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

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*Duilio Arigoni* zum 75. Geburtstag gewidmet

We present optimized reaction conditions for the conversion of 2′-*O*-{[(triisopropylsilyl)oxy]methyl}-(=tom) protected uridine and adenosine nucleosides into the corresponding protected (3-$^{15}$N)-labeled uridine and cytidine and (1-$^{15}$N)-labeled adenosine and guanosine nucleosides **4**, **6**, **12**, and **18**, respectively (*Schemes 1–4*). On a DNA synthesizer, the resulting $^{15}$N-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 $H_2O/D_2O$ 9:1, the $^{15}$N-signals of all base-paired $^{15}$N-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 $^1$H-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 ··· N signals can often be obtained for only relatively short RNA sequences (< 50mers). This serious limitation can be overcome by the introduction of $^{15}$N-labeled nucleotides, allowing the application of heteronuclear NMR experiments that make use of the $^1J$(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 $^{15}$N-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 $^{15}$N-Labeled, 2′-*O*-tom-Protected Phosphoramidites.* Several methods for the selective introduction of $^{15}$N-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 $^{15}$N-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) (=$(MeO)_2$Tr) 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 $^{15}$N-labels in the presence of this lipophilic 2′-*O*-protecting group. Furthermore, we decided to use cheap $^{15}NH_4Cl$ as the sole $^{15}$N-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 $^{15}$N-labeled pyrimidine nucleosides **4** and **6**. Treatment of **1** with $AcOH/H_2O$ (→removal of the $(MeO)_2$Tr group), followed by acetylation of the 3′- and 5′-OH groups with $Ac_2O$ in pyridine gave the diacetylated uridine derivative **2**, which was isolated by chromatography (silica gel) and consecutively treated with $NH_4NO_3/(CF_3CO)_2O$ in $CH_2Cl_2$ 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 $^{15}NH_4Cl$ in the presence of KOH and $Et_3N$ in $MeCN/H_2O$, 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, $^{15}$N-labeled, 2′-*O*-tom-protected uridines was subjected directly to complete deacetylation with NaOH in $THF/MeOH/H_2O$. After extraction and without further purification, the 5′-*O*-$(MeO)_2$Tr group was introduced under standard conditions with

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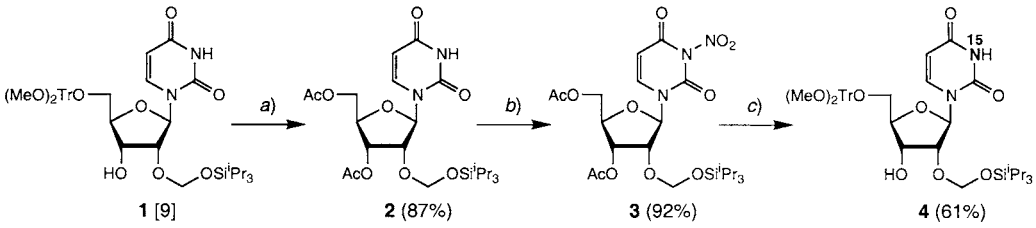
[1]) Bistable RNA sequences adopt two coexisting secondary structures (for other examples, see [11]).

[2]) This clean and efficient reaction sequence allows the straightforward introduction of $^{15}$N 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.

*Scheme 1*

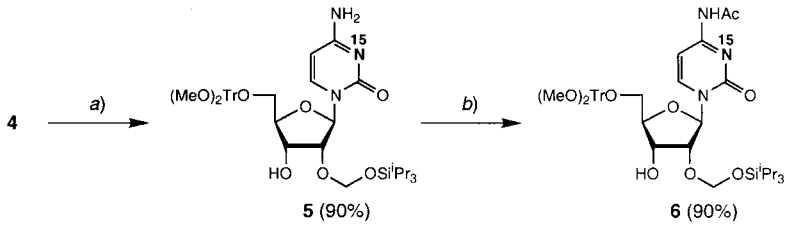



*a*) 1. AcOH, $H_2O$, 25°; 2. $Ac_2O$, *N*,*N*-dimethylpyridin-4-amine (DMAP), pyridine, 25°. *b*) $NH_4NO_3$, $(CF_3CO)_2O$, $CH_2Cl_2$, 4°. *c*) 1. $^{15}NH_4Cl$, KOH, $Et_3N$, MeCN, $H_2O$, 25°; 2. NaOH, THF, MeOH, $H_2O$, 4°; 3. $(MeO)_2TrCl$, pyridine, 25°.

$(MeO)_2TrCl$ in pyridine. The fully protected, $^{15}N$-labeled uridine building block **4** was finally isolated by chromatography (silica gel).

The $^{15}N$-labeled, fully protected cytosine derivative **6** was prepared from the corresponding uridine nucleoside **4** (*Scheme 1*) by first acetylating the 3′-OH group with $Ac_2O$ in pyridine, followed by extractive workup and treatment of the 3′-*O*-acetylated intermediate with $(ClC_6H_4O)P(O)Cl_2$, 1*H*-1,2,4-triazole, and $^iPr_2NEt$ in MeCN (→ formation of the 4-triazolyl derivative)[3]), followed by $NH_3$ in dioxane/$H_2O$ (*Scheme 2*). After extraction and treatment with NaOH in MeOH/THF/$H_2O$, the cytidine nucleoside **5** was isolated by chromatography (silica gel). It was finally transformed into its $N^4$-acetylated, $^{15}N$-labeled derivative **6** by selective *N*-acetylation with $Ac_2O$ in DMF according to *Bhat et al.* [17][4]) and isolated in a good yield by chromatography (silica gel).

*Scheme 2*




*a*) 1. $Ac_2O$, DMAP, pyridine, 25°; 2. 1*H*-1,2,4-triazole, 4-chlorophenyl phosphorodichloridate, $^iPr_2NEt$, MeCN, 25°; 3. $NH_3$, dioxane, MeCN, $H_2O$, 25°; 4. NaOH, THF, MeOH, $H_2O$, 4°. *b*) $Ac_2O$, DMF, 25°.

The two $^{15}N$-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 $MeNH_2$ in $H_2O$/THF and AcOH/$H_2O$, respectively (*Scheme 3*). After precipitation from $CH_2Cl_2$/hexane, the

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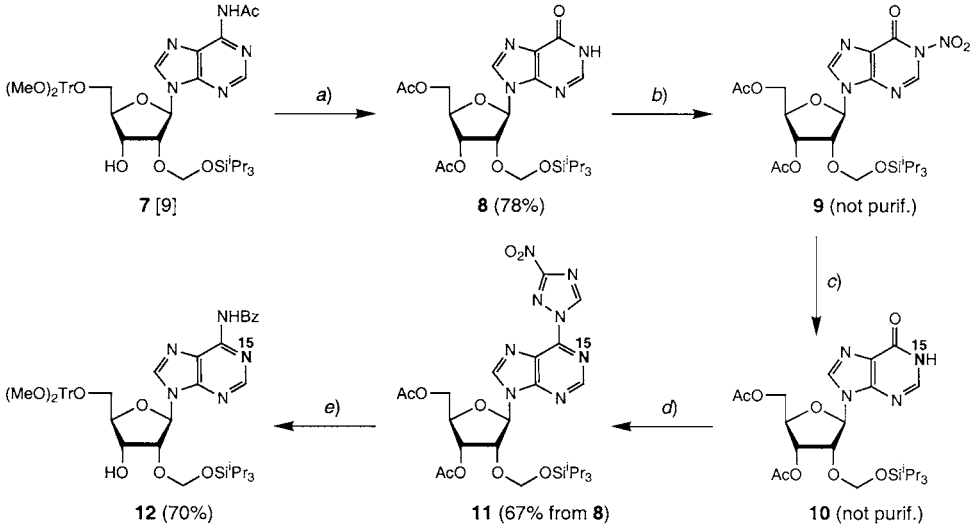
[3]) In preliminary studies, we found that these reaction conditions resulted in a much cleaner and faster product formation than those of the similar reaction performed in pyridine, but without $^iPr_2NEt$ [15], or those involving activation with $TsCl/(PhO)_2P(O)OH$ [16].

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

*Scheme 3*




*a*) 1. $MeNH_2$, THF, $H_2O$, 25°; 2. AcOH, $H_2O$, 25°; 3. $Ac_2O$, DMAP, pyridine, 25°; 4. $NaNO_2$, AcOH, $H_2O$, 25°. *b*) $NH_4NO_3$, $(CF_3CO)_2O$, $CH_2Cl_2$, −20°. *c*) $^{15}NH_4Cl$, KOH, $Et_3N$, MeCN, $H_2O$, 25°. *d*) 3-Nitro-1*H*-1,2,4-triazole, $I_2$, $PPh_3$, $^{i}Pr_2NEt$, toluene, 95°. *e*) 1. $NH_4Cl$, $^{t}BuOK$, $Et_3N$, DMSO, 50°; 2. BzCl, DMAP, pyridine, 25°; 3. NaOH, THF, MeOH, $H_2O$, 4°; 4. $(MeO)_2TrCl$, pyridine, 25°.

intermediate 2′-*O*-tom-protected adenosine was converted with $Ac_2O$ in pyridine into the corresponding 3′,5′-di-*O*-acetyl derivative, which, after evaporation and extraction, was deaminated with $NaNO_2$ in AcOH/$H_2O$ 7:3. The resulting inosine derivative **8** was isolated by crystallization. In analogy to the preparation of the $^{15}N$-labeled uridine nucleoside **3** (*Scheme 1*), but at lower temperature, **8** was treated under strictly anhydrous conditions with $NH_4NO_3/(CF_3CO)_2O$ in $CH_2Cl_2$ [13]. As crude product, the resulting *N*-nitro derivative **9** was treated with 1.3 equiv. of $^{15}NH_4Cl$ in the presence of KOH and $Et_3N$ in MeCN/$H_2O$ [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 $^{15}N$-labeled inosine derivative **10** was treated with $I_2$, $PPh_3$, $^{i}Pr_2NEt$, 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

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[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 $NH_3$, 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/$POCl_3$/$^{i}Pr_2NEt$ in MeCN [20] (very slow reaction); 1*H*-1,2,4-triazole/$(ClC_6H_4O)P(O)Cl_2$/$^{i}Pr_2NEt$ in pyridine [15] (low yield, significant side reactions); 1*H*-tetrazole/TsCl/$(PhO)_2P(O)OH$/$^{i}Pr_2NEt$ in pyridine [21] (incomplete conversion); 3-nitro-1-[(2,4,6-triisopropylphenyl)sulfonyl]-1*H*-1,2,4-triazole/$(PhO)_2P(O)OH$/$^{i}Pr_2NEt$ 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/$(PhO)_2P(O)OH$ 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 $NH_4Cl$, $^t$BuOK, $Et_3N$ in DMSO[6]), followed by precipitation with $H_2O$, 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 derivative[7]) with BzCl in pyridine, then deacetylated with NaOH in THF/MeOH/$H_2O$, and finally converted to its 5′-*O*-$(MeO)_2$Tr derivative **12** with $(MeO)_2$TrCl in pyridine. The fully protected, $^{15}$N-labeled adenosine building block **12** was isolated in a good yield by chromatography (silica gel).

The synthesis of the $^{15}$N-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 $I_2$, $PPh_3$, $^i Pr_2NEt$, and 3-nitro-1*H*-1,2,4-triazole in toluene (*Scheme 4*); the resulting 3-nitro-1*H*-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 $^t$BuOK/$Et_3N$ in DMSO. The so-obtained $^{15}$N-labeled adenosine derivative **14** was precipitated with $H_2O$. 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 $CH_2Cl_2$ 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 $Et_3N$ and MeI to yield a 1-methoxy-$N^6$-cyano-substituted derivative; the latter was treated first with NaOH in THF/$H_2O$ 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-$N^6$-methoxy-substituted purine derivative **16** was formed almost quantitatively, together with small amounts of still partially acetylated derivatives[8]). 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 $N^2$,$N^2$,$N^6$,3′-*O*,5′-*O*-pentaacetyl derivatives was formed, which was filtered through silica gel and then partially deacetylated with NaOH in THF/MeOH/$H_2O$ 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 **17**[9]). Without purification, this purine-2,6-diamine nucleoside was first deaminated with $NaNO_2$ in AcOH/$H_2O$ and then dimethoxytritylated with $(MeO)_2$TrCl in pyridine; finally, the $^{15}$N-labeled, fully protected guanosine

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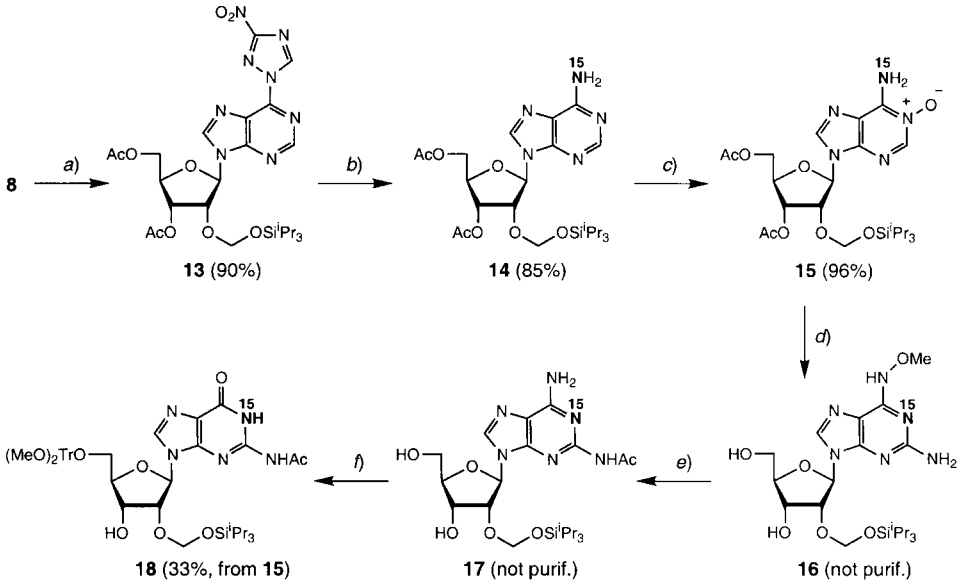
[6]) Conditions developed for the preparation of the analogous compound **14** (*Scheme 4*) with $^{15}NH_4Cl$.

[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*-$(MeO)_2$Tr-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.

*Scheme 4*




*a*) 3-Nitro-1*H*-1,2,4-triazole, $I_2$, $PPh_3$, $^iPr_2NEt$, toluene, 95°. *b*) 1. $^{15}NH_4Cl$, $^tBuOK$, $Et_3N$, DMSO, 50°; 2. $Ac_2O$, DMAP, pyridine, 25°. *c*) 3-Chloroperbenzoic acid, $CH_2Cl_2$, 25° (indicated yield based on consumption). *d*) 1. BrCN, MeOH, 25°; 2. $Et_3N$, DMF; 3. MeI, DMF, 25°; 4. NaOH, THF, $H_2O$, 25°; 5. aq. HCl soln. (→ pH 7), EtOH, 60°. *e*) 1. $Ac_2O$, pyridine, 100°; 2. NaOH, THF, MeOH, $H_2O$, 4°; 3. *Raney*-Ni, THF, MeOH, $H_2O$, 80°. *f*) 1. $NaNO_2$, AcOH, $H_2O$, 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).

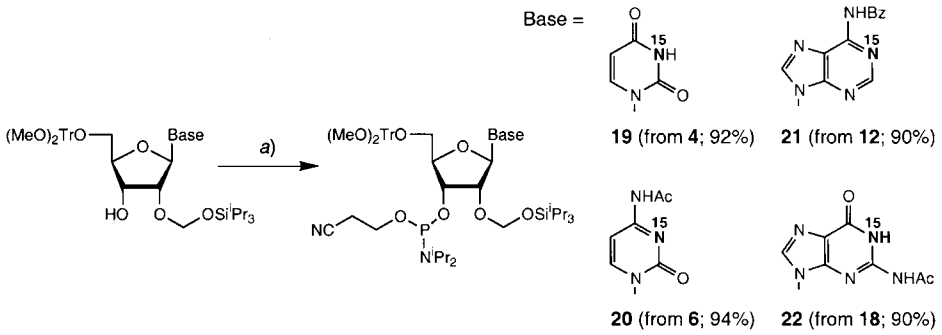
All compounds presented in *Schemes 1–4* were characterized by $^1$H-NMR, $^{13}$C-NMR, and MS (see *Exper. Part*). In the MS of all compounds **1**–**18**, the *m*/*z* value of the most-intense signal was in agreement with the calculated value (with the exception of **3**, which decomposed during analysis).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

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

| | *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** ($N^6$-$^{15}$N) | 2.5 | | 3.3 | 20.7 |
| **15** ($N^6$-$^{15}$N) | | | 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 |

According to our standard procedure, the four $^{15}$N-labeled, 2′-*O*-tom-protected nucleosides **4**, **6**, **12**, and **18** were converted with 2-cyanoethyl diisopropylphosphoramidochloridite/$^i$Pr$_2$NEt into the corresponding phosphoramidite building blocks **19**–**22** (*Scheme 5*), which were isolated in good yields by chromatography (silica gel).

*Scheme 5*




*a*) 2-Cyanoethyl diisopropylphosphoramidochloridite, $^i$Pr$_2$NEt, $CH_2Cl_2$, 25°.

MALDI-MS Analysis of the four $^{15}$N-labeled phosphoramidites **19**–**22** showed the expected *m*/*z* values. The chemical-shift values of their $^1$H- and $^{31}$P-NMR spectra corresponded well with the ones found for their unlabeled analogues [6][8]. The additional *J*(H,N) coupling constants, detected by $^1$H-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 $^{15}$N-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

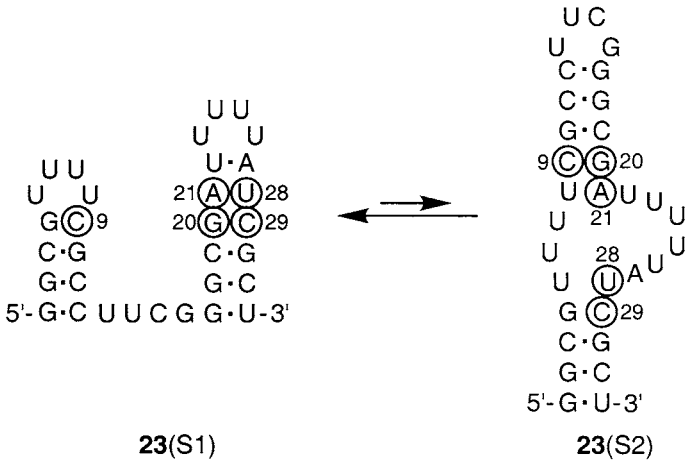



Fig. 1. *Representation of the two coexisting structures (S1) and (S2) adopted by the RNA sequence* **23** (according to [9]). The encircled and numbered nucleosides derive from the $^{15}$N-labeled phosphoramidites **19**–**22** (numbering from 5′ → 3′ end). In 150 mM NaCl and 50 mM sodium phosphate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

any $^{15}$N-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-μM scale from conventional 2′-*O*-tom-protected ribonucleoside phosphoramidites and the $^{15}$N-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 well-separated, chromatographically homogeneous main product (*Fig. 2,a*). After purification by prep. AE-HPLC and desalting (for details, see *Exper. Part*), 30 mg (2.7 μmole; 27% yield, based on the solid support) of chromatographically pure RNA sequence **23** ($NH_4^+$ form, containing 1 mol-% of $Et_3NH^+$, according to $^1$H-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 $^1$H-NMR spectrum (400 MHz, $D_2O$; *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 $H_2O/D_2O$ 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 ($\delta$ 9–15) of spectra obtained from three different 1D experiments are shown. The conventional $^1$H-NMR spectrum, recorded without $^{15}$N-decoupling (*Fig. 3,a*), shows the expected complex

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[10]) Partial assignment was carried out by comparative NH ··· N $^1$H-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.

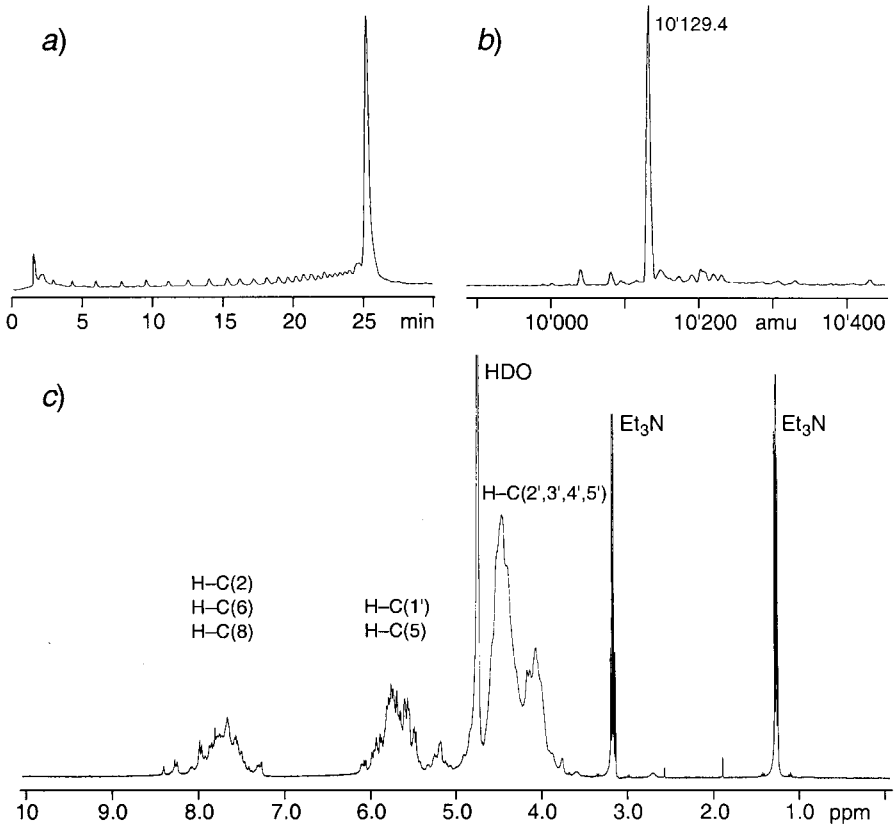


Fig. 2. *Characterization of RNA sequence* **23** (prepared according to [8]): a*) AE-HPLC trace of the crude product* (detection at 260 nm, 15–70% *B* in 30 min); b*) deconvoluted LC-ESI-MS* (neg. mode) *of the purified product* (*M* (calc.) 10129.2 amu; for details, see *Exper. Part*); c*) $^1$H-NMR spectrum* (400 MHz, $D_2O$, *c* = 2 mM) *of purified* **23** ($NH_4^+$ form, containing 1 mol-% of residual $Et_3NH^+$ 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 $\delta$ 12.0–13.5, typical for the 'imino-protons' of G · C base pairs [1]. Upon broad-band $^{15}$N-decoupling (*Fig. 3*,*b*), the *d*s at $\delta$ 14.2, 12.1, and 11.9 ($J$(H,N) ≈ 90 Hz) change to *s*s, 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 *d*s of *Fig. 3*,*a*, appear in the ($^1$H-decoupled) 1D-HSQC spectrum (*Fig. 3*,*c*). These initial 1D-NMR experiments allowed, in a straightforward way, to isolate 'imino-protons' of base-paired, $^{15}$N-labeled uridines and guanosines and showed the occurrence of one A · $^{15}$NU ($\delta$ 14.2) and

[12]) The $\delta$ 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]).

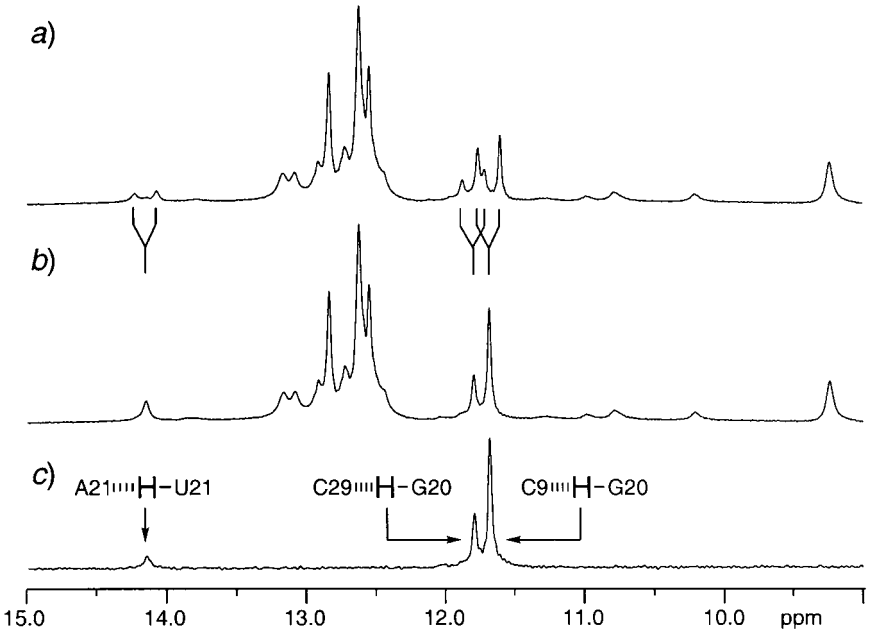


Fig. 3. *'Imino-proton' region of $^1$H-NMR spectra* (600 Hz, $H_2O/D_2O$ 9:1, for conditions, see text): a) *$^{15}$N-decoupled,* b) *$^{15}$N-broad-band-decoupled, and* c) *1D-HSQC spectrum, showing the 'imino-proton' signals of the indicated base pairs*

two C·$^{15}$NG base pairs ($\delta$ 12.1 and 11.9). Based on the two secondary structures of **23** (*Fig. 1*), the signal appearing at $\delta$ 14.3 was assigned to base pair U28·A21-**23**(S1). The other two signals, appearing in the HSQC spectrum at $\delta$ 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 $^1$H,$^{15}$N-correlation, are shown in *Fig. 4*. The atom $^{15}$N(3) of the base pair $^{15}$NU28·A21-**23**(S1) appears at $\delta$ 163, whereas $^{15}$N(1) of both the base pairs $^{15}$NG20·C29-**23**(S1) and $^{15}$NG20·C9-**23**(S2) appear at the same $\delta$ value of 146 (*Fig. 4,a*). Furthermore, the two-bond scalar coupling between H–C(2) and N(1) of A21 ($^2J$(H,N) = 16 Hz) resulted in two resolved cross-peaks at $\delta$(H/N) 7.82/222.1 and $\delta$(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 $^{15}$NA21-**23**(S1) in its base-paired form.

The main purpose of HNN-COSY experiments is the correlation of the H-bond-donor with the H-bond-acceptor N-atom within a base pair, provided that both are $^{15}$N-labeled [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 $^{15}$N-labeled base pairs A21·U28-**23**(S1), G20·C29-**23**(S1), and G20·C9-**23**(S1), and furthermore, the $\delta$ values for the acceptor nucleosides A21, C29, and C9 could be assigned. In contrast to

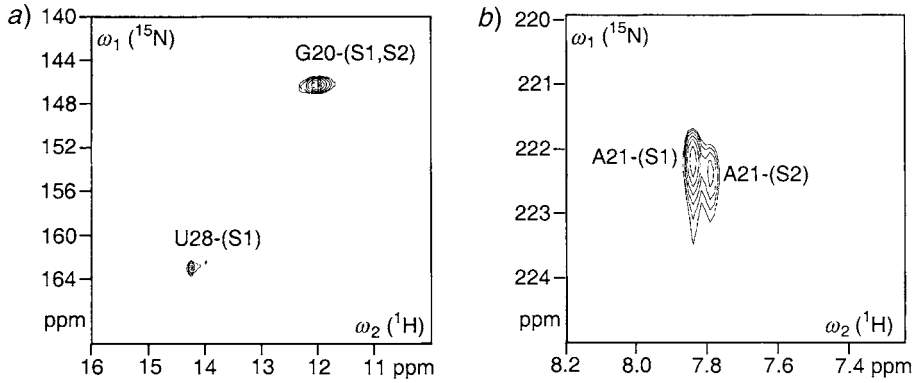


Fig. 4. *Selected regions of a 2D-HSQC spectrum* (600 MHz, $H_2O/D_2O$ 9:1, for conditions, see text): a) *H−N(3),N(3) and H−N(1),N(1) cross-peaks of the base-paired, $^{15}$N-labeled nucleotides U28-(S1) and G20-(S1,S2);* b) *resolved H−C(2),N(1) cross-peaks of the $^{15}$N-labeled nucleotide A21-(S1) and A21-(S2)* (forming base pairs in both structures)

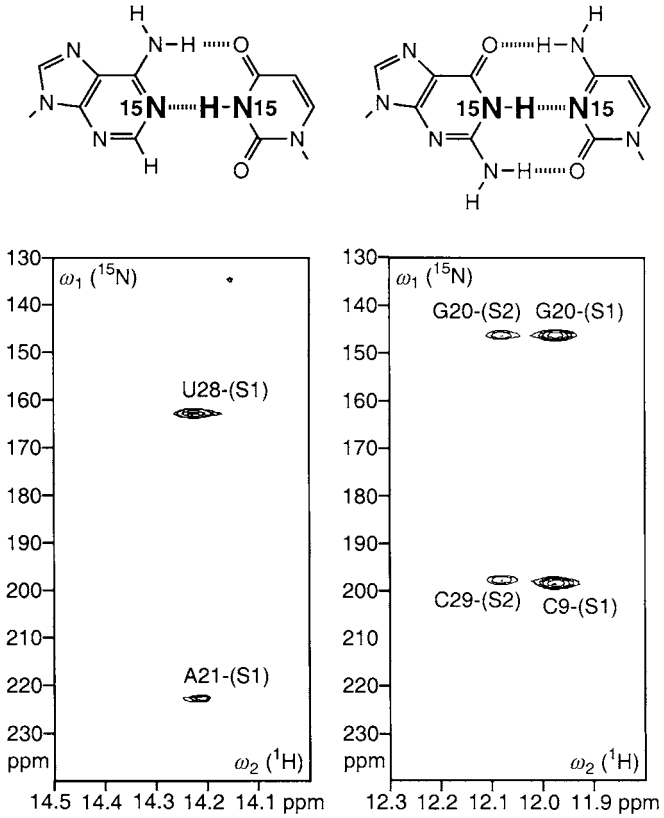


Fig. 5. *Selected regions of a HNN-COSY plot* (600 MHz, N,N-transfer time 20 ms, H,N-transfer time 5 ms; $H_2O/D_2O$ 9:1, for conditions, see text), *showing correlation between $^{15}$N-resonances of doubly $^{15}$N-labeled base pairs*

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

**3. Discussion.** – NMR Analysis of larger RNA sequences (consisting of $>50$ nucleotides) suffers from bad signal separation in the $^1$H- as well as in the $^{15}$N-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 $^{15}$N-labeled, 2′-*O*-tom-protected ribonucleoside phosphoramidite building blocks **19**–**22**, which are precursors of selectively $^{15}$N-labeled 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 $^{15}NH_4Cl$ (our only $^{15}$N-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 $^{15}$N-labeled ribonucleosides, such as (1-$^{15}$N)inosine from **10** and (1-$^{15}$N)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 $^{15}$N-labeled nucleotides. In the $^1$H-NMR spectrum, the 'imino-proton' signals of the G·C base pairs (forming the stem regions of the two coexisting hairpin structures **23**(S1) and **23**(S2)), are crowded in a narrow $\delta$ range (11.9–13.2 ppm), and some signal overlap occurs. The corresponding 1D-HSQC spectrum, however, shows only the three resonances of the $^{15}$N-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, $^{15}$N-chemical-shift values the for base-paired, labeled guanosines and uridines were obtained, but unfortunately, the $^{15}$N-signals of G20, involved in two different base pairs, were isochronous[13]). The coupling between $^{15}$N(1) and H–C(2) of A21, however, resulted in cross-peaks with different $\delta$ values, both in the $^1$H- and the $^{15}$N-dimension. As expected,

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[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 $^{15}$N-labeled, whereas, in the second structure, only the donor base carries a $^{15}$N-label. This labeling pattern would allow to detect both $^{15}$N-resonances in the HSQC experiment, and to assign them by a HNN-COSY experiment.

the HNN-COSY experiment provided correlated $^{15}N$-resonances for the doubly labeled base pairs and allowed the assignment of the $^{15}N$-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 $^{15}N$-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].

We are very grateful to Dr. *Jens Dittmer* and Prof. *G. Bodenhausen* (EPFL) for many helpful discussions and continuous support in carrying out NMR experiments and we thank Prof. *Ronald Micura* (University of Innsbruck) for his highly appreciated advice concerning bistable RNA sequences. This work was generously supported by the EPFL and by the *Swiss National Science Foundation* (Grant Nr. 2000-06890).

## 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 $CH_2Cl_2$ and sat. aq. $NaHCO_3$ soln., drying of the org. layer ($MgSO_4$), and evaporation. TLC: precoated silica-gel plates from *Merck*, stained by dipping into a soln. of anisaldehyde (10 ml), $H_2SO_4$ (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), 6 M urea; eluent *B:* 12 mM *Tris*·HCl (pH 7.4), 0.5 M $NaClO_4$, 6 M urea; detection at 260 nm, elution at 85°. NMR (*Bruker* instruments): chemical shift $\delta$ in ppm rel. to external standards ($^1H$ and $^{13}C$: $Me_4Si$, $^{31}P$: 85% aq. $H_3PO_4$ soln., $^{15}N$: 20 mM $^{15}NH_4Cl$ in 10% aq. HCl soln.); coupling constants *J* in Hz; multiplicities ($^{13}C$) according to DEPT spectra. ESI-MS (pos. mode): *SSQ 710* (*Finnigan*), measurements in MeCN/$H_2O$/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. $Me_2NBu$·$H_2CO_3$ (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*-software[14]).

*3′,5′-Di*-O-*acetyl-2′*-O-*{[(triisopropylsilyl)oxy]methyl}uridine* (**2**). A soln. of **1** [9] (7.32 g, 10 mmol) in AcOH/$H_2O$ 4:1 (180 ml) was stirred for 30 min at r.t. After evaporation (under continuous addition of small portions of $H_2O$ 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 $Ac_2O$ (2.83 ml, 30 mmol). After 1 h at r.t., MeOH (5 ml) was added, and the mixture was evaporated. CC ($SiO_2$, (150 g), hexane/AcOEt 4:1 → 1:4) gave **2** (4.47 g, 87%). Colorless foam. TLC (hexane/AcOEt 2:3): $R_f$ 0.45. $^1H$-NMR (400 MHz, $CDCl_3$): 1.01–1.03 (*m*, $^iPr_3Si$); 2.12, 2.14 (2*s*, 2 MeCO); 4.31–4.36 (*m*, H–C(4′), $CH_2$(5′)); 4.53 (*t*, *J* = 5.7, H–C(2′)); 4.87, 4.95 (2*d*, *J* = 5.1, $OCH_2O$); 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)). $^{13}C$-NMR (100 MHz, $CDCl_3$): 12.0 (*d*, $Me_2CH$); 17.9 (*q*, $Me_2CH$); 20.9 (*q*, *Me*CO); 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*, $OCH_2O$); 103.1 (*d*, C(5)); 140.0 (*d*, C(6)); 150.0 (*s*, C(2)); 162.6 (*s*, C(4)); 170.2 (*s*, Me*C*O). ESI-MS: 515.3 (100, $[M+H]^+$).

*3′,5′-Di*-O-*acetyl-3-nitro-2′*-O-*{[(triisopropylsilyl)oxy]methyl}uridine* (**3**). At 4° and under Ar, $(CF_3CO)_2O$ (6.55 g, 31.2 mmol) was added dropwise to a well-stirred suspension of finely powdered $NH_4NO_3$ (1.25 g,

[14]) Conditions adapted from [27].

15.6 mmol; dried for 48 h at 60°/0.05 mbar) in $CH_2Cl_2$ (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 $CH_2Cl_2$ (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 $SiO_2$ (40 g, $CH_2Cl_2 \rightarrow CH_2Cl_2$/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. $^1$H-NMR (400 MHz, $CDCl_3$): 1.01–1.07 (*m*, $^i$Pr$_3$Si); 2.12, 2.15 (2*s*, MeCO); 4.29–4.40 (*m*, H–C(4′), $CH_2$(5′)); 4.55 (*t*, *J* = 5.7, H–C(2′)); 4.86, 4.96 (2*d*, *J* = 5.0, $OCH_2O$); 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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2CH$); 17.8 (*q*, $Me_2CH$); 20.85, 20.88 (2*q*, *Me*CO); 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*, $OCH_2O$); 102.0 (*d*, C(5)); 139.4 (*d*, C(6)); 145.4 (*s*, C(2)); 155.0 (*s*, C(4)); 170.1 (*s*, Me*C*O). ESI-MS: dec.

*5′-O-(4,4′-Dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}(3-$^{15}$N)uridine* (**4**). Into a tightly stoppered, evacuated flask containing $^{15}NH_4Cl$ (0.552 g, 10.14 mmol) and KOH (0.525 g, 9.36 mmol), consecutively $H_2O$ (20 ml), MeCN (20 ml), $Et_3N$ (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 $CH_2Cl_2/H_2O$, 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 g), hexane/AcOEt 4:1 → 2:3 (+1% $Et_3N$)) afforded **4** (3.20 g, 61%). Colorless, solid foam. TLC (hexane/AcOEt 1:1): $R_f$ 0.55. $^1$H-NMR (400 MHz, $CDCl_3$): 1.07–1.14 (*m*, $^i$Pr$_3$Si); 3.17 (*d*, *J* = 5.7, OH–C(3′)); 3.52 (br. *d*, *J* ≈ 1, $CH_2$(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 (2*d*, *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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 12.0 (*d*, $Me_2CH$); 17.9 (*q*, $Me_2CH$); 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*, $OCH_2O$); 102.3 (*d*, *J*(C,N) = 5.6, C(5)); 113.4 (*d*, arom. C); 127.3, 128.1, 128.3, 130.3 (4*d*, arom. C); 135.2, 135.5 (2*s*, 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)). $^{15}$N-NMR (40 MHz, $CDCl_3$): 133.1. ESI-MS: 756.30 (100, $[M + Na]^+$).

*5′-O-(4,4′-Dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}(3-$^{15}$N)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 $Ac_2O$ (0.34 ml, 3.54 mmol). Workup after 3 h at r.t., co-evaporation with toluene (2 × 20 ml), extraction with $CH_2Cl_2$/10% aq. citric acid and sat. aq. $NaHCO_3$ soln., drying ($MgSO_4$), 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 1*H*-1,2,4-triazole (2.13 g, 30.8 mmol; dried by sublimation) in dry MeCN (12 ml). After 15 min at 4°, $^i$Pr$_2$NEt (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. $NH_3$ soln. (27 ml) and stirred for another 3 h at r.t. Extraction with $CH_2Cl_2$/10% aq. citric acid and sat. aq. $NaHCO_3$ 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 ($SiO_2$ (15 g), $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 19:1) gave **5** (1.17 g, 90%). Colorless foam. TLC (AcOEt): $R_f$ 0.40. $^1$H-NMR (400 MHz, $CDCl_3$): 1.05–1.14 (*m*, $^i$Pr$_3$Si); 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 (2*d*, *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, 4 arom. H); 7.21–7.43 (*m*, 9 arom. H); 8.08 (*d*, *J* = 7.4, H–C(6)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 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*, $OCH_2O$); 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, $[M + 1]^+$).

*N$^4$-Acetyl-5′-O-(4,4′-dimethoxytrityl)-2′-O-{[(triisopropylsilyl)oxy]methyl}(3-$^{15}$N)cytidine* (**6**). A soln. of **5** (1.15 g, 1.57 mmol) in DMF (7.5 ml) was treated with $Ac_2O$ (225 mg, 2.2 mmol) and stirred for 8 h at r.t. Extraction with sat. aq. $NaHCO_3$ soln. and AcOEt/hexane 1:1, followed by CC ($SiO_2$ (12 g) hexane/AcOEt 4:1 → 3:7 (+1% $Et_3N$)) gave **6** (1.10 g, 90%). Colorless solid foam. TLC (hexane/AcOEt 3:7): $R_f$ 0.50. $^1$H-NMR (400 MHz, $CDCl_3$): 1.07–1.14 (*m*, $^i$Pr$_3$Si); 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

($d$, $J$ = 4.9, H–C(2′)); 4.40 ($dt$, $J$ = 8.4, 5.0, H–C(3′)); 5.16, 5.30 (2$d$, $J$ = 4.7, $OCH_2O$); 5.98 ($s$, H–C(1′)); 6.88 ($d$, $J$ = 8.8, 4 arom. H); 7.08 ($d$, $J$ = 7.4, H–C(5)); 7.28–7.44 ($m$, 9 arom. H); 8.49 ($d$, $J$ = 7.4, H–C(6)); 9.11 (br. $s$, NH–C(4)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 12.0 ($d$, $Me_2CH$); 17.9 ($q$, $Me_2CH$); 25.1 ($q$, $MeCO$); 55.4 ($q$, MeO); 61.3 ($t$, C(5′)); 67.9 ($d$, C(2′)); 83.5 ($d$, C(3′)); 83.6 ($d$, C(4′)); 87.2 ($s$, arom. C); 90.2 ($d$, C(1′)); 90.9 ($t$, $OCH_2O$); 96.5 ($d$, $J$(C,N) = 1.0, C(5)); 113.4 ($d$, arom. C); 127.3, 128.2, 128.4, 130.3 (4$d$, arom. C); 135.7, 144.5 (2$s$, arom. C); 145.0 ($d$, C(6)); 155.4 ($s$, $J$(C,N) = 7.7, C(2)); 158.8 ($s$, arom. C); 162.6 ($s$, $J$ = 7.4, C(4)); 170.0 ($s$, Me$CO$). $^{15}$N-NMR (40 MHz, $CDCl_3$): 208.1. ESI-MS: 775.3 (100, $[M+H]^+$).

*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% aq. $MeNH_2$ 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/$H_2O$ 4:1 (650 ml) and stirred for 30 min at r.t. The solvent was evaporated (under addition of a small amount of $H_2O$ towards the end of the evaporation) and the residue co-evaporated with toluene (3 × 200 ml). To a well-stirred soln. of this residue in $CH_2Cl_2$ (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 $Ac_2O$ (8.33 ml, 88.2 mmol). After 30 min at r.t., MeOH (15 ml) was added, the solvent evaporated, and the residue co-evaporated with toluene (3 × 100 ml) and dissolved in AcOH/$H_2O$ 7:3 (560 ml). At r.t., this soln. was treated with five portions of $NaNO_2$ (5 × 40 g, 2.9 mol), which were added within 2.5 h. $H_2O$ (100 ml) was added and the mixture extracted with $CHCl_3$ (500 ml). The org. phase was extracted with $H_2O$ and sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. Recrystallization from AcOEt (400 ml) gave **8** (14.94 g, 78%). Colorless crystals. M.p. 190°. TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.49. $^1$H-NMR (400 MHz, $CDCl_3$): 0.93 ($m$, $^iPr_3Si$); 2.12, 2.17 (2$s$, 2 MeCO); 4.35–4.45 ($m$, H–C(4′), $CH_2$(5′)); 4.86, 4.92 (2$d$, $J$ = 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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 ($d$, $Me_2CH$); 17.8 ($q$, $Me_2CH$); 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 (2$s$, Me$CO$). 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, $(CF_3CO)_2O$ (10.9 g, 52.0 mmol) was added dropwise to a well-stirred suspension of finely powdered $NH_4NO_3$ (2.08 g, 26 mmol; dried for 48 h at 60°/0.05 mbar) in $CH_2Cl_2$ (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 $CH_2Cl_2$ (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 ($SiO_2$ (1 g), $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 19:1): 35 mg (*ca.* 70%) of pure **9**[15]). TLC (hexane/AcOEt 3:7): $R_f$ 0.81. $^1$H-NMR (400 MHz, $CDCl_3$): 0.88–1.09 ($m$, $^iPr_3Si$); 2.12, 2.17 (2$s$, 2 MeCO); 4.38 (br. $t$, $J \approx 4$, $CH_2$(5′)); 4.48 ($dd$, $J$ = 3.8, 7.3, H–C(4′)); 4.85, 4.91 (2$d$, $J$ = 5.1, $OCH_2O$); 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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 ($d$, $Me_2CH$); 17.8 ($q$, $Me_2CH$); 20.9 ($q$, $MeCO$); 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 ($t$, $OCH_2O$); 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 (2$s$, Me$CO$). 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 $^{15}NH_4Cl$ (0.46 g, 8.5 mmol) and KOH (0.43 g, 7.8 mmol), consecutively $H_2O$ (16 ml), MeCN (16 ml), $Et_3N$ (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 $CH_2Cl_2/H_2O$, and co-evaporated with toluene: crude **10** (3.40 g). Colorless solid foam. For characterization, a small amount was purified by prep. TLC. TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.49. $^1$H-NMR (400 MHz, $CDCl_3$): 0.87–1.06 ($m$, $^iPr_3Si$); 2.12, 2.17 (2$s$, 2 MeCO); 4.35–4.45 ($m$, H–C(4′), $CH_2$(5′)); 4.86, 4.92 (2$d$, $J$ = 5.1, $OCH_2O$); 5.07 ($t$, $J \approx 5$, 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 ($d$, $J$(H,N) = 7.5, H–C(2)); 13.08 ($d$, $J$(H,N) = 88.6, H–N(1)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 ($d$, $Me_2CH$); 17.8 ($q$, $Me_2CH$); 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 (4$d$, C(1′)); 89.6 ($t$, $OCH_2O$); 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 (2$s$, Me$CO$). ESI-MS: 540.32 (100, $[M+H]^+$).

*{3′,5′-Di*-O-*acetyl-2′*-O-*[(triisopropylsilyl)oxy]methyl}-β-*D-*ribofuranosyl}-6-(3-nitro-1*H*-1,2,4-triazol-1-yl)(1-$^{15}$N)purine* (**11**). A suspension of crude **10** (3.35 g), 3-nitro-1,2,4-1*H*-triazole (2.60 g, 22.7 mmol), $PPh_3$

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

(4.10 g, 14.9 mmol), and $I_2$ (3.46 g, 13.6 mmol) in toluene (130 ml) was heated to 95° and treated with $^i Pr_2NEt$ (5.60 ml, 32.5 mmol). After stirring for 50 min at 95°, the solvent was evaporated, and CC ($SiO_2$ (100 g), hexane/AcOEt 4:1 → 2:3) gave **11** (2.34 g, 67% based on **8**) as a yellow foam. TLC (AcOEt/hexane 1:1): $R_f$ 0.49. $^1$H-NMR (400 MHz, $CDCl_3$): 0.88–0.91 (*m*, $^i Pr_3Si$); 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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2CH$); 17.8 (*q*, $Me_2CH$); 20.9 (*q*, *Me*CO); 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*, $OCH_2O$); 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*, Me*C*O). 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 $NH_4Cl$ (730 mg, 13.5 mmol), DMSO (21 ml) was added, followed by a soln. of $^t$BuOK (1.45 g, 13.0 mmol) and $Et_3N$ (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. $N_2$). 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 $H_2O$ (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 × 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 $CH_2Cl_2$ and extracted with 10% aq. citric acid soln., followed by sat. aq. $NaHCO_3$ soln. The solid obtained after drying ($MgSO_4$) 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 ($SiO_2$ (50 g), hexane/AcOEt 4:1 → 3:7) gave **12** (1.63 g, 70%). Colorless foam. TLC (AcOEt/hexane 7:3): $R_f$ 0.60. $^1$H-NMR (400 MHz, $CDCl_3$): 1.05–1.15 (*m*, $^i Pr_3Si$); 3.12 (*d*, $J = 3.2$, OH–C(3′)); 3.42 (*dd*, $J = 3.8$, 10.9, H–C(5′)); 3.53 (*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′)); 5.00 (*t*, $J = 5.2$, H–C(2′)); 5.01, 5.18 (2*d*, $J = 4.7$, $OCH_2O$); 6.27 (*d*, $J = 5.3$, H–C(1′)); 6.81–6.83 (*m*, 4 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 (br. *s*, NH–C(6)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2CH$); 17.9 (*q*, $Me_2CH$); 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*, $OCH_2O$); 113.3 (*d*, arom. C); 123.6 (*s*, *J*(C,N) = 1.5, C(5)); 127.1, 128.0, 128.3 (3*d*, arom. C); 129.0, 130.2, 132.9 (3*d*, arom. C), 133.8, 135.8 (2*s*, 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*, Ph*C*O). $^{15}$N-NMR (40 MHz, $CDCl_3$): 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-1*H*-1,2,4-triazol-1-yl)purine* (**13**). A suspension of **8** (6.53 g, 12.1 mmol), 3-nitro-1,2,4-1*H*-triazole (4.85 g, 42.5 mmol), $PPh_3$ (7.64 g, 29.1 mmol), and $I_2$ (6.47 g, 25.5 mmol) in toluene (240 ml) was heated to 95° and treated with $^i Pr_2NEt$ (10.4 ml, 60.7 mmol). After stirring for 50 min at 95°, the solvent was evaporated, and CC ($SiO_2$ (160 g), hexane/AcOEt 4:2 → 2:3) gave **13** (6.92 g, 90%). Yellow foam. TLC (AcOEt/hexane 1:1): $R_f$ 0.49. $^1$H-NMR (400 MHz, $CDCl_3$): 0.88–0.91 (*m*, $^i Pr_3Si$); 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 (*s*, H–C(2)); 9.82 (*s*, H–C(triazole)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2CH$); 17.8 (*q*, $Me_2CH$); 20.9 (*q*, *Me*CO); 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*, $OCH_2O$); 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*, Me*C*O). ESI-MS: 635.38 (100, $[M+H]^+$).

*3′,5′-Di-*O*-acetyl-2′-*O*-{[(triisopropylsilyl)oxy]methyl}(*N$^6$*-*$^{15}$*N)adenosine* (**14**). Through the septum of an evacuated flask containing $^{15}NH_4Cl$ (1.78 g, 32.8 mmol), DMSO (29 ml) was added, followed by a soln. of $^t$BuOK (3.43 g, 30.6 mmol) and $Et_3N$ (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. $N_2$). 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 $H_2O$ (2.5 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 × 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 $Ac_2O$ (0.21 ml, 2.2 mmol). After 30 min at r.t., MeOH (5 ml) was added, and the solvent was evaporated. CC ($SiO_2$ (140 g), $CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH 93:7) gave **14** (4.99 g, 85%). Colorless foam. TLC ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.57. $^1$H-NMR (400 MHz, $CDCl_3$): 0.88–1.01 (*m*, $^i$Pr$_3$Si); 2.11, 2.16 (2*s*, 2 MeCO); 4.37–4.48 (*m*, H–C(4′), $CH_2$(5′)); 4.84, 4.90 (2*d*, *J* = 4.8, $OCH_2O$); 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, $NH_2$(6)); 6.12 (*d*, *J* = 5.7, H–C(1′)); 7.93 (*s*, H–C(8)); 8.34 (*s*, H–C(2)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2CH$); 17.7 (*q*, *Me*$_2$CH); 20.9 (*q*, *Me*CO); 63.6 (*t*, C(5′)); 71.6 (*d*, C(3′)); 76.4 (*d*, C(2′)); 80.7 (*d*, C(4′)); 87.6 (*d*, C(1′)); 89.6 (*t*, $OCH_2O$); 120.4 (*s*, *J*(C,N) = 3.3, C(5)); 139.5 (*d*, C(8)); 150.0 (*s*, C(4)); 153.3 (*d*, *J*(C,N) = 2.5, C(2)); 155.6 (*s*, *J*(C,N) = 20.7, C(6)); 170.2, 170.5 (2*s*, Me*C*O). MALDI-MS: 638.60 (100, $[M+H]^+$).

*3′,5′-Di*-O-*acetyl-2′*-O-*{[(triisopropylsilyl)oxy]methyl}*($N^{6}$-$^{15}$*N)adenosine 1-Oxide* (**15**). A suspension of 70% 3-chloroperbenzoic acid (5.50 g, 22.3 mmol) and anh. $MgSO_4$ (5 g) in $CH_2Cl_2$ (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 $CHCl_3$ (150 ml) and 5% aq. NaI soln. (100 ml). After stirring for 10 min, solid $Na_2S_3O_3$ was added until the red color ($I_2$) disappeared. The org. phase was extracted with sat. aq. $NaHCO_3$ soln. and evaporated. CC ($SiO_2$ (75 g), $CH_2Cl_2 \rightarrow CH_2Cl_2$/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 ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.46. $^1$H-NMR (400 MHz, $CDCl_3$): 0.88–1.01 (*m*, $^i$Pr$_3$Si); 2.10, 2.16 (2*s*, 2 MeCO); 4.34–4.44 (*m*, H–C(4′), $CH_2$(5′)); 4.84, 4.90 (2*d*, *J* = 4.8, $OCH_2O$); 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, $NH_2$–C(6)); 8.04 (*s*, H–C(8)); 8.69 (*s*, H–C(2)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.8 (*d*, $Me_2$*C*H); 17.7 (*q*, *Me*$_2$CH); 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*, $OCH_2O$); 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*, Me*C*O). MALDI-MS: 638.60 (100, $[M+H]^+$).

$N^6$-*Methoxy-9-[2′*-O-*{[(triisopropylsilyl)oxy]methyl}-β-*D-*ribofuranosyl](1-*$^{15}$*N)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 $Et_3N$ (1.54 ml, 10.97 mmol; freshly filtered over $Al_2O_3$) 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 ($CH_2Cl_2$/MeOH 9:1): $R_f$ 0.40. $^1$H-NMR (400 MHz, $CDCl_3$): 0.88–1.06 (*m*, $^i$Pr$_3$Si); 3.11 (*s*, OH–C(3′)); 3.74 (*m*, $CH_2$(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 (2*d*, *J* = 5.1, $OCH_2O$); 4.92 (*dd*, *J* = 4.7, 7.5, H–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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2$*C*H); 17.8 (*q*, *Me*$_2$CH); 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(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-*$^{15}$*N)purine-2,6-diamine* (**17**). A soln. of crude **16** (2.4 g) was dissolved in $Ac_2O$/pyridine 1:2 (22 ml) and heated for 3 h at 100°. Evaporation and co-evaporation with toluene (2 × 50 ml) gave a residue, which was dissolved in $CH_2Cl_2$ (40 ml), adsorbed on $SiO_2$ (12 g), and filtered through a pad of $SiO_2$ (12 g) (hexane/AcOEt 1:1 → 9:1, then AcOEt/MeOH 99:1). The 1:1 mixture of peracetylated intermediates (2.11 g, TLC (hexane/AcOEt 1:9): $R_f$ 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/$H_2O$ 1:1 (27 ml) and treated under $H_2$ (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 × 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 ($CH_2Cl_2$/acetone 1:1): $R_f$ 0.51. $^1$H-NMR (400 MHz, $CDCl_3$): 0.90–1.09 (*m*, $^i$Pr$_3$Si); 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 (2*d*, *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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2$*C*H); 17.8, (*q*, *Me*$_2$CH); 25.2 (*q*, *Me*CO); 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*, $OCH_2O$); 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*, Me*C*O). ESI-MS: 512.30 (100, $[M+H]^+$).

N$^2$-*Acetyl-5′*-O-*(4,4′-dimethoxytrityl)-2′*-O-*{[(triisopropylsilyl)oxy]methyl}(1-$^{15}$N)guanosine* (**18**). At r.t., a soln. of crude **17** (1.2 g) in AcOH/$H_2O$ 7:3 (73 ml) was treated with 5 portions of $NaNO_2$ (5 × 5 g, 36 mmol), which were added within 2 h. $H_2O$ (20 ml) was added and the mixture extracted with $CHCl_3$ (150 ml). The org. phase was extracted with $H_2O$ and sat. aq. $NaHCO_3$ soln., dried ($MgSO_4$), and evaporated. The residue was co-evaporated with toluene (2 × 20 ml), dried (14 h, 0.05 mbar), redissolved in pyridine (22 ml), and treated with $(MeO)_2TrCl$ (1.78 g, 5.27 mmol). After 1 h at r.t., workup and CC ($SiO_2$ (35 g), hexane/AcOEt 1:1 → 1:9, then AcOEt/MeOH 99:1) gave **18** (1.18 g, 33% from **15**). Off-white foam. TLC ($CH_2Cl_2$/MeOH 19:1): $R_f$ 0.55. $^1$H-NMR (400 MHz, $CDCl_3$): 0.98–1.10 (*m*, $^i$Pr$_3$Si); 1.46 (*s*, MeCO); 3.08 (*d*, *J* = 3.2, OH–C(3′)); 3.12 (*dd*, *J* = 2.9, 10.7, H–C(5′)); 3.53 (*dd*, *J* ≈ 2, 10.6, H′–C(5′)); 3.74, 3.78 (2*s*, 2 MeO); 4.22 (*q*, *J* = 1.7, H–C(4′)); 4.60 (br. *dd*, *J* = 4.8, 1.6, H–C(3′)); 4.93, 5.11 (2*d*, *J* = 4.6, $OCH_2O$); 5.06 (*dd*, *J* = 5.2, 6.9, H–C(2′)); 5.90 (*d*, *J* = 7.3, 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)). $^{13}$C-NMR (100 MHz, $CDCl_3$): 11.9 (*d*, $Me_2CH$); 17.9 (*q*, *Me*$_2$CH); 23.6 (*q*, *Me*CO); 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*, $OCH_2O$); 113.4 (*d*, arom. C); 122.6 (*s*, *J*(C,N) = 8.3, C(5)); 127.3, 128.1, 128.2, 130.1 (4*d*, arom. C); 135.7, 136.2 (2*s*, 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*, Me*C*O). $^{15}$N-NMR (40 MHz, $CDCl_3$): 129.4. ESI-MS: 815.29 (100, $[M+H]^+$).

*5′*-O-*(4,4′-Dimethoxytrityl)-2′*-O-*{[(triisopropylsilyl)oxy]methyl}(3-$^{15}$N)uridine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**19**). A soln. of **4** (1.20 g, 1.64 mmol) in $CH_2Cl_2$ (6.6 ml) was treated consecutively with $^i$Pr$_2$NEt (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 ($SiO_2$ (25 g), hexane/AcOEt 9:1 → 3:7 (+2% $Et_3N$)): **19** (1.53 g, 92%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 1:1): $R_f$ 0.65. $^1$H-NMR (400 MHz, $CDCl_3$): 1.02–1.05 (*m*, $^i$Pr$_3$Si); 1.16–1.17 (*m*, (*Me*$_2$CH)$_2$N); 2.39 (*t*, *J* = 6.4, 1 H, $CH_2CN$); 2.64 (*dt*, *J* = 2.7, 6.3, 1 H, $CH_2CN$); 3.39 (*dt*, *J* = 2.6, 9.5, H–C(5′)); 3.51–3.70 (*m*, 4 H ($Me_2CH)_2$N, H′–C(5′), $POCH_2$); 3.79, 3.80 (2*s*, 2 MeO); 3.82–3.97 (*m*, 1 H, $POCH_2$); 4.19, 4.27 (2*q*, *J* = 2.4, H–C(4′)); 4.40–4.48 (*m*, H–C(2′), H–C(3′)); 4.99–5.06 (*m*, $OCH_2O$); 5.32, 5.36 (2*dd*, *J* = 8.1, *J*(H,N) = 2.7, H–C(5)); 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)). $^{31}$P-NMR (162 MHz, $CDCl_3$): 150.8, 151.3. MALDI-MS: 934.87 (100, $[M+H]^+$).

N$^4$-*Acetyl-5′*-O-*(4,4′-dimethoxytrityl)-2′*-O-*{[(triisopropylsilyl)oxy]methyl}(3-$^{15}$N)cytidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**20**). As described for **19**, with **6** (1.20 g, 1.56 mmol), $CH_2Cl_2$ (6.3 ml), $^i$Pr$_2$NEt (0.60 ml, 3.90 mmol), and cyanoethyl diisopropylphosphoramidochloridite (444 mg, 1.88 mmol). CC ($SiO_2$ (30 g), hexane/AcOEt 1:1 → AcOEt (+2% $Et_3N$)) gave **20** (1.42 g, 94%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): $R_f$ 0.75. $^1$H-NMR (400 MHz, $CDCl_3$): 0.98–1.08 (*m*, $^i$Pr$_3$Si); 1.13, 1.16 (2*d*, *J* = 6.7, (*Me*$_2$CH)$_2$N); 2.21, 2.22 (2*s*, MeCO); 2.38 (*t*, *J* = 6.4, 1 H, $CH_2CN$); 2.60 (*q*, *J* = 6.1, 1 H, $CH_2CN$); 3.42–3.69 (*m*, 1 H of $POCH_2$, ($Me_2CH)_2$N, H–C(5′)); 3.81, 3.82 (2*s*, 2 MeO); 3.92 (*m*, 1 H, $POCH_2$); 4.27–4.42 (*m*, H–C(2′), H–C(4′)); 4.51 (*m*, H–C(3′)); 5.15–5.22 (*m*, $OCH_2O$); 6.15 (*d*, *J* = 1.3, 0.5 H, H–C(1′)); 6.16 (*d*, *J* = 1.8, 0.5 H, H–C(1′)); 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)). $^{31}$P-NMR (162 MHz, $CDCl_3$): 150.6, 151.9. MALDI-MS: 975.68 (100, $[M+H]^+$).

N$^6$-*Benzoyl-5′*-O-*(4,4′-dimethoxytrityl)-2′*-O-*{[(triisopropylsilyl)oxy]methyl}(1-$^{15}$N)adenosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**21**). As described for **19**, with **12** (1.40 g, 1.20 mmol), $CH_2Cl_2$ (6.4 ml), $^i$Pr$_2$NEt (0.68 ml, 4.00 mmol), and cyanoethyl diisopropylphosphoramidochloridite (456 mg, 1.92 mmol). CC ($SiO_2$ (28 g), hexane/AcOEt 9:1 → 3:7 (+2% $Et_3N$)) gave **21** (1.54 g, 90%; 1:1 mixture of diastereoisomers)). Colorless foam. TLC (hexane/AcOEt 1:1): $R_f$ 0.70. $^1$H-NMR (400 MHz, $CDCl_3$): 0.86–0.93 (*m*, $^i$Pr$_3$Si); 1.08, 1.18, 1.20 (3*d*, *J* = 6.6, ($Me_2CH)_2$N); 2.38 (*t*, *J* = 6.5, 1 H, $CH_2CN$); 2.64 (*dt*, *J* = 2.5, 6.4, 1 H, $CH_2CN$); 3.34 (*dt*, *J* = 4.3, 10.5, 1 H, $POCH_2$); 3.51–3.72 (*m*, 1 H of $POCH_2$, (Me*CH*)$_2$N, H–C(5′)); 3.76, 3.77 (2*s*, 2 MeO); 3.83–3.99 (*m*, 1 H, $POCH_2$); 4.38, 4.43 (2*q*, *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 (2*t*, *J* = 5.3, H–C(2′)); 6.19, 6.21 (2*d*, *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 (2*s*, H–C(8)); 8.67, 8.71 (2*d*, *J*(H,N) = 10.1, H–C(2)); 9.04 (br. *s*, NH–C(6)). $^{31}$P-NMR (162 MHz, $CDCl_3$) 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-$^{15}$N)guanosine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**22**). A as described for **19**, with **18** (1.10 g, 1.35 mmol), $CH_2Cl_2$

(5.5 ml), $^{i}Pr_2NEt$ (0.59 ml, 3.4 mmol), and cyanoethyl diisopropylphosphoramidochloridite (384 mg, 1.62 mmol). CC ($SiO_2$ (25 g), hexane/AcOEt 1:1 → AcOEt, then AcOEt/MeOH 99:1 → 19:1 (+2% $Et_3N$)) gave **22** (1.23 g, 90%; 1:1 mixture of diastereoisomers). Colorless foam. TLC (hexane/AcOEt 3:7): $R_f$ 0.55. $^1$H-NMR (400 MHz, $CDCl_3$): 0.92–0.93 (*m*, $^{i}Pr_3Si$); 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, $CH_2CN$); 2.69–2.82 (*m*, 1 H, $CH_2CN$); 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′), $POCH_2$); 3.68 (*m*, 0.5 H, $POCH_2$); 3.757, 3.760, 3.768, 3.773 (4*s*, 2 MeO); 3.92–4.05 (*m*, 1.5 H, $POCH_2$); 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, $OCH_2O$); 4.89, 4.99 (2*d*, *J* = 5.3, 1 H, $OCH_2O$); 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)). $^{31}$P-NMR (162 MHz, $CDCl_3$): 149.8, 150.4. MALDI-MS: 1015.46 (100, $[M+H]^+$).

*RNA Sequence r(GpGpCpGpUpUpUpUp$^{(3\text{-}^{15}N)}$CpGpCpCpUpUpCpGpGpGpCp$^{(1\text{-}^{15}N)}$Gp$^{(1\text{-}^{15}N)}$ApUpUpUpUpUpA p$^{(3\text{-}^{15}N)}$Up$^{(3\text{-}^{15}N)}$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 $MeNH_2$ in $H_2O$ and 8M $MeNH_2$ 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 $Bu_4NF \cdot 3\,H_2O$ 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 (→ 30 ml), treated with 1M aq. $Et_3N \cdot AcOH$ (pH 7, 5 ml), and applied to 2 *Sepak* cartridges (*Waters*): after elution of the salts with 0.1M aq. $Et_3N \cdot AcOH$ (pH 7, 10 ml), followed by $H_2O$ (20 ml), **23** ($Et_3NH^+$ form) was eluted with MeCN/$H_2O$ 1:1 (5 ml). After evaporation, the residue was twice dissolved in $H_2O$ (2 × 1.5 ml) and then treated with $(NH_4)HCO_3$ (2 × 8 mg). The mixture was evaporated, the residue dissolved in $H_2O$ (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 $l \cdot mol^{-1} \cdot cm^{-1}$); $NH_4^+$ form, containing 1 mol-% of $Et_3NH^+$ (according to $^1$H-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*). $^1$H-NMR (400 MHz, $D_2O$, *c* = 2 mM): see *Fig. 2*, *c*.

## REFERENCES


[1] K. Wüthrich, 'NMR of Proteins and Nucleic Acids', Wiley-Interscience, New York, 1986.

[2] C. Höbartner, M. O. Ebert, B. Jaun, R. Micura, *Angew. Chem.* **2002**, *41*, 605; Y. Tanaka, T. Hori, M. Tagaya, T. Sakamoto, Y. Kurihara, M. Katahira, S. Uesugi, *Nucleic Acids Res.* **2002**, *30*, 766; J. Hearst, in 'Nucleic Acids: Structures, Properties and Function' , Eds. V. A. Bloomfield, D. A. Crothers, and I. Crothers Jr., University Science Books, Sausalito, CA, 2000, p. 45; M. Wu, I. Tinoco Jr., *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11555; H. A. Heus, O. C. Uhlenbeck, A. Pardi, *Nucleic Acids Res.* **1990**, *18*, 1103; O. Odai, H. Kodama, H. Hirokai, T. Sakata, T. Tanaka, S. Uesugi, *Nucleic. Acids Res.* **1990**, *18*, 5955; N. B. Leontis, P. B. Moore, *Biochemistry* **1986**, *25*, 3916.

[3] A. J. Dingley, S. Grzesiek, *J. Am. Chem. Soc.* **1998**, *120*, 8293.

[4] J. Dittmer, C. H. Kim, G. Bodenhausen, *J. Biomol. NMR* **2003**, *26*, 256; C. H. Kim, I. Tinoco Jr., *J. Mol. Biol.* **2001**, *307*, 827; E. T. Mollova, A. Pardi, *Curr. Opin. Struct. Biol.* **2000**, *10*, 298.

[5] I. Kim, P. J. Lukavsky, J. D. Puglisi, *J. Am Chem. Soc.* **2002**, *124*, 9338; J. Xu, J. Lapham, D. M. Crothers, *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 44; F. Aboul-ela, E. P. Nikonowicz, A. Pardi, *FEBS Lett.* **1994**, *347*, 261.

[6] X. Zhang, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1998**, *120*, 6625; K. Shin, S. Toko, O. Akira Mei, T. Shin-ichi, O. Akira Sho, K. Masatsune, *Nucleic Acids Symp. Ser.* **1996**, *35*, 93.

[7] S. Pitsch, P. A. Weiss, L. Jenny, 'Ribonucleoside Derivative and Method for Preparing the Same', US Patent 5,986,084, Nov. 16, 1999.

[8] X. Wu, S. Pitsch, *Nucleic Acids Res.* **1998**, *26*, 4315; S. Pitsch, P. A. Weiss, X. Wu, D. Ackermann, T. Honegger, *Helv. Chim. Acta* **1999**, *82*, 1753.

[9] S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz, X. Wu, *Helv. Chim. Acta* **2001**, *84*, 3773.

[10] C. Höbartner, R. Micura, *J. Mol. Biol.* **2003**, *325*, 421.

[11] C. Flamm, I. L. Hofacker, S. Maurer-Stroh, P. F. Stadler, M. Zehl, *RNA* **2001**, *7*, 254; E. A. Schultes, D. P. Bartel, *Science (Washington, D.C.)* **2000**, *289*, 448; C. Flamm, W. Fontana, I. Hofacker, P. Schuster, *RNA* **2000**, *6*, 325.
[12] A. R. Pagano, H. Zhao, A. Shallop, R. A. Jones, *J. Org. Chem*. **1998**, *63*, 3213; C. Bleasdale, S. B. Ellwood, B. T. Golding, P. K. Slaich, O. J. Taylor, W. P. Watson, *J. Chem. Soc., Perkin Trans. 1* **1994**, 2859; L. De Napoli, A. Messere, D. Montesarchio, G. Picialli, C. Santacroce, M. Varra, *J. Chem. Soc., Perkin Trans. 1* **1994**, 923; J. Adler, W. Powell, R. Wolfenden, *J. Am. Chem. Soc.* **1990**, *112*, 1247; X. Gao, R. A. Jones, *J. Am. Chem. Soc.* **1987**, *109*, 1275; C. H. Niu, *Anal. Biochem.* **1984**, *139*, 404.
[13] X. Ariza, V. Bou, J. Vilarrasa, *J. Am. Chem. Soc.* **1995**, *117*, 3665.
[14] B. Goswami, R. A. Jones, *J. Am. Chem. Soc.* **1991**, *113*, 644.
[15] W. L. Sung, *J. Org. Chem.* **1982**, *47*, 3623.
[16] C. B. Reese, A. Ubasawa, *Nucleic Acids Symp. Ser.* **1980**, *7*, 5.
[17] V. Bhat, B. G. Ugarkar, V. A. Sayeed, K. Grimm, N. Kosora, P. A. Domenico, E. Stocker, *Nucleosides Nucleotides* **1989**, *8*, 179.
[18] X. Lin, M. J. Robins, *Org. Lett.* **2000**, *22*, 3497.
[19] K. Kamaike, M. Takahashi, K. Utsugi, K. Tomizuka, Y. Ishido, *Tetrahedron Lett.* **1995**, *36*, 91.
[20] A. C. Niemann, M. Meyer, T. Engeloch, O. Botta, A. Hädener, P. Strazewski, *Helv. Chim. Acta* **1995**, *78*, 421.
[21] X. Ariza, J. Villarrasa, *J. Org. Chem.* **2000**, *65*, 2827.
[22] C. B. Reese, A. Ubasawa, *Tetrahedron Lett.* **1980**, *21*, 2265.
[23] M. MacCoss, E. K. Ryu, R. S. White, R. L. Last, *J. Org. Chem.* **1980**, *45*, 788.
[24] S. Grzesiek, A. Bax, *J. Am. Chem. Soc*. **1993**, *115*, 12593.
[25] M. Piotto, V. Saudek, V. Sklenàr, *J. Biomol. NMR* **1992**, *2*, 661.
[26] J. Dittmer, P. Wenter, S. Pitsch, unpublished results.
[27] B. Mayr, G. Hölzl, K. Eder, M. R. Buchmeiser, C. G. Huber, *Anal. Chem.* **2002**, *74*, 6080.
[28] B. S. Furniss, A. J. Hannaford, P. W. G. Smith, A. R. Tatchell, 'Vogel's Textbook of Practical Organic Chemistry', Longman, Harlow, 1989, p. 450.

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