Synthesis of Selectively ¹⁵N-Labeled 2'-O-{[(Triisopropylsilyl)oxy]methyl}(=tom)-Protected Ribonucleoside Phosphoramidites and Their Incorporation into a Bistable 32Mer RNA Sequence

by Philipp Wenter and Stefan Pitsch*

Laboratory of Nucleic Acid Chemistry, Ecole Polytechnique Fédérale de Lausanne (EPFL), EPFL-BCH, CH-1015 Lausanne

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We present optimized reaction conditions for the conversion of 2'-O-{[(triisopropylsily])oxy]methyl}-(=tom) protected uridine and adenosine nucleosides into the corresponding protected (3-¹⁵N)-labeled uridine and cytidine and (1-¹⁵N)-labeled adenosine and guanosine nucleosides **4**, **6**, **12**, and **18**, respectively (*Schemes 1-4*). On a DNA synthesizer, the resulting ¹⁵N-labeled 2'-O-tom-protected phosphoramidite building blocks **19–22** were efficiently incorporated into five selected positions of a bistable 32mer RNA sequence **23** (known to adopt two different structures) (*Fig. 1*). By 2D-HSQC and HNN-COSY experiments in H₂O/D₂O 9:1, the ¹⁵N-signals of all base-paired ¹⁵N-labeled nucleotides could be identified and attributed to one of the two coexisting structures of **23**.

1. Introduction. - We are interested in a detailed and quantitative investigation of the RNA folding mechanism, including thermodynamic and kinetic aspects. On the secondary-structure level, folding events lead to changes in the H-bond-mediated Watson - Crick base-pairing pattern. These base-pair contacts can be easily monitored by ¹H-NMR spectroscopy in H_2O because, upon base-pair formation, the H-N(3) of uridines (paired with adenosines) and the H-N(1) of guanosines (paired with cytosines) are protected from fast exchange with the solvent, and are, therefore, detectable. The observation of these characteristic NH ... N signals ('imino-proton' signals [1]), appearing at $\delta 9-15$, allows a sensitive characterization of base-pair existence and dynamics [2]. However, due to signal overlap, detailed information from these $NH \cdots N$ signals can often be obtained for only relatively short RNA sequences (< 50 mers). This serious limitation can be overcome by the introduction of ¹⁵N-labeled nucleotides, allowing the application of heteronuclear NMR experiments that make use of the ${}^{1}J(H,N)$ scalar coupling and the two-bond 'trans'-H-bond scalar coupling $^{2h}J(N,N)$ [3][4]. For the study of folding and refolding processes of rather large systems, such as ribozymes or tRNAs, we are planning to introduce such labeled nucleotides only at representative base pairs within defined secondary-structure motifs. Furthermore, it is sufficient for such NMR studies, to label only the N(3) position of the pyrimidine and the N(1) position of the purine nucleotides, respectively.

Site-specifically isotope-labeled RNA sequences were prepared by transcription/ ligation strategies [5] or by automated chemical synthesis [6]. Whereas enzymatic methods are ideal for the preparation of large sequences containing stretches of labeled nucleotides, chemical synthesis offers the unique possibility to introduce labeled nucleotides anywhere in the sequence, without constraints of position or labeling pattern. We recently introduced a reliable RNA-synthesis method based on $\{2'-O-[(triisopropylsilyl)oxy]methyl\}(=tom)$ -protected ribonucleoside phosphoramidites that allows the preparation of RNA sequences under DNA-coupling conditions [7–9]. In the above-mentioned context, we report here the synthesis of four phosphoramidite analogues, each containing a single ¹⁵N-label (N(3) of uridine/cytosine and N(1) of guanosine/adenosine), their incorporation into a known, bistable 32mer RNA sequence [10]¹), and preliminary NMR experiments.

2. Results. – 2.1. Synthesis of ¹⁵N-Labeled, 2'-O-tom-Protected Phosphoramidites. Several methods for the selective introduction of ¹⁵N-labels into position N(3) of pyrimidine and position N(1) of purine nucleosides, and some examples for the incorporation of such labeled ribonucleosides into RNA sequences have been reported already [12–14]. In all these approaches, first the ¹⁵N-labeled nucleotides were prepared by nucleobase-transformation reactions or nucleoside formation with appropriately labeled nucleobases, followed by introduction of the nucleobase-protecting groups, the 5'-O-(4,4'-dimethoxytrityl) (=(MeO)₂Tr) group, the 2'-O-(tert-butyl)dimethylsilyl (=tbdms) group, and finally the 3'-(2-cyanoethyl diisopropylphosphoramidite) moiety. This strategy includes many steps and the isolation/purification of very polar intermediates. In our approach, we decided to start from 2'-O-tom-protected ribonucleosides and to carry out the introduction of the ¹⁵N-labels in the presence of this lipophilic 2'-O-protecting group. Furthermore, we decided to use cheap ¹⁵NH₄Cl as the sole ¹⁵N-source and to choose/optimize reaction conditions carefully, to avoid the use of large excesses of reagents.

The easily accessible 2'-O-tom-protected uridine nucleoside **1** [9] was chosen as starting material for the synthesis of the ¹⁵N-labeled pyrimidine nucleosides **4** and **6**. Treatment of **1** with AcOH/H₂O (\rightarrow removal of the (MeO)₂Tr group), followed by acetylation of the 3'- and 5'-OH groups with Ac₂O in pyridine gave the diacetylated uridine derivative **2**, which was isolated by chromatography (silica gel) and consecutively treated with NH₄NO₃/(CF₃CO)₂O in CH₂Cl₂ under strictly anhydrous conditions according to *Ariza et al.* [13] (*Scheme 1*). After fast and careful extraction, followed by filtration on silica gel, the resulting *N*-nitro derivative **3** was treated immediately with 1.3 equiv. of ¹⁵NH₄Cl in the presence of KOH and Et₃N in MeCN/H₂O, again following reported conditions [13]²). During this reaction, partial loss of the acetyl groups was observed; therefore, after extraction, the mixture of differently acetylated, ¹⁵N-labeled, 2'-O-tom-protected uridines was subjected directly to complete deacetylation with NaOH in THF/MeOH/H₂O. After extraction and without further purification, the 5'-O-(MeO)₂Tr group was introduced under standard conditions with

¹⁾ Bistable RNA sequences adopt two coexisting secondary structures (for other examples, see [11]).

²) This clean and efficient reaction sequence allows the straightforward introduction of ¹⁵N at position N(3) of uridine and N(1) of inosine derivatives. Efficient formation of the *N*-nitro derivative proceeds only under strictly anhydrous conditions and in the absence of light; the *N*-nitro derivatives cannot be stored for a prolonged period of time and should be subjected immediately to the next step.





a) 1. AcOH, H₂O, 25°; 2. Ac₂O, *N*,*N*-dimethylpyridin-4-amine (DMAP), pyridine, 25°. *b*) NH₄NO₃, (CF₃CO)₂O, CH₂Cl₂, 4°. *c*) 1. ¹⁵NH₄Cl, KOH, Et₃N, MeCN, H₂O, 25°; 2. NaOH, THF, MeOH, H₂O, 4°; 3. (MeO)₂TrCl, pyridine, 25°.

(MeO)₂TrCl in pyridine. The fully protected, ¹⁵N-labeled uridine building block **4** was finally isolated by chromatography (silica gel).

The ¹⁵N-labeled, fully protected cytosine derivative **6** was prepared from the corresponding uridine nucleoside **4** (*Scheme 1*) by first acetylating the 3'-OH group with Ac₂O in pyridine, followed by extractive workup and treatment of the 3'-O-acetylated intermediate with (ClC₆H₄O)P(O)Cl₂, 1*H*-1,2,4-triazole, and ¹Pr₂NEt in MeCN (\rightarrow formation of the 4-triazolyl derivative)³), followed by NH₃ in dioxane/H₂O (*Scheme 2*). After extraction and treatment with NaOH in MeOH/THF/H₂O, the cytidine nucleoside **5** was isolated by chromatography (silica gel). It was finally transformed into its *N*⁴-acetylated, ¹⁵N-labeled derivative **6** by selective *N*-acetylation with Ac₂O in DMF according to *Bhat et al.* [17]⁴) and isolated in a good yield by chromatography (silica gel).



a) 1. Ac₂O, DMAP, pyridine, 25°; 2. 1*H*-1,2,4-triazole, 4-chlorophenyl phosphorodichloridate, ⁱPr₂NEt, MeCN, 25°; 3. NH₃, dioxane, MeCN, H₂O, 25°; 4. NaOH, THF, MeOH, H₂O, 4°. *b*) Ac₂O, DMF, 25°.

The two ¹⁵N-labeled purine nucleosides **12** and **18** were prepared from the readily available 2'-*O*-tom-protected adenosine nucleoside **7** [9]. First, the *N*-acetyl protecting group and the 5'-*O*-(MeO)₂Tr group of **7** were removed with MeNH₂ in H₂O/THF and AcOH/H₂O, respectively (*Scheme 3*). After precipitation from CH₂Cl₂/hexane, the

³) In preliminary studies, we found that these reaction conditions resulted in a much cleaner and faster product formation than those of the similar reaction performed in pyridine, but without ⁱPr₂NEt [15], or those involving activation with TsCl/(PhO)₂P(O)OH [16].

⁴) This reaction proceeds very cleanly (without acetylation of the 3'-OH group), provided that pure starting material is used.



a) 1. MeNH₂, THF, H₂O, 25°; 2. AcOH, H₂O, 25°; 3. Ac₂O, DMAP, pyridine, 25°; 4. NaNO₂, AcOH, H₂O, 25°. *b*) NH₄NO₃, (CF₃CO)₂O, CH₂Cl₂, -20°. *c*) ¹⁵NH₄Cl, KOH, Et₃N, MeCN, H₂O, 25°. *d*) 3-Nitro-1*H*-1,2,4triazole, I₂, PPh₃, ⁱPr₂NEt, toluene, 95°. *e*) 1. NH₄Cl, ⁱBuOK, Et₃N, DMSO, 50°; 2. BzCl, DMAP, pyridine, 25°; 3. NaOH, THF, MeOH, H₂O, 4°; 4. (MeO)₂TrCl, pyridine, 25°.

intermediate 2'-O-tom-protected adenosine was converted with Ac₂O in pyridine into the corresponding 3',5'-di-O-acetyl derivative, which, after evaporation and extraction, was deaminated with NaNO₂ in AcOH/H₂O 7:3. The resulting inosine derivative **8** was isolated by crystallization. In analogy to the preparation of the ¹⁵N-labeled uridine nucleoside **3** (*Scheme 1*), but at lower temperature, **8** was treated under strictly anhydrous conditions with NH₄NO₃/(CF₃CO)₂O in CH₂Cl₂ [13]. As crude product, the resulting *N*-nitro derivative **9** was treated with 1.3 equiv. of ¹⁵NH₄Cl in the presence of KOH and Et₃N in MeCN/H₂O [13]²). This reaction proceeded much faster than with the analogous uridine nucleoside **3**, and without concomitant loss of acetyl groups. After extraction, but without further purification, the thus-obtained ¹⁵N-labeled inosine derivative **10** was treated with I₂, PPh₃, ⁱPr₂NEt, and 3-nitro-1*H*-1,2,4-triazole in toluene, in analogy to *Lin* and *Robins* [18]⁵). The nitro-triazole derivative **11** was

⁵) These very efficient reaction conditions were originally developed for the introduction of imidazole and cyclic secondary amines at C(6) of purine nucleosides [18]; we found, however, that these conditions allowed also smooth preparation of the corresponding 3-nitro-1*H*-1,2,4-triazole derivative, which, for substitution reactions with NH₃, is the superior leaving group [19]. Our first, unsuccessful or inefficient attempts to prepare similarly activated purine derivatives from **8** included the following conditions: 1*H*-1,2,4-triazole/POCl₃/Pr₂NEt in MeCN [20] (very slow reaction); 1*H*-1,2,4-triazole/(ClC₆H₄O)P(O)Cl₂/¹Pr₂NEt in pyridine [15] (low yield, significant side reactions); 1*H*-tetrazole/TsCl/(PhO)₂P(O)OH/¹Pr₂NEt in pyridine [21] (incomplete conversion); 3-nitro-1-[(2,4,6-triazone/NCl/PhC)₂P(O)OH/¹Pr₂NEt in pyridine [22] (fast formation of N(1)- and *O*⁶-sulfonylated products (1:1), followed by slow conversion of the latter into the corresponding 3-nitro-1*H*-1,2,4-triazole/derivative; 3-nitro-1*H*-1,2,4-triazole/TsCl/(PhO)₂P(O)OH/¹Pr₂NE(1) (PhO)₂P(O)OH in pyridine [19] (incomplete conversion).

isolated in a good yield of 67% (based on **8**) after evaporation and chromatography (silica gel). Treatment of this activated purine nucleoside under carefully optimized conditions with NH₄Cl, 'BuOK, Et₃N in DMSO⁶), followed by precipitation with H₂O, resulted in quantitative isolation of the 3',5'-di-*O*-acetylated, 2'-*O*-tom-protected adenosine. Without intermediate purification, this nucleoside was first transformed to its *N*⁶-benzoyl derivative⁷) with BzCl in pyridine, then deacetylated with NaOH in THF/MeOH/H₂O, and finally converted to its 5'-*O*-(MeO)₂Tr derivative **12** with (MeO)₂TrCl in pyridine. The fully protected, ¹⁵N-labeled adenosine building block **12** was isolated in a good yield by chromatography (silica gel).

The synthesis of the ¹⁵N-labeled guanosine nucleoside 18 was carried out from inosine intermediate 8 (Scheme 3). In analogy to the preparation of 11, 8 was first treated with I₂, PPh₃, Pr₂NEt, and 3-nitro-1*H*-1,2,4-triazole in toluene (*Scheme 4*); the resulting 3-nitro-1H-1,2,4-triazole derivative **13** was isolated by chromatography (silica gel) and subsequently treated with 3 equiv. of $^{15}NH_4Cl$ in the presence of 'BuOK/Et₃N in DMSO. The so-obtained ¹⁵N-labeled adenosine derivative **14** was precipitated with H₂O. According to TLC analysis, a partially deacetylated derivative was formed during this step (ca. 5%); therefore, crude 14 was reacetylated with Ac_2O in pyridine, and finally purified by chromatography (silica gel). Transformation into the corresponding N(1)-oxide 15 was achieved with 3-chloroperbenzoic acid in CH₂Cl₂ according to MacCoss et al. [23]. The product was isolated in a yield of 79% by chromatography (silica gel); additionally, 18% of unreacted 14 was recovered. Following (with some adaptations) the well-known method of Goswami and Jones [14], the N-oxide 15 was treated first with BrCN in MeOH; a DMF solution of the resulting dihydrooxadiazole derivative was then treated consecutively with Et₃N and MeI to yield a 1-methoxy-N⁶cyano-substituted derivative; the latter was treated first with NaOH in THF/H₂O and, finally, after neutralizing with aq. HCl solution and addition of EtOH, kept at 60° . In the course of this Dimroth rearrangement, the 2-amino-N6-methoxy-substituted purine derivative 16 was formed almost quantitatively, together with small amounts of still partially acetylated derivatives⁸). After extraction, this product mixture was directly subjected to an exhaustive acetylation with Ac_2O in pyridine at 100° . Under these conditions, a 1:1 mixture of the corresponding N^2 , N^6 , 3'-O, 5'-O-tetraacetyl and N², N², N⁶, 3'-O, 5'-O-pentaacetyl derivatives was formed, which was filtered through silica gel and then partially deacetylated with NaOH in THF/MeOH/H₂O to yield the N^2 -acetyl- N^6 -methoxy-2'-O-tom-substituted purine nucleoside. The latter was treated as crude product with Raney-Ni in THF/MeOH/ H_2O , resulting in cleavage of the N-O bond and clean formation of 179). Without purification, this purine-2,6-diamine nucleoside was first deaminated with NaNO2 in AcOH/H2O and then dimethoxytritylated with (MeO)₂TrCl in pyridine; finally, the ¹⁵N-labeled, fully protected guanosine

⁶⁾ Conditions developed for the preparation of the analogous compound 14 (Scheme 4) with ¹⁵NH₄Cl.

⁷) For routine RNA synthesis, we are using N^6 -acetylated adenosine building blocks [8][9]. In the here presented reaction sequence, however, it was more convenient to prepare the N^6 -benzoyl derivative.

⁸⁾ This reaction sequence (formation of N(1)-oxide, *Dimroth* rearrangement) was also successfully applied to corresponding transformations starting from 2'-O-tom-protected adenosine or 5'-O-(MeO)₂Tr-2'-O-tom-protected adenosine; in the context of the preceding and subsequent reactions, however, it was more convenient to use the diacetylated adenosine derivative 14.

⁹⁾ Unfortunately, a significant part of the product remains adsorbed on the Raney-Ni.



a) 3-Nitro-1*H*-1,2,4-triazole, I₂, PPh₃, ⁱPr₂NEt, toluene, 95°. *b*) 1. ¹⁵NH₄Cl, 'BuOK, Et₃N, DMSO, 50°; 2. Ac₂O, DMAP, pyridine, 25°. *c*) 3-Chloroperbenzoic acid, CH₂Cl₂, 25° (indicated yield based on consumption). *d*) 1. BrCN, MeOH, 25°; 2. Et₃N, DMF; 3. MeI, DMF, 25°; 4. NaOH, THF, H₂O, 25°; 5. aq. HCl soln. (\rightarrow pH 7), EtOH, 60°. *e*) 1. Ac₂O, pyridine, 100°; 2. NaOH, THF, MeOH, H₂O, 4°; 3. *Raney*-Ni, THF, MeOH, H₂O, 80°. *f*) 1. NaNO₂, AcOH, H₂O, 25°; 2. (MeO)₂TrCl, pyridine, 25°.

nucleoside **18** was isolated in a yield of 33% (based on **15**, ten consecutive steps) after chromatography (silica gel).

All compounds presented in Schemes 1-4 were characterized by ¹H-NMR, ¹³C-NMR, and MS (see *Exper. Part*). In the MS of all compounds 1-18, the m/zvalue of the most-intense signal was in agreement with the calculated value (with the exception of 3, which decomposed during analysis). The chemical-shift values δ of the fully protected, ¹⁵N-labeled nucleosides 4, 6, 12, and 18 were identical to the reported values of the unlabeled analogues, both in ¹H- and ¹³C-NMR analyses [7][9]. Nucleosides 4, 6, 12, and 18 (precursors of phosphoramidites 19-22, see below) were additionally characterized by H-decoupled ¹⁵N-NMR spectroscopy, and each showed one signal, corresponding to the ¹⁵N-label (δ 133.1 (4), 208.1 (6), 227.2 (12), and 129.4 (18)). Many of the ¹⁵N-containing compounds exhibited additional J(H,N) couplings (¹H-NMR), and all showed additional J(C,N) couplings (¹³C-NMR). The following values were recorded: ${}^{1}J(H,N) = 88.6 - 91.1$ Hz for pyrimidine and purine nucleosides **4**, **10**, **14**, **15**, and **18**, ${}^{3}J(H-C(5),N(3)) = 2.1$ Hz for uridine derivative **4**, and $^{2}J(H-C(2),N(1)) = 7.5$, 16.1, and 15.9 Hz, for the inosine and adenosine derivatives 10, 11, and 12 respectively. The coupling constants J(C,N) of the ¹⁵N-labeled nucleosides 4-6, 10-12, and 14-18 as derived from ¹³C-NMR spectra are summarized in the Table.



	J(C,N) [Hz]			
	C(2)	C(4)	C(5)	C(6)
4 (3- ¹⁵ N)	17.0	8.4	5.6	
$5(3^{-15}N)$	7.4	6.3	4.2	
$6(3^{-15}N)$	7.7	7.4	1.0	
$10(1^{-15}N)$	8.4		7.0	9.8
11 $(1^{-15}N)$	2.5		3.2	7.0
$12(1^{-15}N)$	3.0	2.4	1.5	5.2
14 $(N^{6}-15N)$	2.5		3.3	20.7
15 $(N^{6}-15N)$			1.3	22.5
16 (3- ¹⁵ N)	8.5	5.0	2.0	
17 (3- ¹⁵ N)	6.7	7.0	1.1	
18 (3- ¹⁵ N)	14.2		8.3	10.5

Table 1. Observed Couplings (J(C,N)) between Nucleobase ¹³C- and ¹⁵N-Atoms, as Obtained from ¹³C-NMR Spectra of ¹⁵N-Containing Nucleosides (see Exper. Part)

According to our standard procedure, the four ¹⁵N-labeled, 2'-O-tom-protected nucleosides **4**, **6**, **12**, and **18** were converted with 2-cyanoethyl diisopropylphosphoramidochloridite/ $^{1}Pr_{2}NEt$ into the corresponding phosphoramidite building blocks **19**– **22** (*Scheme 5*), which were isolated in good yields by chromatography (silica gel).



a) 2-Cyanoethyl diisopropylphosphoramidochloridite, ⁱPr₂NEt, CH₂Cl₂, 25°.

MALDI-MS Analysis of the four ¹⁵N-labeled phosphoramidites **19**–**22** showed the expected m/z values. The chemical-shift values of their ¹H- and ³¹P-NMR spectra corresponded well with the ones found for their unlabeled analogues [6][8]. The additional J(H,N) coupling constants, detected by ¹H-NMR, included ¹J=83.8 and 82.5 Hz for **19** and **22**, respectively, ²J=10.1 Hz for **21**, and ³J=2.7 Hz for **19**.

2.2. Synthesis of a 32Mer RNA Sequence. As a first example for our planned NMR studies, we chose to prepare the bistable 32mer RNA sequence 23 and to incorporate the ¹⁵N-labeled nucleoside building blocks 19-22 at the positions U28, C9, C29, A21, and G20 (counted from the 5'-end). The same bistable RNA sequence (not containing



Fig. 1. Representation of the two coexisting structures (S1) and (S2) adopted by the RNA sequence 23 (according to [9]). The encircled and numbered nucleosides derive from the ¹⁵N-labeled phosphoramidites 19–22 (numbering from 5' → 3' end). In 150 mM NaCl and 50 mM sodium phosphate (pH 7.4) at 23°, an equilibrium ratio 23(S1)/23(S2) of 7:3 was determined (see below, *Fig. 3*).

any ¹⁵N-labels) was first designed, prepared, and characterized by *Höbartner* and *Micura* [10] and is known to adopt the two different folding motifs shown in *Fig.* 1^{10}).

The sequence 23 was prepared on a 10- μ M scale from conventional 2'-O-tomprotected ribonucleoside phosphoramidites and the ¹⁵N-labeled analogues 19–22 according to our standard protocols [9]. The anion-exchange (=AE) HPLC trace of the crude product was in agreement with good coupling efficiences and showed a wellseparated, chromatographically homogeneous main product (*Fig. 2, a*). After purification by prep. AE-HPLC and desalting (for details, see *Exper. Part*), 30 mg (2.7 μ mole; 27% yield, based on the solid support) of chromatographically pure RNA sequence 23 (NH₄⁺ form, containing 1 mol-% of Et₃NH⁺, according to ¹H-NMR (*Fig. 2, c*)) were obtained. The LC-ESI-MS (neg. mode) of the purified sequence 23 showed the correct mass and indicated a high degree of purity (*Fig. 2, b*; for details see *Exper. Part*). The ¹H-NMR spectrum (400 MHz, D₂O; *Fig. 2, c*) demonstrated the spectral complexity of this 32mer RNA sequence.

2.3. *NMR Experiments*. The subsequently described NMR experiments with RNA sequence **23** were carried out at c = 2 mm in H₂O/D₂O 9:1, at 150 mm NaCl, and 50 mm sodium phosphate (pH 7.0) at 23°¹¹). Spectra were recorded at 600 MHz (*Bruker* instrument), and a combined flip-back [24]/WATERGATE [25] pulse sequence was used for water suppression. In *Fig. 3*, the 'imino-proton' regions (δ 9–15) of spectra obtained from three different 1D experiments are shown. The conventional ¹H-NMR spectrum, recorded without ¹⁵N-decoupling (*Fig. 3, a*), shows the expected complex

¹⁰) Partial assignment was carried out by comparative NH ··· N ¹H-NMR spectroscopy of small reference hairpins [10].

¹¹) Preliminary measurements at other temperatures showed a strong temperature dependence of the equilibrium distribution between 23(S1) and 23(S2). At higher temperatures, 23(S2), and at lower temperatures, 23(S1) was favored.



Fig. 2. Characterization of RNA sequence 23 (prepared according to [8]): a) AE-HPLC trace of the crude product (detection at 260 nm, 15–70% B in 30 min); b) deconvoluted LC-ESI-MS (neg. mode) of the purified product (M (calc.) 10129.2 amu; for details, see Exper. Part); c) ¹H-NMR spectrum (400 MHz, D₂O, c = 2 mM) of purified 23 (NH₄⁺ form, containing 1 mol-% of residual Et₃NH⁺ ions)

pattern of signals, each corresponding to a proton involved in a individual base pair of the two coexisting structures. Most of the signals appear in a narrow range between δ 12.0–13.5, typical for the 'imino-protons' of G · C base pairs [1]. Upon broad-band ¹⁵Ndecoupling (*Fig. 3,b*), the *ds* at δ 14.2, 12.1, and 11.9 (*J*(H,N) \approx 90 Hz) change to *ss*, resulting in a spectrum which qualitatively corresponds to the spectrum of the analogous, unlabeled, bistable RNA sequence reported by *Höbartner* and *Micura* [10]¹²). As expected from the decoupling experiment, only the 3 *ds* of *Fig. 3,a*, appear in the (¹H-decoupled) 1D-HSQC spectrum (*Fig. 3,c*). These initial 1D-NMR experiments allowed, in a straightforward way, to isolate 'imino-protons' of base-paired, ¹⁵Nlabeled uridines and guanosines and showed the occurrence of one A · ¹⁵NU (δ 14.2) and

¹²) The δ values observed for RNA sequence **23** and the unlabeled analogue [10] are almost identical. However, the ratio **23**(S1)/**23**(S2) was 7:3 in our, and 1:1 in the reported measurement. This difference likely originates from the medium (150 mM NaCl and 50 mM sodium phosphate vs. 25 mM sodium arsenate [10]).



Fig. 3. 'Imino-proton' region of ¹H-NMR spectra (600 Hz, H₂O/D₂O 9:1, for conditions, see text): a) ¹⁵N-decoupled, b) ¹⁵N-broad-band-decoupled, and c) 1D-HSQC spectrum, showing the 'imino-proton' signals of the indicated base pairs

two C·¹⁵NG base pairs (δ 12.1 and 11.9). Based on the two secondary structures of **23** (*Fig. 1*), the signal appearing at δ 14.3 was assigned to base pair U28 · A21-**23**(S1). The other two signals, appearing in the HSQC spectrum at δ 12.1 and 11.9, originate both from G20; they were assigned to G20 · C9-**23**(S2) and G20 · C29-**23**(S1), respectively, according to their integral ratio.

Two relevant parts of the 2D-HSQC spectrum of the RNA sequence **23**, displaying the heteronuclear ¹H,¹⁵N-correlation, are shown in *Fig. 4*. The atom ¹⁵N(3) of the base pair ¹⁵NU28 · A21-**23**(S1) appears at δ 163, whereas ¹⁵N(1) of both the base pairs ¹⁵NG20 · C29-**23**(S1) and ¹⁵NG20 · C9-**23**(S2) appear at the same δ value of 146 (*Fig. 4, a*). Furthermore, the two-bond scalar coupling between H–C(2) and N(1) of A21 (²*J*(H,N) = 16 Hz) resulted in two resolved cross-peaks at δ (H/N) 7.82/222.1 and δ (H/ N) 7.76/222.3, corresponding to both structures **23**(S1) and **23**(S2), respectively (*Fig. 4, b*). By an additional HNN-COSY experiment (see below, *Fig. 5*), the former cross-peak could be assigned to ¹⁵NA21-**23**(S1) in its base-paired form.

The main purpose of HNN-COSY experiments is the correlation of the H-bonddonor with the H-bond-acceptor N-atom within a base pair, provided that both are ¹⁵Nlabeled [3]. The HNN-COSY experiment with our RNA sequence **23** was carried out with a relaxation-optimized N,N-transfer time of 20 ms and a H,N-transfer time of 5 ms (*Fig.* 5). It provided a correlation for the three doubly ¹⁵N-labeled base pairs A21 · U28-**23**(S1), G20 · C29-**23**(S1), and G20 · C9-**23**(S1), and furthermore, the δ values for the acceptor nucleosides A21, C29, and C9 could be assigned. In contrast to



Fig. 4. Selected regions of a 2D-HSQC spectrum (600 MHz, H₂O/D₂O 9:1, for conditions, see text): a) H-N(3),N(3) and H-N(1),N(1) cross-peaks of the base-paired, ¹⁵N-labeled nucleotides U28-(S1) and G20-(S1,S2); b) resolved H-C(2),N(1) cross-peaks of the ¹⁵N-labeled nucleotide A21-(S1) and A21-(S2) (forming base pairs in both structures)



Fig. 5. Selected regions of a HNN-COSY plot (600 MHz, N,N-transfer time 20 ms, H,N-transfer time 5 ms; H_2O/D_2O 9:1, for conditions, see text), showing correlation between ¹⁵N-resonances of doubly ¹⁵N-labeled base pairs

G20, which displays the same chemical shift for both structures, its two pairing partners show a detectable difference and appear at δ 198.0 (C9-**23**(S2)) and δ 198.5 (C29-**23**(S1)).

3. Discussion. – NMR Analysis of larger RNA sequences (consisting of > 50 nucleotides) suffers from bad signal separation in the ¹H- as well as in the ¹⁵N-dimension, and assignment of these signals is often difficult. For investigations with RNA sequences where a complete structure elucidation is not required, the site-specific introduction of labeled nucleotides can overcome this limitation in a straightforward way.

In this context, we prepared the ¹⁵N-labeled, 2'-O-tom-protected ribonucleoside phosphoramidite building blocks **19–22**, which are precursors of selectively ¹⁵Nlabeled RNA sequences. The excellent stability of the 2'-O-tom protecting group towards a variety of different reaction conditions allowed us to perform all reactions in its presence, and we never detected any trace of 2'-O-deprotected by-products. As a consequence of its very lipophilic character, all 2'-O-tom-protected intermediates were well-soluble in organic solvents, extractable, and easy to purify by chromatography (silica gel). By carefully optimizing existing reaction conditions and developing new ones, we were able to use the relatively cheap ¹⁵NH₄Cl (our only ¹⁵N-source) in small quantities (1.3 and 3.0 equiv. for the pyrimidine and the purine nucleosides, resp.). Our convergent strategy allowed to reduce the number of steps and necessary purifications to a minimum. The here presented route can also be applied, in principle, to the straightforward synthesis of 2'-O-tom-protected phosphoramidites of other ¹⁵N-labeled ribonucleosides, such as (1-¹⁵N)inosine from **10** and (1-¹⁵N)purine-2,6-diamine ribonucleoside from **17**, respectively.

As a model system for initial NMR experiments, we have prepared the bistable RNA sequence 23, containing five ¹⁵N-labeled nucleotides. In the ¹H-NMR spectrum, the 'imino-proton' signals of the $G \cdot C$ base pairs (forming the stem regions of the two coexisting hairpin structures 23(S1) and 23(S2)), are crowded in a narrow δ range (11.9–13.2 ppm), and some signal overlap occurs. The corresponding 1D-HSQC spectrum, however, shows only the three resonances of the ¹⁵N-labeled, base-paired uridines and guanosines; therefore, an unambiguous assignment of these signals to U28 (forming an A · U base pair in 23(S1)) and G20 (forming C · G base pairs in 23(S1) and **23**(S2)) was possible. At the same time, it provided clear evidence that G20 is involved in the formation of two different base pairs, and U28 in only one; this is consistent with the two coexisting secondary structures, which were proposed upon comparison with spectra of reference compounds [9]. From the 2D-HSQC experiments, ¹⁵N-chemicalshift values the for base-paired, labeled guanosines and uridines were obtained, but unfortunately, the ¹⁵N-signals of G20, involved in two different base pairs, were isochronous¹³). The coupling between ${}^{15}N(1)$ and H-C(2) of A21, however, resulted in cross-peaks with different δ values, both in the ¹H- and the ¹⁵N-dimension. As expected,

¹³) A synthetic approach to overcome such limitations would be the introduction of labeled base pairs in such a way that, in one structure, both the donor and acceptor base are ¹⁵N-labeled, whereas, in the second structure, only the donor base carries a ¹⁵N-label. This labeling pattern would allow to detect both ¹⁵Nresonances in the HSQC experiment, and to assign them by a HNN-COSY experiment.

the HNN-COSY experiment provided correlated ¹⁵N-resonances for the doubly labeled base pairs and allowed the assignment of the ¹⁵N-signals of cytidines C9 and C29, and of adenosine A21.

Here, we present an application of site-specifically labeled RNA sequences as a tool for assignment and probing for putative secondary-structure motifs. However, additional, more sophisticated NMR experiments, involving the ¹⁵N-dimension and sensitive to dynamics, could be carried out with such RNA sequences, eventually resulting in a detailed understanding of the dynamics of selected base pairs (representative for certain secondary structures). Such preliminary experiments with RNA sequence **23** revealed so far that the rate-constant *k* for the interconversion of the two secondary structures of **23** is probably below 1 sec⁻¹ [26].

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Experimental Part

General. Reagents and solvents (highest purity) were from various suppliers and used without further purification, unless otherwise stated. $^{15}NH_4Cl$ ($^{15}N > 99\%$) was from Spectra Stable Isotopes (Columbia, MD). Unlabeled, 2'-O-tom-protected ribonucleoside phosphoramidites and supports were prepared according to [9]. Workup implies distribution of the reaction mixture between CH₂Cl₂ and sat. aq. NaHCO₃ soln., drying of the org. layer (MgSO₄), and evaporation. TLC: precoated silica-gel plates from Merck, stained by dipping into a soln. of anisaldehyde (10 ml), H₂SO₄ (10 ml), and AcOH (2 ml) in EtOH (180 ml) and subsequent heating with a heat gun. CC (column chromatography): silica gel 60 (230-400 mesh) from Fluka. Anion-exchange (AE) HPLC: DNAPAC PA-100 (9.0 × 250 mm; Dionex), flow 2.5 ml/min; eluent A: 12 mM Tris HCl (pH 7.4), 6M urea; eluent B: 12 mM Tris · HCl (pH 7.4), 0.5 M NaClO₄, 6 M urea; detection at 260 nm, elution at 85°. NMR (Bruker instruments): chemical shift δ in ppm rel. to external standards (¹H and ¹³C: Me₄Si, ³¹P: 85% ag. H₃PO₄ soln., ¹⁵N: 20 mM ¹⁵NH₄Cl in 10% aq. HCl soln.); coupling constants J in Hz; multiplicities (¹³C) according to DEPT spectra. ESI-MS (pos. mode): SSQ 710 (Finnigan), measurements in MeCN/H2O/AcOH 50:50:1. MALDI-MS (pos. mode): Axima CFR Plus (Kratos/Shimadzu); matrix: 2,4,6-trihydroxyacetophenone, diammonium citrate; m/z (rel. intensity in %). LC-ESI-MS (neg. mode): Q-Tof-Ultima (Micromass/Waters) coupled to Cap-LC (Waters); injection: 2 µl aq. sample (c (RNA) = 2.5 µM, c (EDTA) = 1 mM); chromatography on Xterra RP-C18 column (Waters, 5 μm, 0.32 × 50 mm; flow 8 μl/min; eluent A: 25 mM aq. Me₂NBu· H₂CO₃ (pH 8.4); eluent B: MeCN; elution at 60° , sheath flow 25 µl/min (MeCN)); gradient $A \rightarrow A/B$ 1:1 (15 min); deconvolution by MaxEnt1-software¹⁴).

3',5'-Di-O-acetyl-2'-O-{[(triisopropylsily])oxy]methyl]uridine (2). A soln. of 1 [9] (7.32 g, 10 mmol) in AcOH/H₂O 4:1 (180 ml) was stirred for 30 min at r.t. After evaporation (under continuous addition of small portions of H₂O towards the end), followed by co-evaporation with toluene (2 × 100 ml), the residue was dissolved in pyridine (40 ml) and treated with DMAP (366 mg, 3 mmol) and Ac₂O (2.83 ml, 30 mmol). After 1 h at r.t., MeOH (5 ml) was added, and the mixture was evaporated. CC (SiO₂, (150 g), hexane/AcOEt 4:1 \rightarrow 1:4) gave 2 (4.47 g, 87%). Colorless foam. TLC (hexane/AcOEt 2:3): *R*₁ 0.45. ¹H-NMR (400 MHz, CDCl₃): 1.01– 1.03 (*m*, ¹Pr₃Si); 2.12, 2.14 (2s, 2 MeCO); 4.31–4.36 (*m*, H–C(4'), CH₂(5')); 4.53 (*t*, *J* = 5.7, H–C(2')); 4.87, 4.95 (2*d*, *J* = 5.1, OCH₂O); 5.19 (*dd*, *J* = 3.8, 5.7, H–C(3')); 5.75 (*d*, *J* = 8.3, H–C(5)); 6.02 (*d*, *J* = 6.0, H–C(1')); 7.40 (*d*, *J* = 8.3, H–C(6)); 8.59 (br. *s*, H–N(3)). ¹³C-NMR (100 MHz, CDCl₃): 12.0 (*d*, Me₂CH); 17.9 (*q*, Me₂CH); 20.9 (*q*, MeCO); 63.6 (*t*, C(5')); 70.6 (*d*, C(3')); 75.8 (*d*, C(2')); 80.2 (*d*, C(4')); 88.4 (*d*, C(1')); 89.2 (*t*, OCH₂O); 103.1 (*d*, C(5)); 140.0 (*d*, C(6)); 150.0 (*s*, C(2)); 162.6 (*s*, C(4)); 170.2 (*s*, MeCO). ESI-MS: 515.3 (100, [*M* + H]⁺).

3',5'-Di-O-acetyl-3-nitro-2'-O-f[(triisopropylsilyl)oxy]methyl]uridine (**3**). At 4° and under Ar, (CF₃CO)₂O (6.55 g, 31.2 mmol) was added dropwise to a well-stirred suspension of finely powdered NH₄NO₃ (1.25 g,

¹⁴) Conditions adapted from [27].

15.6 mmol; dried for 48 h at 60°/0.05 mbar) in CH₂Cl₂ (20 ml). After 1 h at r.t., a clear soln. was obtained, to which a soln. of **2** (4.03 g, 7.8 mmol; dried for 48 h at 60°/0.05 mbar) in CH₂Cl₂ (20 ml) was added slowly. The reaction was kept for 20 min at r.t. under exclusion of light. Workup at 4°, followed by filtration through SiO₂ (40 g, CH₂Cl₂ \rightarrow CH₂Cl₂/AcOEt 3:2) gave **3** (4.01 g, 92%). Colorless foam (used immediately for the next step). TLC (hexane/AcOEt 1:1): R_f 0.71. ¹H-NMR (400 MHz, CDCl₃): 1.01 – 1.07 (m, ¹Pr₃Si); 2.12, 2.15 (2s, MeCO); 4.29 – 4.40 (m, H–C(4'), CH₂(5')); 4.55 (t, J = 5.7, H–C(2')); 4.86, 4.96 (2d, J = 5.0, OCH₂O); 5.19 (br. t, J ≈ 4.4, H–C(3')); 5.88 (d, J = 8.44, H–C(5)); 5.99 (d, J = 5.5, H–C(1')); 7.47 (d, J = 8.44, H–C(6)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.8 (q, Me_2 CH); 20.85, 20.88 (2q, MeCO); 63.3 (t, C(5')); 70.2 (d, C(3')); 75.9 (d, C(2')); 80.6 (d, C(4')); 88.2 (d, C(1')); 89.3 (t, OCH₂O); 102.0 (d, C(5)); 139.4 (d, C(6)); 145.4 (s, C(2)); 155.0 (s, C(4)); 170.1 (s, MeCO). ESI-MS: dec.

5'-O-(4,4'-Dimethoxytrityl)-2'-O-[[(triisopropylsilyl)oxy]methyl](3-15N)uridine (4). Into a tightly stoppered, evacuated flask containing ¹⁵NH₄Cl (0.552 g, 10.14 mmol) and KOH (0.525 g, 9.36 mmol), consecutively H₂O (20 ml), MeCN (20 ml), Et₃N (1.43 ml, 10.14 mmol), and a soln. of **3** (4.01 g, 7.18 mmol) in MeCN (40 ml) were added. The mixture was stirred for 7 d at r.t. and then extracted with CH₂Cl₂/H₂O, and the residue obtained after evaporation was treated at 4° with THF/MeOH 5:4 (292.5 ml) and 2M aq. NaOH (32.5 ml). After 30 min at 4°, AcOH (3.72 ml) was added, and the mixture was concentrated to 100 ml. Workup and drying (14 h, 0.05 mbar) gave a colorless residue, which was dissolved in pyridine (32 ml) and treated with (MeO)₂TrCl (5.29 g, 15.6 mmol). Workup after 45 min at r.t. and CC $(SiO_2(100 \text{ g}), \text{hexane/AcOEt} 4:1 \rightarrow 2:3 (+1\% \text{ Et}_3\text{N}))$ afforded 4 (3.20 g, 61%). Colorless, solid foam. TLC (hexane/AcOEt 1:1): Rf 0.55. 1H-NMR (400 MHz, $CDCl_3$): 1.07-1.14 (*m*, ⁱPr₃Si); 3.17 (*d*, *J* = 5.7, OH-C(3')); 3.52 (br. *d*, *J* \approx 1, CH₂(5')); 3.80 (*s*, 2 MeO); 4.11 (m, H-C(4')); 4.26 (t, J=4.1, H-C(2')); 4.46 (q, J=5.5, H-C(3')); 5.03, 5.23 $(2d, J=4.7, OCH_2O);$ 5.29 (dd, J = 9.5, J(H,N) = 2.1, H - C(5)); 6.03 (d, J = 3.1, H - C(1')); 6.84 (d, J = 8.6, 4 arom. H); 7.24 - 7.39(*m*, 9 arom. H); 7.94 (*d*, *J* = 8.1, H-C(6)); 8.34 (*d*, *J*(H,N) = 90.6, H-N(3)). ¹³C-NMR (100 MHz, CDCl₃): 12.0 (d, Me₂CH); 17.9 (q, Me₂CH); 55.4 (q, MeO); 62.3 (t, C(5')); 69.5 (d, C(2')); 83.0 (d, C(3')); 83.8 (d, C(4')); 87.3 (*s*, arom. C); 88.0 (*d*, C(1')); 90.8 (*t*, OCH₂O); 102.3 (*d*, J(C,N) = 5.6, C(5)); 113.4 (*d*, arom. C); 127.3, 128.1, 128.3, 130.3 (4d, arom. C); 135.2, 135.5 (2s, arom. C); 140.3 (d, C(6)); 150.0 (s, J(C,N) = 17.0, C(2)); 158.9 (s, arom. C); 162.9 (s, J(C,N) = 8.4, C(4)). ¹⁵N-NMR (40 MHz, CDCl₃): 133.1. ESI-MS: 756.30 (100, [M+ Na]+).

5'-O-(4,4'-Dimethoxytrityl)-2'-O-{[(triisopropylsilyl)oxy]methyl}(3-15N)cytidine (5). A soln. of 4 (1.30 g, 1.77 mmol) in pyridine (7.1 ml) was treated with DMAP (43 mg, 0.35 mmol) and Ac₂O (0.34 ml, 3.54 mmol). Workup after 3 h at r.t., co-evaporation with toluene $(2 \times 20 \text{ ml})$, extraction with CH₂Cl₂/10% aq. citric acid and sat. aq. NaHCO3 soln., drying (MgSO4), and evaporation gave a yellow solid foam (1.25 g), which was carefully dried (14 h at 60°/0.05 mbar) and dissolved in MeCN (6 ml). Meanwhile, under Ar and at 4°, 4-chlorophenyl phosphorodichloridate (1.30 g, 5.31 mmol) was added dropwise to a suspension of finely powdered 1H-1,2,4triazole (2.13 g, 30.8 mmol; dried by sublimation) in dry MeCN (12 ml). After 15 min at 4°, ⁱPr₂NEt (4.5 ml, 26.6 mmol) was added, and after 40 min at r.t., the mixture was again cooled to 4° and treated with the MeCN soln. obtained before (6 ml, containing 1.25 g of the intermediate nucleoside). After 6 h at r.t., the soln. was diluted with dioxane (18 ml), treated with 25% aq. NH3 soln. (27 ml) and stirred for another 3 h at r.t. Extraction with CH2Cl2/10% aq. citric acid and sat. aq. NaHCO3 soln. gave a solid yellow foam (1.15 g), which was dissolved in THF/MeOH 5:4 (66 ml), cooled to 4°, and treated with 2N NaOH (7.3 ml). After 30 min at 4°, the soln. was treated with AcOH (0.85 ml) and concentrated to 30 ml. Workup and CC (SiO₂ (15 g), CH₂Cl₂ \rightarrow CH₂Cl₂/MeOH 19:1) gave 5 (1.17 g, 90%). Colorless foam. TLC (AcOEt): R_f 0.40. ¹H-NMR (400 MHz, CDCl₃): 1.05 - 1.14 (*m*, ⁱPr₃Si); 3.39 (*d*, *J* = 7.9, OH - C(3')); 3.48 (*dd*, *J* = 11.0, 2.7, H - C(5')); 3.57 (*dd*, *J* = 11.0, 1.5, H'-C(5'); 3.79 (s, 2 MeO); 4.06 (td, J = 6.2, 8.2, H-C(4')); 4.22 (br. d, J = 4.9, H-C(2')); 4.37 $(m, H-C(3')); 5.12, 5.26 (2d, J=4.7, OCH_2O); 5.1 (d, J=7.2, C(5)); 5.96 (br. s, H-C(1')); 6.84 (d, J=8.7, C(5)); 5.96 (br. s, H-C(1')); 5.96 (br. s, H-C(1'))$ 4 arom. H); 7.21-7.43 (*m*, 9 arom. H); 8.08 (*d*, *J* = 7.4, H-C(6)). ¹³C-NMR (100 MHz, CDCl₃): 12.0 $(d, Me_2CH); 17.9 (q, Me_2CH); 55.3 (q, MeO); 61.6 (t, C(5')); 68.2 (d, C(2')); 83.1 (d, C(3')); 84.0 (d, C(4'));$ 86.9 (s, arom. C); 89.7 (d, C(1')); 90.9 (t, OCH₂O); 94.1 (s, arom. C); 113.3 (d, arom. C); 127.1, 128.0, 128.4 (3*d*, arom. C); 130.3 (*d*, *J*(C,N) = 4.2, C(5)); 135.5, 135.7, 141.5 (3*s*, arom. C); 144.7 (*d*, C(6)); 155.7 (*s*, *J*(C,N) = 7.4, C(2)); 158.7 (*s*, arom. C); 165.9 (*s*, *J*(C,N) = 6.3, C(4)). ESI-MS: 733.35 (100, [*M*+1]⁺).

N⁴-Acetyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-[[(triisopropylsilyl)oxy]methyl](3-¹⁵N)cytidine (**6**). A soln. of **5** (1.15 g, 1.57 mmol) in DMF (7.5 ml) was treated with Ac₂O (225 mg, 2.2 mmol) and stirred for 8 h at r.t. Extraction with sat. aq. NaHCO₃ soln. and AcOEt/hexane 1:1, followed by CC (SiO₂ (12 g) hexane/AcOEt 4:1 \rightarrow 3:7 (+1% Et₃N)) gave **6** (1.10 g, 90%). Colorless solid foam. TLC (hexane/AcOEt 3:7): R_f 0.50. ¹H-NMR (400 MHz, CDCl₃): 1.07–1.14 (*m*, ⁱPr₃Si); 2.24 (*s*, MeCO); 3.35 (*d*, *J* = 8.4, OH–C(3')); 3.55 (*dd*, *J* = 11.1, 2.3, H–C(5')); 3.61 (*dd*, *J* = 11.1, 2.3, H'–C(5')); 3.83 (*s*, 2 MeO); 4.11 (*td*, *J* = 2.0, 8.7, H–C(4')); 4.24

 $\begin{array}{l} (d, J = 4.9, \mathrm{H-C(2')}); 4.40 \ (dt, J = 8.4, 5.0, \mathrm{H-C(3')}); 5.16, 5.30 \ (2d, J = 4.7, \mathrm{OCH_2O}); 5.98 \ (s, \mathrm{H-C(1')}); 6.88 \ (d, J = 8.8, 4 \ \mathrm{arom. H}); 7.08 \ (d, J = 7.4, \mathrm{H-C(5)}); 7.28 - 7.44 \ (m, 9 \ \mathrm{arom. H}); 8.49 \ (d, J = 7.4, \mathrm{H-C(6)}); 9.11 \ (\mathrm{br.} s, \mathrm{NH-C(4)}). \ ^{13}\mbox{C-NMR} \ (100 \ \mathrm{MHz}, \ \mathrm{CDCl_3}); 12.0 \ (d, \mathrm{Me_2CH}); \ 17.9 \ (q, Me_2\mathrm{CH}); \ 25.1 \ (q, Me\mathrm{CO}); 55.4 \ (q, \mathrm{MeO}); 61.3 \ (t, \mathrm{C(5')}); 67.9 \ (d, \mathrm{C(2')}); 83.5 \ (d, \mathrm{C(3')}); 83.6 \ (d, \mathrm{C(4')}); 87.2 \ (s, \mathrm{arom. C}); 90.2 \ (d, \mathrm{C(1')}); 90.9 \ (t, \mathrm{OCH_2O}); 96.5 \ (d, J(\mathrm{C,N}) = 1.0, \mathrm{C(5)}); 113.4 \ (d, \mathrm{arom. C}); 127.3, 128.2, 128.4, 130.3 \ (4d, \mathrm{arom. C}); 135.7, 144.5 \ (2s, \mathrm{arom. C}); 145.0 \ (d, \mathrm{C(6)}); 155.4 \ (s, J(\mathrm{C,N}) = 7.7, \mathrm{C(2)}); 158.8 \ (s, \mathrm{arom. C}); 162.6 \ (s, J = 7.4, \mathrm{C(4)}); 170.0 \ (s, \mathrm{MeCO}). \ ^{15}\mbox{NMR} \ (40 \ \mathrm{MHz}, \mathrm{CDCl_3}): 208.1. \ \mathrm{ESI-MS}: 775.3 \ (100, \ [M+H]^+). \end{array}$

3',5'-Di-O-acetyl-2'-O-{[(triisopropylsilyl)oxy]methyl]inosine (8). A soln. of 7 [9] (28.16 g, 35.29 mmol) in 41% ag. MeNH₂ soln./THF 5:4 (765 ml) was left for 20 min at r.t. and concentrated to 400 ml. After workup, the residue was dissolved in AcOH/H2O 4:1 (650 ml) and stirred for 30 min at r.t. The solvent was evaporated (under addition of a small amount of H₂O towards the end of the evaporation) and the residue co-evaporated with toluene ($3 \times 200 \text{ ml}$). To a well-stirred soln. of this residue in CH₂Cl₂ (200 ml), hexane (400 ml) was added slowly. Filtration after 30 min gave a white solid, which was dissolved in pyridine (100 ml). After evaporation, the residue was again dissolved in pyridine (175 ml) and treated with DMAP (0.43 g, 3.5 mmol) and Ac₂O (8.33 ml, 88.2 mmol). After 30 min at r.t., MeOH (15 ml) was added, the solvent evaporated, and the residue coevaporated with toluene $(3 \times 100 \text{ ml})$ and dissolved in AcOH/H₂O 7:3 (560 ml). At r.t., this soln. was treated with five portions of NaNO₂ (5×40 g, 2.9 mol), which were added within 2.5 h. H₂O (100 ml) was added and the mixture extracted with CHCl₃ (500 ml). The org. phase was extracted with H₂O and sat. aq. NaHCO₃ soln., dried (MgSO₄), and evaporated. Recrystallization from AcOEt (400 ml) gave 8 (14.94 g, 78%). Colorless crystals. M.p. 190°. TLC (CH₂Cl₂/MeOH 9:1): R_f 0.49. ¹H-NMR (400 MHz, CDCl₃): 0.93 (m, ¹Pr₃Si); 2.12, 2.17 $(2s, 2 \text{ MeCO}); 4.35 - 4.45 (m, H - C(4'), CH_2(5')); 4.86, 4.92 (2d, J = 5.1, OCH_2O); 5.07 (br. t, J \approx 5, H - C(2'));$ 5.42 (dd, J = 3.8, 5.1, H - C(3')); 6.13 (d, J = 5.9, H - C(1')); 7.99 (s, H - C(8)); 8.17 (s, H - C(2)); 13.08 (s, H-N(1)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.8 (q, Me₂CH); 20.9 (q, MeCO); 63.5 (t, C(5')); 71.4 (d, C(3')); 76.8 (d, C(2')); 80.8 (d, C(4')); 87.6 (d, C(1')); 89.6 $(t, OCH_2O);$ 125.7 (s, C(5));139.0 (d, C(8)); 145.2 (d, C(2)); 149.0 (s, C(4)); 159.3 (s, C(6)); 170.2, 170.5 (2s, MeCO). ESI-MS: 539.35 (100, $[M + H]^+$).

3',5'-Di-O-acetyl-1-nitro-2'-O-[[(triisopropylsilyl)oxy]methyl]inosine (**9**). At 4° and under Ar, (CF₃CO)₂O (10.9 g, 52.0 mmol) was added dropwise to a well-stirred suspension of finely powdered NH₄NO₃ (2.08 g, 26 mmol; dried for 48 h at 60°/0.05 mbar) in CH₂Cl₂ (35 ml). After 1 h at r.t., a clear soln. was obtained, to which at -20° a soln. of **8** (3.50 g, 78 mmol; dried for 48 h at 60°/0.05 mbar) in CH₂Cl₂ (35 ml). After 1 h at r.t., a clear soln. was obtained, to which at -20° a soln. of **8** (3.50 g, 78 mmol; dried for 48 h at 60°/0.05 mbar) in CH₂Cl₂ (30 ml) was added slowly. The reaction was kept for 20 min at -20° under exclusion of light. Workup at 4° gave crude **9** (3.60 g) as a yellow oil, which was used immediately for the next step. For characterization, 50 mg were subjected to CC (SiO₂ (1 g), CH₂Cl₂ \rightarrow CH₂Cl₂/MeOH 19:1): 35 mg (*ca.* 70%) of pure **9**¹⁵). TLC (hexane/AcOEt 3:7): *R*_f 0.81. ¹H-NMR (400 MHz, CDCl₃): 0.88 - 1.09 (*m*, ¹Pr₃Si); 2.12, 2.17 (2*s*, 2 MeCO) ; 4.38 (br. *t*, *J* \approx 4, CH₂(5')); 4.48 (*dd*, *J* = 3.8, 7.3, H-C(4')); 4.85, 4.91 (2*d*, *J* = 5.1, OCH₂O); 4.96 (*t*, *J* = 5.7, H-C(2')); 5.37 (*dd*, *J* = 3.8, 5.0, H-C(3')); 6.10 (*d*, *J* = 6.0, H-C(1')); 8.02 (*s*, H-C(8)); 8.62 (*s*, H-C(2)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (*d*, Me₂CH); 17.8 (*q*, *Me*₂CH); 20.9 (*q*, *Me*CO); 63.4 (*t*, C(5')); 71.2 (*d*, C(3')); 76.8 (*d*, C(2')); 81.1 (*d*, C(4')); 87.7 (*d*, C(1')); 89.6 (*t*, OCH₂O); 125.6 (*s*, C(5)); 140.1 (*d*, C(8)); 140.9 (*d*, C(2)); 145.4 (*s*, C(6)); 149.5 (*s*, C(4)), 170.1, 170.3 (*cs*, MeCO). ESI-MS: 584.38 (100, [*M* + H]⁺).

3',5'-Di-O-acetyl-2'-O-{[(triisopropylsilyl)oxy]methyl]($1^{-15}N$)inosine (**10**). Into a tightly stoppered, evacuated flask containing ${}^{15}NH_4C1$ (0.46 g, 8.5 mmol) and KOH (0.43 g, 7.8 mmol), consecutively H₂O (16 ml), MeCN (16 ml), Et₃N (1.20 ml, 8.5 mmol), and a soln. of crude **9** (3.55 g, *ca*. 75 mmol) in MeCN (32 ml) were added. The mixture was stirred for 6 h at r.t., extracted with CH₂Cl₂/H₂O, and co-evaporated with toluene: crude **10** (3.40 g). Colorless solid foam. For characterization, a small amount was purified by prep. TLC. TLC (CH₂Cl₂/MeOH 9:1): R_f 0.49. ¹H-NMR (400 MHz, CDCl₃): 0.87–1.06 (m, ¹Pr₃Si); 2.12, 2.17 (2s, 2 MeCO); 4.35–4.45 (m, H–C(4'), CH₂(5')); 4.86, 4.92 (2d, J = 5.1, OCH₂O); 5.07 (t, J ≈ 5, H–C(2')); 5.42 (dd, J = 3.8, 5.1, H–C(3')); 6.13 (d, J = 5.9, H–C(1')); 7.99 (s, H–C(8)); 8.17 (d, J(H,N) = 7.5, H–C(2)); 13.08 (d, J(H,N) = 88.6, H–N(1)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.8 (q, Me_2 CH); 20.9 (q, MeCO); 63.5 (t, C(5')); 71.4 (d, C(3')); 76.8 (d, C(2')); 80.8 (d, C(4')); 87.6 (4d, C(1')); 89.6 (t, OCH₂O); 125.7 (s, J(C,N) = 7.0, C(5)); 139.0 (d, C(8)); 145.2 (d, J(C,N) = 8.4, C(2)); 149.0 (s, C(4)); 159.3 (s, J(C,N) = 9.8, C(6)), 170.2, 170.5 (2s, MeCO). ESI-MS: 540.32 (100, [M + H]⁺).

 $\{3',5'$ -Di-O-acetyl-2'-O-{[(triisopropylsilyl)oxy]methyl]- β -D-ribofuranosyl]-6-(3-nitro-1H-1,2,4-triazol-1-yl)($I_{-}^{15}N$)purine (11). A suspension of crude 10 (3.35 g), 3-nitro-1,2,4-1H-triazole (2.60 g, 22.7 mmol), PPh₃

¹⁵) During this CC, partial decomposition of **9** occurred (under formation of polar products).

(4.10 g, 14.9 mmol), and I₂ (3.46 g, 13.6 mmol) in toluene (130 ml) was heated to 95° and treated with ⁱPr₂NEt (5.60 ml, 32.5 mmol). After stirring for 50 min at 95°, the solvent was evaporated, and CC (SiO₂ (100 g), hexane/AcOEt 4:1 \rightarrow 2:3) gave **11** (2.34 g, 67% based on **8**) as a yellow foam. TLC (AcOEt/hexane 1:1): R_f 0.49. ¹H-NMR (400 MHz, CDCl₃): 0.88-0.91 (*m*, ⁱPr₃Si); 2.13, 2.19 (2*s*, 2 MeCO); 4.45 (br. *dd*, $J \approx 4, 8, CH_2(5')$); 4.51 (*m*, H-C(4')); 4.88, 4.94 (2*d*, $J = 4.9, OCH_2O$); 5.21 (br. *t*, $J \approx 5, H - C(2')$); 5.47 (*dd*, J = 3.9, 5.2, H - C(3')); 6.30 (*d*, J = 5.7, H - C(1')); 8.45 (*s*, H-C(8)); 9.0 (*d*, J(H,N) = 16.1, H - C(2)); 9.82 (*s*, H - C(triazole)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (*d*, Me₂CH); 17.8 (*q*, Me₂CH); 20.9 (*q*, MeCO); 63.3 (*t*, C(5')); 71.2 (*d*, C(3')); 76.8 (*d*, C(2')); 81.2 (*d*, C(4')); 88.2 (*d*, C(1')); 89.7 (*t*, OCH₂O); 123.9 (*s*, C(4)); 143.9 (*s*, J(C,N) = 7.0, C(6)); 145.8 (*d*, C(8)); 147.4 (*d*, C(triazole)); 152.6 (*d*, J(C,N) = 2.5, C(2)); 154.4 (*s*, J(C,N) = 3.2, C(5)); 164.1 (*s*, C(triazole)); 170.1, 170.4 (2*s*, MeCO). ESI-MS: 636.33 (100, $[M + H]^+$).

 N^{6} -Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-{[(triisopropylsilyl)oxy]methyl}(1-^{15}N)adenosine (12).Through the septum of an evacuated flask containing NH₄Cl (730 mg, 13.5 mmol), DMSO (21 ml) was added, followed by a soln. of 'BuOK (1.45 g, 13.0 mmol) and Et₃N (1.90 ml, 13.5 mmol) in DMSO (22 ml). The soln. was stirred for 10 min at r.t. and cooled to -200° (liq. N₂). The flask was then quickly opened, solid **11** (1.75 g, 2.7 mmol) was added to the frozen soln., and the flask was placed in an autoclave. After 14 h at 50°, the mixture was added slowly to well-stirred H₂O (1 l) of 4° . After 1 h at 4° , the suspension was filtered over *Celite*, and the solid was washed with H_2O . The filter was then placed on top of a flask and rinsed with acetone (5 \times 50 ml). Evaporation of the filtrate and workup gave a white solid, which was dissolved in pyridine (10 ml). After evaporation, the residue was again dissolved in pyridine (11 ml) and treated with DMAP (200 mg, 1.6 mmol) and BzCl (2.28 g, 16.2 mmol) for 14 h at r.t. After workup and co-evaporation with toluene (2 × 10 ml), the residue was dissolved in CH2Cl2 and extracted with 10% aq. citric acid soln., followed by sat. aq. NaHCO3 soln. The solid obtained after drying (MgSO₄) and evaporation was dissolved in THF/MeOH 5:4 (100 ml) and treated at 4° with 2N NaOH (11 ml). After 30 min at 4°, AcOH (1.40 ml) was added, and the mixture was concentrated to 40 ml. The solid obtained after workup was dried (14 h, 0.05 mbar), dissolved in pyridine (14 ml), treated with (MeO)₂TrCl (1.83 g, 5.4 mmol), and kept for 30 min at r.t. Workup and CC (SiO₂ (50 g), hexane/AcOEt 4:1 \rightarrow 3:7) gave 12 (1.63 g, 70%). Colorless foam. TLC (AcOEt/hexane 7:3): R_f 0.60. ¹H-NMR $(400 \text{ MHz}, \text{CDCl}_3): 1.05 - 1.15 (m, {}^{1}\text{Pr}_3\text{Si}); 3.12 (d, J = 3.2, \text{OH} - \text{C}(3')); 3.42 (dd, J = 3.8, 10.9, \text{H} - \text{C}(5')); 3.53 (dd,$ $(dd, J = 3.8, 10.9, H' - C(5')); 3.73 (s, 2 MeO); 4.33 (dd, J = 3.7, 7.4, H - C(4')); 4.60 (br. dd, J \approx 3, 7, H - C(3')); dd, J \approx 3, 7, H - C(3')); dd, J \approx 3, 7, H - C(3');$ $5.00(t, J = 5.2, H - C(2')); 5.01, 5.18(2d, J = 4.7, OCH_2O); 6.27(d, J = 5.3, H - C(1')); 6.81 - 6.83(m, 4 \text{ arom. H});$ 7.22 - 7.65 (m, 12 arom. H); 8.05 (d, J = 7.6, 2 arom. H); 8.23 (s, H - C(8)); 8.74 (d, J(H,N) = 15.9, H - C(2)); 9.04 + C(2); 9.04 + C((br. s, NH-C(6)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.9 (q, Me₂CH); 55.4 (q, MeO); 63.4 (t, C(5')); 71.1 (d, C(2')); 82.3 (d, C(3')); 84.5 (d, C(4')); 86.7 (s, arom. C); 87.3 (d, C(1')); 91.0 (t, OCH₂O); 113.3 (d, arom. C); 123.6 (s, J(C,N) = 1.5, C(5)); 127.1, 128.0, 128.3 (3d, arom. C); 129.0, 130.2, 132.9 (3d, arom. C), 133.8, 135.8 (2s, arom. C); 142.0 (d, C(8)); 144.6 (s, arom. C); 149.6 (s, J(C,N) = 5.2, C(6)); 151.8 (s, J(C,N) = 2.4, C(4)); 152.8 (d, J(C,N) = 3.0, C(2)); 158.7 (s, arom. C), 164.7 (s, PhCO). ¹⁵N-NMR (40 MHz, CDCl₃): 227.2. ESI-MS: 861.27 (100, $[M + H]^+$).

9-[3',5'-Di-O-acetyl-2'-O-[[(triisopropylsilyl)oxy]methyl]-β-D-ribofuranosyl]-6-(3-nitro-1H-1,2,4-triazol-1-yl)purine (13). A suspension of 8 (6.53 g, 12.1 mmol), 3-nitro-1,2,4-1H-triazole (4.85 g, 42.5 mmol), PPh₃ (7.64 g, 29.1 mmol), and I₂ (6.47 g, 25.5 mmol) in toluene (240 ml) was heated to 95° and treated with ⁱPr₂NEt (10.4 ml, 60.7 mmol). After stirring for 50 min at 95°, the solvent was evaporated, and CC (SiO₂ (160 g), hexane/ACOEt 4:2 → 2:3) gave 13 (6.92 g, 90%). Yellow foam. TLC (AcOEt/hexane 1:1): R_1 0.49. ¹H-NMR (400 MHz, CDCl₃): 0.88 – 0.91 (*m*, ⁱPr₃Si); 2.13, 2.19 (2*s*, 2 MeCO); 4.45 (br. *dd*, *J* ≈ 4, 8, CH₂(5')); 4.51 (*m*, H−C(4')); 4.88, 4.94 (2*d*, *J* = 4.9, OCH₂O); 5.21 (br. *t*, *J* ≈ 5, H−C(2')); 5.47 (*dd*, *J* = 3.9, 5.2, H−C(3')); 6.30 (*d*, *J* = 5.7, H−C(1')); 8.45 (s, H−C(8)); 9.0 (s, H−C(2)); 9.82 (s, H−C(triazole)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (*d*, Me₂CH); 17.8 (*q*, Me₂CH); 20.9 (*q*, MeCO); 63.3 (*t*, C(5')); 71.2 (*d*, C(3')); 76.8 (*d*, C(2')); 81.2 (*d*, C(4')); 88.2 (*d*, C(1')); 89.7 (*t*, OCH₂O); 123.9 (*s*, C(4)); 143.9 (*s*, C(6)); 145.8 (*d*, C(8)); 147.4 (*d*, C(triazole)); 152.6 (*d*, C(2)); 154.4 (*s*, C(5)); 164.1 (*s*, C(triazole)); 170.1, 170.4 (2*s*, MeCO). ESI-MS: 635.38 (100, [M+H]⁺).

3',5'-Di-O-*acetyl*-2'-O-{[(*triisopropylsily*])oxy]*methyl*](N⁶-¹⁵N)*adenosine* (14). Through the septum of an evacuated flask containing ¹⁵NH₄Cl (1.78 g, 32.8 mmol), DMSO (29 ml) was added, followed by a soln. of 'BuOK (3.43 g, 30.6 mmol) and Et₃N (4.60 ml, 32.8 mmol) in DMSO (28 ml). The soln. was stirred for 10 min at r.t. and cooled to -200° (liq. N₂). The flask was then quickly opened, solid 13 (6.93 g, 10.92 mmol) was added to the frozen soln., and the flask was placed in an autoclave. After 14 h at 50°, the mixture was added slowly to well-stirred H₂O (2.51) of 4°. After 1 h at 4°, the suspension was filtered over *Celite*, and the solid was washed with H₂O. The filter was then placed on top of a flask and rinsed with acetone (5 × 100 ml). Evaporation of the filtrate and workup gave a white solid, which was dissolved in pyridine (10 ml). After evaporation, the residue

was dissolved in pyridine (55 ml) and treated with DMAP (27 mg, 0.22 mmol) and Ac₂O (0.21 ml, 2.2 mmol). After 30 min at r.t., MeOH (5 ml) was added, and the solvent was evaporated. CC (SiO₂ (140 g), CH₂Cl₂ \rightarrow CH₂Cl₂/MeOH 93:7) gave **14** (4.99 g, 85%). Colorless foam. TLC (CH₂Cl₂/MeOH 9:1): R_t 0.57. ¹H-NMR (400 MHz, CDCl₃): 0.88–1.01 (*m*, Pr₃Si); 2.11, 2.16 (2*s*, 2 MeCO); 4.37–4.48 (*m*, H–C(4'), CH₂(5')); 4.84, 4.90 (2*d*, *J* = 4.8, OCH₂O); 5.21 (*t*, *J* = 5.76, H–C(2')); 5.48 (*dd*, *J* = 3.2, 5.3, H–C(3')); 5.76 (*d*, *J*(H,N) = 89.6, NH₂(6)); 6.12 (*d*, *J* = 5.7, H–C(1')); 7.93 (*s*, H–C(8)); 8.34 (*s*, H–C(2)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (*d*, Me₂CH); 17.7 (*q*, Me₂CH); 20.9 (*q*, MeCO); 63.6 (*t*, C(5')); 71.6 (*d*, C(3')); 76.4 (*d*, C(2')); 80.7 (*d*, C(4')); 87.6 (*d*, *J*(C,N) = 2.5, C(2)); 155.6 (*s*, *J*(C,N) = 20.7, C(6)); 170.2, 170.5 (2*s*, MeCO). MALDI-MS: 638.60 (100, [*M*+H]⁺).

3',5'-Di-O-acetyl-2'-O-{[(triisopropylsilyl)oxy]methyl](N⁶-¹⁵N)adenosine 1-Oxide (**15**). A suspension of 70% 3-chloroperbenzoic acid (5.50 g, 22.3 mmol) and anh. MgSO₄ (5 g) in CH₂Cl₂ (55 ml) was stirred for 1 h at r.t. and then filtered into a flask containing solid **14** (3.00 g, 5.57 mmol). The soln. was stirred for 14 h at r.t. and then diluted with CHCl₃ (150 ml) and 5% aq. NaI soln. (100 ml). After stirring for 10 min, solid Na₂S₃O₃ was added until the red color (I₂) disappeared. The org. phase was extracted with sat. aq. NaHCO₃ soln. and evaporated. CC (SiO₂ (75 g), CH₂Cl₂ \rightarrow CH₂Cl₂/MeOH 93:7) gave **15** (2.43 g, 79%) as a pink foam and **14** (0.54 g, 18%) as a colorless foam. **15**: TLC (CH₂Cl₂/MeOH 9:1): R_f 0.46. ¹H-NMR (400 MHz, CDCl₃): 0.88–1.01 (*m*, ⁱPr₃Si); 2.10, 2.16 (2*s*, 2 MeCO); 4.34–4.44 (*m*, H–C(4'), CH₂(5')); 4.84, 4.90 (2*d*, *J* = 4.8, OCH₂O); 5.13 (*t*, *J* = 5.4, H–C(2')); 5.40 (*dd*, *J* = 3.5, 5.2, H–C(3')); 6.11 (*d*, *J* = 6.1, H–C(1')); 7.50 (br. *d*, *J*(H,N) = 91.0, NH₂–C(6)); 8.04 (*s*, H–C(8)); 8.69 (*s*, H–C(2)). ¹³C-NMR (100 MHz, CDCl₃): 11.8 (*d*, Me₂CH); 17.7 (*q*, *Me*₂CH); 20.9 (*q*, *Me*CO); 63.4 (*t*, C(5')); 71.3 (*d*, C(3')); 76.5 (*d*, C(2')); 80.8 (*d*, C(4')); 87.7 (*d*, C(1')); 89.6 (*t*, OCH₂O); 120.0 (*s*, *J*(C,N) = 1.3, C(5)); 142.1 (*d*, C(8)); 142.4 (*s*, C(4)); 144.2 (*d*, C(2)); 148.5 (*s*, *J*(C,N) = 22.5, C(6)); 170.1, 170.4 (2*s*, MeCO). MALDI-MS: 638.60 (100, [*M* + H]⁺).

 N^{6} -Methoxy-9-[2'-O-{[(triisopropylsily])oxy]methyl]- β -D-ribofuranosyl](1-¹⁵N)purine-2,6-diamine (16). To a soln. of 15 (2.43 g, 4.39 mmol) in MeOH (110 ml), BrCN (558 mg, 5.27 mmol) was added, and the mixture was stirred for 3 h at r.t. After removing the solvent, the residue (pink foam) was dried (2 h, 0.05 mbar), redissolved in DMF (17 ml), and treated with Et₃N (1.54 ml, 10.97 mmol; freshly filtered over Al₂O₃) for 45 min at r.t. Then MeI (1.49 g, 10.53 mmol) was added, and the soln. was stirred for 4 h at r.t. After evaporation, the residue was dried (14 h, 0.05 mbar), resuspended in THF (36 ml), and treated with 0.5M aq. NaOH (36 ml) for 40 min at r.t. Then the pH was adjusted to 7.4 with 1M aq. HCl, EtOH (85 ml) was added, and the mixture was stirred for 6 h at 60°. After concentration to 50 ml, workup, and drying (14 h, 0.05 mbar), crude 16 (2.4 g) was obtained as yellow foam. For characterization, a small amount was purified by prep. TLC. TLC (CH₂Cl₂/MeOH 9:1): R_f 0.40. ¹H-NMR (400 MHz, CDCl₃): 0.88-1.06 (*m*, ⁱPr₃Si); 3.11 (*s*, OH-C(3')); 3.74 (*m*, CH₂(5')); 3.93 (*s*, MeO); 3.96 (br. d, J = 12.4, H - C(4')); 4.52 (d, J = 5.13, H - C(3')); 4.81, 5.02 ($2d, J = 5.1, OCH_2O$); 4.92 (dd, J = 4.7, 7.5, 7.5, 7.5); 4.81, 5.02 ($2d, J = 5.1, OCH_2O$); 4.92 (dd, J = 4.7, 7.5, 7.5); 4.91 (dd, J = 4.7, 7.5, 7.5); 4.92 (dd, J = 4.7, 7.5); 4.92 (dd, J = 4.7, 7.5, 7.5); 4.92 (dd, J = 4.7, 7.5); 4.92 (dH-C(2'); 5.01 (br. d, J = 5.3, OH-C(5')); 5.80 (d, J = 8.0, H-C(1')); 7.01 (br. s, $NH_2(2)$); 7.57 (s, H-C(8)); 9.26 (br. s, NH-C(6)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.8 (q, Me₂CH); 63.7 (t, C(5')); 65.0 (q, MeO); 72.3 (d, C(3')); 81.2 (d, C(2')); 87.9 (d, C(4')); 89.6 (d, C(1')); 90.6 $(t, OCH_2O);$ 114.4 (s, J(C, N) = 2.0, C(N)); 90.6 $(t, OCH_2O);$ 114.4 (s, J(C, N)) = 0.0;C(5); 139.1 (*d*, C(8)); 149.3 (*s*, C(4)); 156.5 (*s*, J = 8.5, C(2)); 159.1 (*s*, J(C,N) = 5.0, C(6)). ESI-MS: 500.35 $(100, [M + H]^+).$

 N^2 -Acetyl-9-[2'-O-{[(triisopropylsilyl)oxy]methyl}- β -D-ribofuranosyl](1-¹⁵N)purine-2,6-diamine (17). A soln. of crude 16 (2.4 g) was dissolved in Ac₂O/pyridine 1:2 (22 ml) and heated for 3 h at 100°. Evaporation and co-evaporation with toluene $(2 \times 50 \text{ ml})$ gave a residue, which was dissolved in CH₂Cl₂ (40 ml), adsorbed on SiO₂ (12 g), and filtered through a pad of SiO₂ (12 g) (hexane/AcOEt $1:1 \rightarrow 9:1$, then AcOEt/MeOH 99:1). The 1:1 mixture of peracetylated intermediates (2.11 g, TLC (hexane/AcOEt 1:9): Rf 0.49 and 0.66) was dissolved in THF/MeOH 5:4 (165 ml), cooled to 4° and treated with 2M aq. NaOH (18 ml). After 10 min at 4°, AcOH (2.1 ml) was added and the soln. concentrated to 80 ml. After workup, the N^2 -acetylated intermediate was dissolved in THF/H₂O 1:1 (27 ml) and treated under H₂ (balloon) with a suspension of Raney-Ni (5.3 g, prepared according to [28]) in MeOH (9 ml). After 1 h at 80°, the hot soln. was filtered, the residue was rinsed with hot MeOH/THF 1:1 (5 \times 20 ml), and the combined filtrates were evaporated. Workup gave crude 17 (1.2 g). Colorless foam. For characterization, a small amount was purified by prep. TLC. TLC (CH₂Cl₂/acetone 1:1): R_f 0.51. ¹H-NMR (400 MHz, CDCl₃): 0.90–1.09 (m, ⁱPr₃Si); 2.43 (s, MeCO); 3.18 (s, OH–C(3')); 3.77 $(m, CH_2(5')); 3.96$ (br. d, J = 2.4, H - C(4')); 4.55 (d, J = 4.4, H - C(3')); 4.86, 5.04 ($2d, J = 4.8, OCH_2O$); 4.90 (dd, J = 4.9, 6.9, H - C(2')); 5.60 (br. s, OH - C(5')); 5.90 (d, J = 7.0, H - C(1')); 6.21 (br. s, $NH_2 - C(6)); 7.74$ (s, H-C(8)); 8.55 (br. s, NH-C(2)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.8, (q, Me₂CH); 25.2 (q, MeCO); 63.1 (t, C(5')); 71.8 (d, C(3')); 81.5 (d, C(2')); 87.2 (d, C(4')); 89.0 (d, C(1')); 90.7 (t, OCH₂O); 117.9 (s, J(C,N) = 1.1, C(5)); 140.3 (d, C(8)); 149.6 (s, C(4)); 152.7 (s, J(C,N) = 6.7, C(2)); 156.3 (s, J(C,N) = 7.0, C(6)); 171.2 (s, MeCO). ESI-MS: 512.30 (100, [M + H]⁺).

N²-Acetyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-{[(triisopropylsilyl)oxy]methyl](1-¹⁵N)guanosine (18). At r.t., a soln. of crude 17 (1.2 g) in AcOH/H₂O 7:3 (73 ml) was treated with 5 portions of NaNO₂ (5×5 g, 36 mmol), which were added within 2 h. H₂O (20 ml) was added and the mixture extracted with CHCl₃ (150 ml). The org. phase was extracted with H₂O and sat. aq. NaHCO₃ soln., dried (MgSO₄), and evaporated. The residue was coevaporated with toluene $(2 \times 20 \text{ ml})$, dried (14 h, 0.05 mbar), redissolved in pyridine (22 ml), and treated with $(MeO)_2$ TrCl (1.78 g, 5.27 mmol). After 1 h at r.t., workup and CC (SiO₂ (35 g), hexane/AcOEt 1:1 \rightarrow 1:9, then AcOEt/MeOH 99:1) gave 18 (1.18 g, 33% from 15). Off-white foam. TLC (CH₂Cl₂/MeOH 19:1): R_f 0.55. ¹H-NMR (400 MHz, CDCl₃): 0.98 - 1.10 (*m*, ¹Pr₃Si); 1.46 (*s*, MeCO); 3.08 (*d*, *J* = 3.2, OH – C(3')); 3.12 (*dd*, *J* = 3.2, OH – C(3')); 3.12.9, 10.7, H-C(5'); $3.53 (dd, J \approx 2, 10.6, H'-C(5')$; 3.74, 3.78 (2s, 2 MeO); 4.22 (q, J = 1.7, H-C(4')); 4.60 (br. C(4')); 4.60 (br. C $dd, J = 4.8, 1.6, H - C(3'); 4.93, 5.11 (2d, J = 4.6, OCH_2O); 5.06 (dd, J = 5.2, 6.9, H - C(2')); 5.90 (d, J = 7.3, 1.6, H - C(3')); 5.91 (d, J = 7.3, 1.$ H-C(1')); 6.76-6.78 (m, 4 arom. H); 7.18-7.54 (m, 9 arom. H); 7.81 (s, H-C(8)); 7.84 (br. s, NH-C(2)); 11.79 (d, J(H,N) = 91.1, H-N(1)). ¹³C-NMR (100 MHz, CDCl₃): 11.9 (d, Me₂CH); 17.9 (q, Me₂CH); 23.6 (q, MeCO); 55.4 (q, MeO); 63.9 (t, C(5')); 70.9 (d, C(2')); 81.4 (d, C(3')); 84.5 (d, C(4')); 86.4 (d, C(1')); 86.8 (s, arom. C); 91.1 (t, OCH₂O); 113.4 (d, arom. C); 122.6 (s, J(C,N) = 8.3, C(5)); 127.3, 128.1, 128.2, 130.1 (4d, arom. C); 135.7, 136.2 (2s, arom. C); 139.3 (d, C(8)); 145.2 (s, arom. C); 146.9 (s, J(C,N) = 14.2, C(2)); 148.3 (s, C(4)); 155.61 (*s*, *J*(C,N) = 10.5, C(6)), 158.9 (*s*, arom. C); 171.5 (*s*, MeCO). ¹⁵N-NMR (40 MHz, CDCl₃): 129.4. ESI-MS: 815.29 (100, $[M + H]^+$).

5'-O-(4,4'-Dimethoxytrityl)-2'-O-[[(triisopropylsilyl)oxy]methyl](3-¹⁵N)uridine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**19**). A soln. of **4** (1.20 g, 1.64 mmol) in CH₂Cl₂ (6.6 ml) was treated consecutively with ¹Pr₂NEt (0.70 ml, 4.10 mmol) and cyanoethyl diisopropylphosphoramidochloridite (466 mg, 1.97 mmol). After stirring for 14 h at r.t., the mixture was subjected to CC (SiO₂ (25 g), hexane/AcOEt 9:1 \rightarrow 3:7 (+2% Et₃N)): **19** (1.53 g, 92%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): R_1 0.65. ¹H-NMR (400 MHz, CDCl₃): 1.02-1.05 (*m*, ¹Pr₃Si); 1.16-1.17 (*m*, (*Me*₂CH)₂N); 2.39 (*t*, *J* = 6.4, 1 H, CH₂CN); 2.64 (*dt*, *J* = 2.7, 6.3, 1 H, CH₂CN); 3.39 (*dt*, *J* = 2.6, 9.5, H-C(5')); 3.51-3.70 (*m*, 4H (Me₂CH)₂N, H'-C(5'), POCH₂); 3.79, 3.80 (2*s*, 2 MeO); 3.82-3.97 (*m*, 1 H, POCH₂); 4.19, 4.27 (2*q*, *J* = 2.4, H-C(4')); 4.40-4.48 (*m*, H-C(2'), H-C(3')); 6.12 (*d*, *J* = 4.7, 0.5 H, H-C(1')); 6.13 (*d*, *J* = 4.8, 0.5 H, H-C(1')); 6.82-6.86 (*m*, 4 arom. H); 7.23-7.42 (*m*, 9 arom. H); 7.81, 7.88 (2*d*, *J* = 8.1, H-C(6)); 8.16 (br. *d*, *J*(H,N) = 83.8, H-N(3)). ³¹P-NMR (162 MHz, CDCl₃): 150.8, 151.3.

N⁴-Acetyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-[[(triisopropylsilyl)oxy]methyl](3-¹⁵N)cytidine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**20**). As described for **19**, with **6** (1.20 g, 1.56 mmol), CH₂Cl₂ (6.3 ml), ⁱPr₂NEt (0.60 ml, 3.90 mmol), and cyanoethyl diisopropylphosphoramidochloridite (444 mg, 1.88 mmol). CC (SiO₂ (30 g), hexane/AcOEt 1:1 → AcOEt (+2% Et₃N)) gave **20** (1.42 g, 94%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.75. ⁱH-NMR (400 MHz, CDCl₃): 0.98–1.08 (*m*, ⁱPr₃Si); 1.13, 1.16 (2*d*, *J* = 6.7, (*Me*₂CH)₂N); 2.21, 2.22 (2*s*, MeCO); 2.38 (*t*, *J* = 6.4, 1 H, CH₂CN); 2.60 (*q*, *J* = 6.1, 1 H, CH₂CN); 3.42 – 3.69 (*m*, 1 H of POCH₂, (Me₂CH)₂N, H−C(5')); 3.81, 3.82 (2*s*, 2 MeO); 3.92 (*m*, 1 H, POCH₂); 4.27–4.42 (*m*, H−C(2'), H−C(4')); 6.83–6.87 (*m*, 4 arom. H); 6.94, 7.01 (2*d*, *J* = 7.4, H−C(5)); 7.26–7.44 (*m*, 9 arom. H); 8.37, 8.48 (2*d*, *J* = 7.5, H−C(6)); 9.11, 9.18 (2 br. *s*, NH−C(4)). ³¹P-NMR (162 MHz, CDCl₃): 15.6, 151.9. MALDI-MS: 975.68 (100, [*M* + 1]+).

N⁶-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-[[(triisopropylsilyl)oxy]methyl](1-¹⁵N)adenosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (**21**). As described for **19**, with **12** (1.40 g, 1.20 mmol), CH₂Cl₂ (6.4 ml), ¹Pr₂NEt (0.68 ml, 4.00 mmol), and cyanoethyl diisopropylphosphoramidochloridite (456 mg, 1.92 mmol). CC (SiO₂ (28 g), hexane/AcOEt 9:1 \rightarrow 3:7 (+2% Et₃N)) gave **21** (1.54 g, 90%; 1:1 mixture of diastereoisomers)). Colorless foam. TLC (hexane/AcOEt 1:1): R_t 0.70. ¹H-NMR (400 MHz, CDCl₃): 0.86–0.93 (m, ¹Pr₃Si); 1.08, 1.18, 1.20 (3d, J = 6.6, (Me₂CH)₂N); 2.38 (t, J = 6.5, 1 H, CH₂CN); 2.64 (dt, J = 2.5, 6.4, 1 H, CH₂CN); 3.34 (dt, J = 4.3, 10.5, 1 H, POCH₂); 3.51–3.72 (m, 1 H of POCH₂, (MeCH)₂N, H–C(5')); 3.76, 3.77 (2s, 2 MeO); 3.83–3.99 (m, 1 H, POCH₂); 4.38, 4.43 (2q, J = 3.9, H-C(4')); 4.67–4.75 (m, H-C(3')); 4.95, 4.97, 5.01 (3 $d, J = 5.0, OCH_2O$); 5.19, 5.21 (2t, J = 5.3, H-C(2')); 6.19, 6.21 (2d, J = 5.5, H-C(1')); 6.75–6.79 (m, 4 arom. H); 7.17–7.61 (m, 12 arom. H); 8.01 (d, J = 7.3, 2 arom. H); 8.17, 8.19 (2s, H-C(8)); 8.67, 8.71 (2d, J(H,N) = 10.1, H-C(2)); 9.04 (br. s, NH-C(6)). ³¹P-NMR (162 MHz, CDCl₃) 150.8, 151.6. MALDI-MS: 1061.48 (100, [M + H]+).

 N^2 -Acetyl-5'-O-(4,4'-dimethoxytrityl)-2'-O-{[(triisopropylsilyl)oxy]methyl](1-¹⁵N)guanosine 3'-(2-Cyanoethyl Diisopropylphosphoramidite) (22). A as described for 19, with 18 (1.10 g, 1.35 mmol), CH₂Cl₂

(5.5 ml), ¹Pr₂NEt (0.59 ml, 3.4 mmol), and cyanoethyl diisopropylphosphoramidochloridite (384 mg, 1.62 mmol). CC (SiO₂ (25 g), hexane/AcOEt 1:1 \rightarrow AcOEt, then AcOEt/MeOH 99:1 \rightarrow 19:1 (+2% Et₃N)) gave **22** (1.23 g, 90%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): R_f 0.55. ¹H-NMR (400 MHz, CDCl₃): 0.92 - 0.93 (*m*, ¹Pr₃Si); 1.02 - 1.19 (4*d*, *J* = 6.8, (*Me*₂CH)₂N); 1.70, 1.82 (2*s*, MeCO); 2.17 - 2.35 (*m*, 1 H, CH₂CN); 2.69 - 2.82 (*m*, 1 H, CH₂CN); 3.22 (*dd*, *J* = 3.6, 10.7, 0.5 H, H-C(5')); 3.26 (*dd*, *J* = 5.3, 10.6, 0.5 H, H-C(5')); 3.46 - 3.63 (*m*, 3.5 H, (MeCH)₂N, H-C(5'), POCH₂); 3.68 (*m*, 0.5 H, POCH₂); 3.757, 3.760, 3.768, 3.773 (4*s*, 2 MeO); 3.92 - 4.05 (*m*, 1.5 H, POCH₂); 4.23 (br. *q*, *J* ≈ 2, 0.5 H, H-C(4')); 4.33 (br. *t*, *J* ≈ 3, 0.5 H, H-C(4')); 4.53 (*ddd*, *J* = 1.8, 4.7, 12.1, 0.5 H, H-C(3')); 4.62 (*m*, 0.5 H, H-C(3')); 4.92 (*s*, 1 H, OCH₂O); 4.89, 4.99 (2*d*, *J* = 5.3, 1 H, OCH₂O); 5.02 (*dd*, *J* = 5.0, 7.4, 0.5 H, H-C(2')); 5.06 (*t*, *J* = 5.8, 0.5 H, H-C(2')); 5.86 (*d*, *J* = 5.5, 0.5 H, H-C(1')); 5.97 (*d*, *J* = 7.3, 0.5 H, H-C(1')); 6.77 - 6.82 (*m*, 4 arom. H); 7.20 - 7.53 (*m*, 9 arom. H); 7.73, 7.80 (2*s*, H-C(8')); 8.19, 8.51 (2 br. *s*, NH-C(2)); 11.7 (br. *d*, *J*(H,N) = 82.5, H-N(1)). ³¹P-NMR (162 MHz, CDCl₃): 149.8, 150.4. MALDI-MS: 1015.46 (100, [*M* + H]⁺).

 $UpUpA p^{(3.15N)}Up^{(3.15N)}CpGpCpU)$ (23). The assembly was carried out on a Gene Assembler Plus (Pharmacia), from 320 mg of solid support (loading 32 µmol/g) and 2'-O-tom-protected ribonucleoside phosphoramidites according to [9]. The solid support was subsequently removed from the cartridge and treated with a 1:1 mixture of 12M MeNH₂ in H₂O and 8M MeNH₂ in EtOH (4 ml) for 6 h at 35°. By centrifugation, the supernatant soln. was separated from the solid support and evaporated, and the residue was dissolved in 1M Bu₄NF · 3 H₂O soln. in THF (4 ml). After 14 h at 30°, 1M Tris · HCl buffer (pH 7.4, 4 ml) was added. The soln. was concentrated to 4 ml and desalted on 4 NAP columns (Pharmacia) according to the manufacturer's instructions. The crude RNA sequence (Fig. 2,a) was purified by AE-HPLC (15-70% B in 60 min, 10 injections). The fractions containing pure 23 were pooled (\rightarrow 30 ml), treated with 1M aq. Et₃N · AcOH (pH 7, 5 ml), and applied to 2 Sepak cartridges (Waters): after elution of the salts with 0.1M aq. Et₃N·AcOH (pH 7, 10 ml), followed by H₂O (20 ml), 23 (Et₃NH⁺ form) was eluted with MeCN/H₂O 1:1 (5 ml). After evaporation, the residue was twice dissolved in $\rm H_2O~(2\times1.5~ml)$ and then treated with (NH_4)HCO_3~(2\times8~mg). The mixture was evaporated, the residue dissolved in H₂O (2 ml), and the soln. desalted on two NAP columns according to the manufacturer's instructions: aq. soln of 23 (30 mg (determined by UV spectroscopy, ε (260 nm) 323800 l·mol⁻¹·cm⁻¹); NH₄ form, containing 1 mol-% of Et₃NH⁺ (according to ¹H-NMR); 2.7 µmole, 27% yield (based on solid support)). AE-HPLC (15-70% B in 30 min): t_R 25.2 min (Fig. 2,a). LC-ESI-MS (neg. mode): t_R 11.5 min, 10129.4 amu (after deconvolution, Fig. 2, b). ¹H-NMR (400 MHz, D_2O , c = 2 mM): see Fig. 2, c.

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