Supporting Information).

Scheme 3.

Synthesis of 2'-SCF₃ modified guanosine phosphoramidite **10** carrying *t*Bu/Boc nucleobase protection. Reagents and conditions: a) DMT-Cl (1.2 equiv), 4-(dimethylamino)pyridine (0.1 equiv), EtN(*i*Pr)₂ (1.3 equiv), pyridine, 16 h, RT, 83% of **9**; b) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (2.25 equiv), EtN(CH₃)₂ (10 equiv), CH₂Cl₂, 3.5 h, RT, 78% of **10**.

Scheme 4.

Synthesis of 2'-(3-phthalimidopropoxymethyl) modified guanosine phosphoramidite **13** carrying *t*Bu/Boc nucleobase protection. Reagents and conditions: a) i. *t*Bu₂SnCl₂ (1.05 equiv), EtN(*i*Pr)₂ (3.3 equiv), CH₂Cl₂, RT, 1 h; ii. *N*-[3-(chloromethoxy)propyl]phthalimide **11** (1.2 equiv), EtN(*i*Pr)₂ (5.2 equiv), 50°C, 6 h, 51% of **12**; b) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (3 equiv), EtN(*i*Pr)₂ (8.9 equiv), CH₂Cl₂, 3 h, RT, 84% of **13**.

Scheme 5.

Synthesis of 2'-azidoguanosine phosphodiester **18** carrying *t*Bu/Boc nucleobase protection. Reagents and conditions: a) i. trifluoromethanesulfonyl chloride (2.9 equiv), 4-(dimethylamino)pyridine (1.5 equiv), triethylamine (2.3 equiv), CH_2Cl_2 , 0°C , 40 min; ii. NaN_3 (5 equiv) in DMF, RT, overnight, 98% of **15** over two steps; b) HF-pyridine, CH_2Cl_2 , 0°C , 2 h, 79% of **16**; c) DMT-Cl (1.4 equiv), 4-(dimethylamino)pyridine (0.1 equiv), pyridine, RT, 20 h, 72% of **17**; d) 1,2,4-triazole (5.4 equiv), triethylamine (4.9 equiv), 2-chlorophenyldichlorophosphate (2.4 equiv), *N*-methylimidazole (3.9 equiv), THF, RT, 45 min, 78% of **18**. Compound **14** was synthesized in analogy to ref. [21, 39].

Table 1

Selection of RNAs synthesized by using the *t*Bu/Boc-protected guanosine phosphoramidites **2**, **4**, **10**, and **13** (indicated by G*).

RNA sequence 5' to 3' ^[a]	nt ^[b]	Phosphoramidite compound	<i>M</i> _{w(calcd)} [a.m.u.]	<i>M</i> _{w(obs)} [a.m.u.]
UUA G*CG	6	2	1875.19	1874.85
UG*G* G*G*U	6	2	1931.22	1930.90
CG*C G*AA UUC G*CG	12	2	3810.36	3810.17
UAU CUU AUU G*G*C AG*A G*AC CUG	21	2	6674.05	6673.85
UUA G*CG	6	4	1875.19	1875.07
CG*A UCG* AU	8	4	2509.58	2509.35
AUC AG*G* UG*C AA	11	4	3513.21	3513.21
GCC GCC (2'-SCF ₃ G)*U GGU A	13	10	4255.63	4255.59
UGG U(2'-SCF ₃ G)*A AUG AAG CCA CAG G	19	10	6247.88	6247.52
UGC UCC UAG UAC GAG AGG ACC G(2'-O-(CH ₂) ₃ NH ₂ G)*A GUA	27	13	8799.46	8799.32

^[a] In addition to G* amidites (*t*Bu/Boc), 2'-*O*-*t*BDMS nucleoside phosphoramidites were used for the first four sequences, and 2'-*O*-TOM nucleoside phosphoramidites were used for the subsequent six RNAs.

^[b] Number of nucleotides.