35. Synthesis of Oligoribonucleotides Containing Isoguanosine

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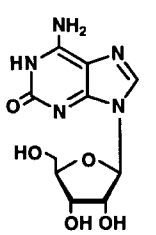
Oligoribonucleotides containing isoguanosine (= 1,2-dihydro-2-oxoadenosine; isoG; **1**) were prepared. The building block **2** was synthesized using the (dimethylamino)methylidene residue as NH_2 protecting group. The monomethoxytrityl as well as dimethoxytrityl group were introduced at OH–C(5′) (→**5** and **6**). Silylation of **5** with triisopropylsilyl chloride formed the 2′-*O*-blocked derivative **7** almost exclusively. Reaction with PCl_3/1,2,4-1*H*-triazole furnished the phosphonate **2** which was used in solid-phase synthesis of the oligoribonucleotides **10** and **11**. RNAse T_1 hydrolyzed U-A-G-U-U-isoG-U-U-A-G (**10**) at the 3′-site of G but not of isoG. The self-complementary oligomer (A-U-isoG-U)$_3$ (**11**) formed a duplex which was less stable than that of (A-U)$_6$.

Isoguanosine (1,2-dihydro-2-oxoadenosine; isoG; **1**) is a naturally occurring ribonucleoside which was isolated from croton beans (crotonoside) [1] and a marine nudibranch molusk [2]. It was synthesized by *Davoll* from 2-aminoadenosine [3]. The N^3-methyl derivative (doridosine) is also naturally occurring [4], and a regioisomeric isoguanine N^1-riboside was prepared recently [5]. Isoguanosine shows the same tendency to aggregate in solutions as guanosine does [6]. However, the aggregates must have a different structure [7]. The UV spectra of isoguanosine show a strong solvent dependence [8]. This was discussed on the basis of various tautomeric structures being formed in solvents of different polarity [9]. This may result in the ambiguous base pairing during duplex and triplex formation. Parallel and antiparallel duplexes may be formed when isoguanosine forms base pairs with regular RNA constituents. Oligoribonucleotides of **1** were already prepared by enzymatic polymerization of isoGDP with polynucleotide phosphorylase [7] or isoGTP with T7 RNA polymerase [10]. Recently, our laboratory reported on the synthesis of oligo-2′-deoxyribonucleotides containing 2′-deoxyisoguanosine [11]. Now we describe the synthesis of the oligoribonucleotide building block **2** allowing the incorporation of an isoguanosine moiety into any position of a synthetic RNA fragment [12].



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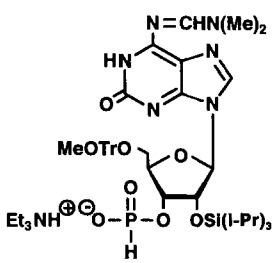

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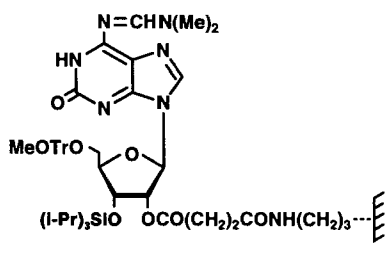

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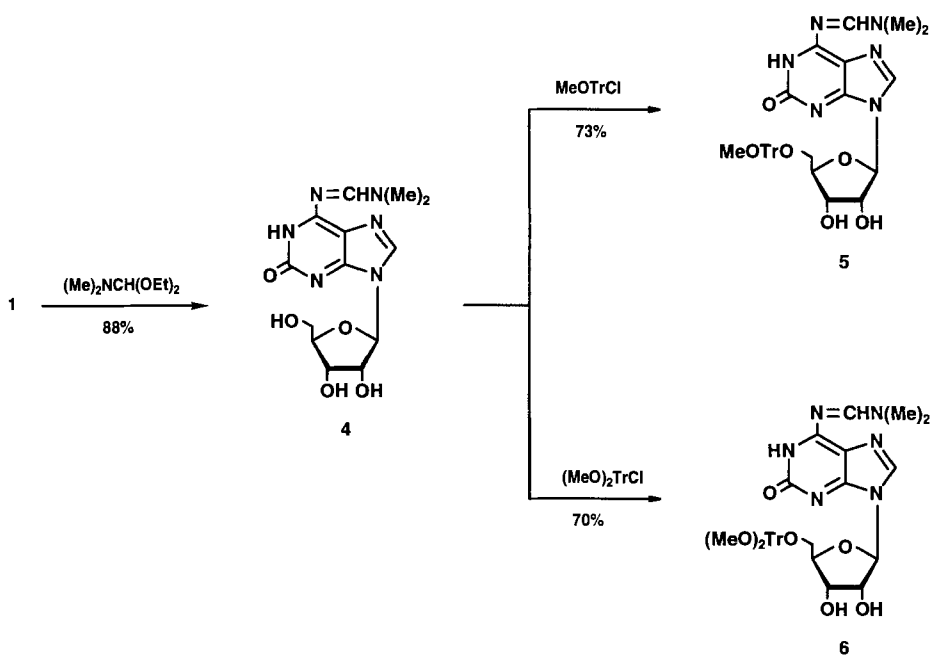
Results and Discussions. – *Monomers.* Isoguanosine (isoG; **1**) was prepared from guanosine *via* 2-aminoadenosine according to a procedure first developed by *Davoll* [3] and using the improved protocol of *Vorbrüggen* and *Krolikiewicz* [13] instead of the formerly described synthesis of 2-aminoadenosine [14] [15]. Thus, silylation of guanosine with hexamethyldisilazane in the presence of Me_3SiCl was followed by amination in a steel vessel affording the 2-aminoadenosine in 68% yield. In the subsequent selective displacement of the 2-amino group according to [3], a smaller excess of $NaNO_2$ was used and the product purified on a *Serdolit* column giving **1** in 73% yield. The latter could also be obtained without chromatographic workup by crystallization from H_2O. However, in this case, the yield was only 56%, the remaining material being retained in the mother liquors, presumably as a result of aggregate formation under the participation of monovalent cations.

Reaction of **1** with *N,N*-dimethylformamide diethyl acetal in DMF gave the crystalline amidine **4** in 88% yield. The introduction of the (dimethylamino)methylidene group [16] was already used in the case of the 2′-deoxyisoguanosine [11]. Acyl protection of **1** is difficult and can destabilize the N-glycosylic bond, whereas an amidine residue has a stabilizing effect [16]. To test the stability of the N^6-protecting group of **4** against bases, it was hydrolyzed in 25% aqueous NH_3 solution at 40°. A half-life time of 6 min was determined, which is short and, therefore, suitable for oligoribonucleotide synthesis. Next, the 5′-OH group was blocked by tritylation, a reaction which was not without problems. Indeed the primary OH group showed low reactivity, the reaction was incomplete with an equimolar amount of reagent, and bis-tritylation occurred with excess of

Scheme 1



reagent. As a consequence, strict reaction conditions (excess of 1.5 mol-equiv. of $(MeO)_2Tr$ Cl) were used. Thus, treatment of **4** with 4-methoxytriphenylmethyl chloride (MeOTrCl) or 4,4′-dimethoxytriphenylmethyl chloride ($(MeO)_2TrCl$) furnished **5** in 73% and **6** in 70% yield, respectively. The position of tritylation was confirmed by ^{13}C-NMR spectroscopy (downfield shifts for C(5′); see **5** and **6** *vs.* **4** in *Table 1*).

Table 1. *^{13}C-NMR Chemical Shifts of Isoguanosine Derivatives in (D_6)DMSO at 25°*

	C(2)[a])	C(4)[a])	C(5)	C(6)	C(8)	MeN
1	152.2	–	109.8	155.9	138.4	–
2	158.3	156.6	113.3	154.6	138.8	34.4, 41.2
4	157.3	156.2	113.6	154.7	140.8	34.4, 40.6
5	157.8	156.6	113.3	154.6	139.7	34.5, 40.6
6	157.8	156.6	113.3	154.6	138.8	34.5, 40.6
7	158.0	156.5	113.3	154.4	139.7	34.5, 41.2
8	158.0	156.5	113.3	154.5	139.7	34.5, 41.2
9	157.3	156.7	113.3	154.6	140.4	34.4, 41.2
	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	MeO
1	87.8	73.0	70.9	85.6	61.7	–
2	85.8	74.2	73.0	83.5	63.8	55.1
4	87.7	72.7	70.6	82.7	61.8	–
5	87.1	73.1	70.3	82.7	64.0	55.1
6	87.1	73.1	70.3	82.7	63.9	55.1
7	87.0	74.8	70.6	83.2	63.8	55.1
8	86.5	72.5	72.4	83.9	63.7	55.1
9	85.6	74.1	70.1	82.9	63.0	55.1

[a]) Tentative.

Commercially available RNA building blocks contain the (*tert*-butyl)dimethylsilyl ((*t*-Bu)Me_2Si) residue as 2′-OH-protecting group [17]. Earlier, our laboratory introduced the triisopropylsilyl residue and reported on the selectivity of this group on 7-deazapurine nucleosides [18]. Now, the silylation was studied on compound **5**. Treatment of **5** with $AgNO_3$ and (i-Pr)$_3$SiCl afforded 2′-*O*-silyl derivative **7** in 71% yield, while the 3′-*O*-regioisomer **8** was isolated in only 6% yield (*Scheme 2*). Using 1*H*-imidazole instead of $AgNO_3$, the reaction was less selective. The structure of **7** and **8** was confirmed by ^1H- and ^{13}C-MMR spectroscopy (^1H-NMR: OH–C(2′) of **8** shifted more downfield than OH–C(3′) of **7** (see *Exper. Part*); ^{13}C-NMR: characteristic downfield shift for C(2′) of **7** and C(3′) of **8** (*Table 1*)).

The isomerization of (*t*-Bu)Me_2Si residues is a severe problem in oligoribonucleotide synthesis and causes part of the drawbacks of the use of silyl protecting groups for 2′-OH protection. The phenomenon was studied by *Ogilvie* and *Entwistle* on regular ribonucleosides [19]. When compound **5** was reacted with (i-Pr)$_3$SiCl and the reaction monitored by TLC, the 2′-*O*-regioisomer **7** was formed first. On increasing the reaction time, more and more **8** appeared, demonstrating that **7** is the kinetically controlled product which equilibrates afterwards. The migration of the (i-Pr)$_3$Si residue of **7** was studied in several solvents at room temperature. In general, the isomerization is much faster in protic than in

Scheme 2

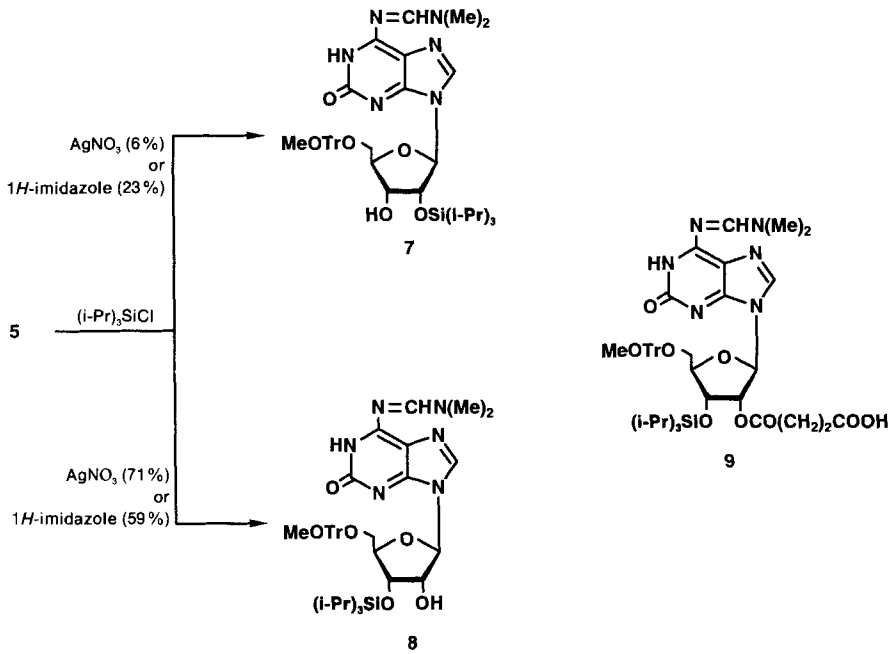


Table 2. *Yield of the 3′-O-Regioisomer* **8** *Formed by Isomerization of* **7** *in Various Solvents*

Time [h]	Yields of **8** [%]			
	CH_2Cl_2	AcOEt/MeOH	Pyridine (abs.)	Pyridine (with H_2O)
1.5	0	4.8	5.1	34.3
2.5	0	7.9	6.0	34.9
24	0	14.0	7.2	54.1
30	0	16.1	8.0	54.4
46	0	18.7	11.7	54.5
70	0	21.4	12.0	56.3

nonprotic solvents [20]. Thus, even after 70 h at room temperature, no **8** could be detected in a CH_2Cl_2 (*p.a.*) solution of **7**, but high amounts of **8** were formed in technical-grade pyridine containing traces of H_2O (*Table 2*). In abs. pyridine, the formation of **8** was less pronounced.

Phosphoramidites have become the common building blocks in oligodeoxyribonucleotide synthesis. However, the reaction cycle is faster in the case of phosphonates. Several authors observed that ribonucleoside phosphonates are very efficient building blocks in oligoribonucleotide synthesis [21]. The monomers are stable against oxidation and hydrolysis [22], and the excess of reagent can be recovered after the synthesis and recycled. As a consequence, 3′-phosphonate **2** was prepared from 2′-*O*-silyl derivative **7** by the action of PCl_3/1,2,4-1*H*-triazole in CH_2Cl_2 [23]. It was characterized by ^{1}H-, ^{13}C-,

and ^{31}P-NMR spectroscopy as well as by elemental analysis. Starting from isoguanosine (**1**) the overall yield of **2** was 37%. Succinylation of the 3′-*O*-silyl derivative **8** in the presence of 4-(dimethylamino)pyridine gave acid **9** which was subsequently activated to the 4-nitrophenyl ester which was then coupled to amino-functionalized controlled-pore glass [24] yielding **3**.

Oligoribonucleotides Containing **1**. Ribonucleic acids can adopt unusual structures. Single-stranded regions are involved in non-*Watson-Crick* base pairing with regular or modified bases. These interactions lead to the folding of the RNA. As a consequence, the incorporation of modified oligoribonucleotides in RNA fragments and the evaluation of the structures being formed is of great value. To test the utility of 3′-phosphonate **2** for such a purpose, it was used in the solid-phase synthesis together with the 3′-phosphonates of the regular RNA building blocks. The phosphonates of A, G, and U were prepared according to [23] and protected, at 5′-OH with a $(MeO)_2$Tr residue and at 2′-OH with a (*t*-Bu)Me_2Si group. The NH_2 group of G was blocked with the (dimethylamino)-methylidene group and that of A with the benzoyl residue. The synthesis was carried out on a 1-μmol scale on an *ABI* synthesizer. The cycle time for detritylation, coupling, activation, and oxidation followed the recently published protocol [25]. The protected oligoribonucleotides corresponding to **10** and **11** were cleaved from the solid support and the base moieties deprotected with 25% NH_3 solution/EtOH 3:1. The silyl groups were cleaved with Bu_4NF/THF (16 h, room temperature). Desalting with an anion-exchange cartridge resulted in almost pure oligomers. Further purification was carried out by reversed-phase HPLC and another desalting to yield, after lyophilization the oligoribonucleotides **10** and **11** as white powders. Their composition was determined upon hydrolysis with snake-venom phosphodiesterase followed by treatment with alkaline phosphatase. The digestion products were detected and quantified by HPLC (*Fig. 1*). It is noteworthy that the coupling yield of phosphonate **2** was reduced if an isoguanosine

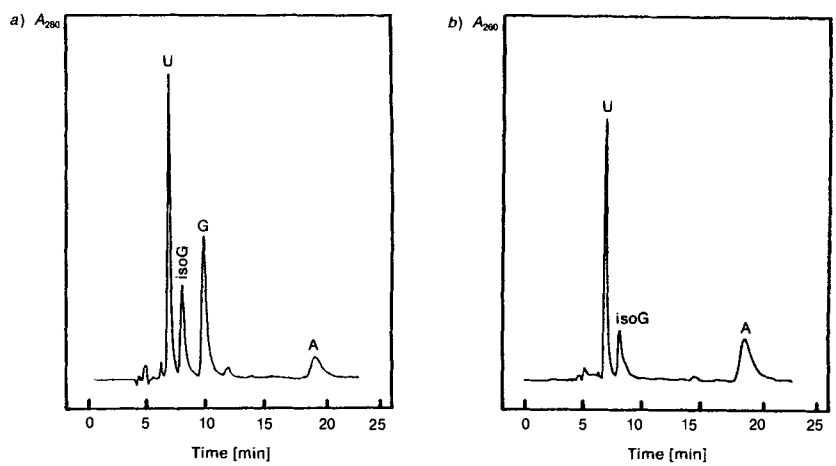


Fig. 1. *HPLC Profiles of enzymatically hydrolyzed oligomers* **10** *and* **11**: a) *from* **10**; b) *from* **11**. *RP-18* Column (*LiChrosorb*, 7 μm, 250 × 4.9 mm); 5% MeCN in 0.1M NH_4(AcO) flow rate 0.6 ml/min. The hydrolysis was performed by tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase. t_R 6.50 (uridine; U), 7.65 (**1**; isoG), 9.52 (guanosine; G), and 19.34 min (adenosine; A).

residue was already incorporated at the 3′-end of the growing oligonucleotide chain. Therefore, problems occur synthesizing longer oligonucleotides.

U-A-G-U-U-isoG-U-U-A-G **10**

A-U-isoG-U-A-U-isoG-U-A-U-isoG-U **11**

As isoguanosine is related to guanosine and adenosine, the behaviour of isoG-containing oligoribonucleotides against RNAse T_1 was tested. The enzyme digests selectively the phosphodiester bond at the 3′-site of guanosine residues [26]. Treatment of **10** with RNAse T_1 afforded two hydrolysis products which were identified by HPLC and tandem hydrolysis with snake-venom phosphodiesterase/alkaline phosphatase. It became apparent that RNAse T_1 is unable to cleave an oligonucleotide at the 3′-site of isoG as only the trimer U-A-G and the heptamer U-U-isoG-U-U-A-G (*Fig. 2*) were formed. It became

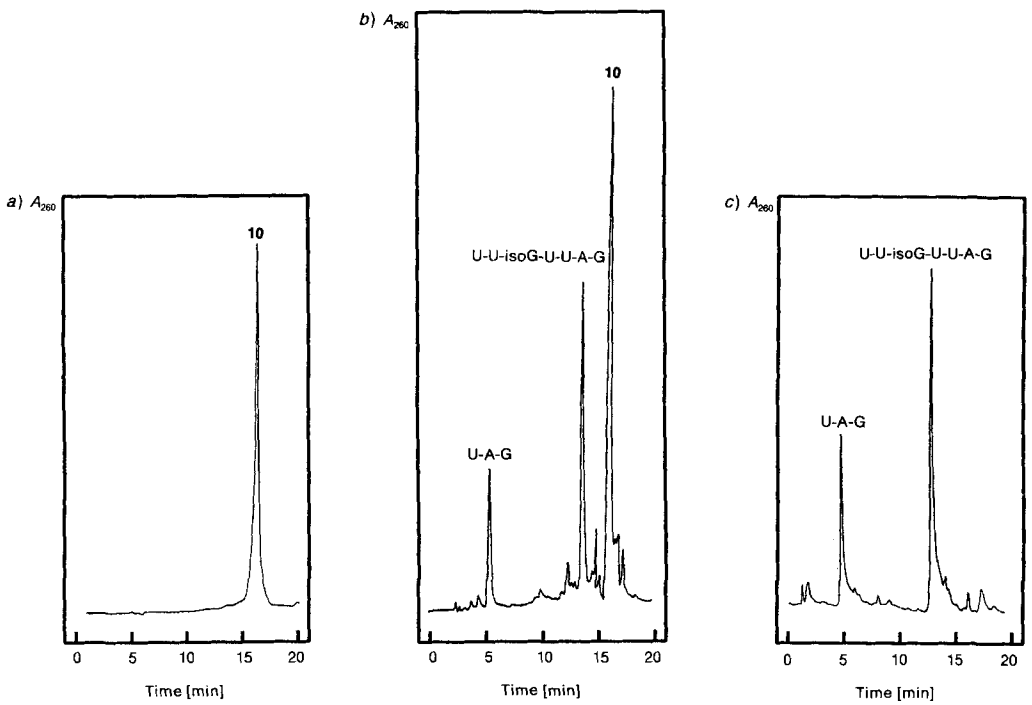


Fig. 2. *HPLC Profile* a) *of educt* **10** *and* b) *of a sample after 7.5 min and* c) *15 min treatment with RNAse* T_1 (see *Exper. Part*). *RP-18* Column (*LiChrosorb*, 7 µm, 250 × 4.9 mm); linear gradient of 3–16% MeCN in 0.1M $NH_4(AcO)$ (30 min), flow rate 1 ml/min. t_R 5.2 (U-A-G), 13.6 (U-U-isoG-U-U-A-G), and 16.1 min (**10**).

obvious that in this case, isoG behaved similarly to A and not to G. The RNAse T_1 reaction is also useful to confirm the correct (3′–5′)-phosphodiester linkages in **10** as the enzyme is not able to cleave (2′–5′)-linked oligonucleotides. According to the HPLC pattern, no educt **10** was left after enzymatic hydrolysis confirming that isomerization of the phosphodiester moiety had not taken place.

For the characterization of the isoG-U base pair every second adenosine residue of an alternating $(A\text{-}U)_6$ was replaced by an isoG residue leading to compound **11**. The T_m value of the duplex of **11** was 19±1° which was lower than that of $(A\text{-}U)_6$ (34°). This phenomenon is probably caused by the 2-oxo group in isoG which sterically interferes with the oxo group of U, a phenomenon which was also observed in the case of corresponding oligodeoxyribonucleotides [11]. In principle, antiparallel as well as parallel strand orientation is possible in the duplex of **11** (*Fig. 3*). As the oligonucleotide contains already three A residues, the antiparallel orientation is most likely. This is supported by the finding that $isoG_dTP$ is incorporated in a DNA template primer complex opposite to T_d [10]. More detailed investigations on the isoguanosine base-pairing capabilities and its aggregation in solution are in progress.

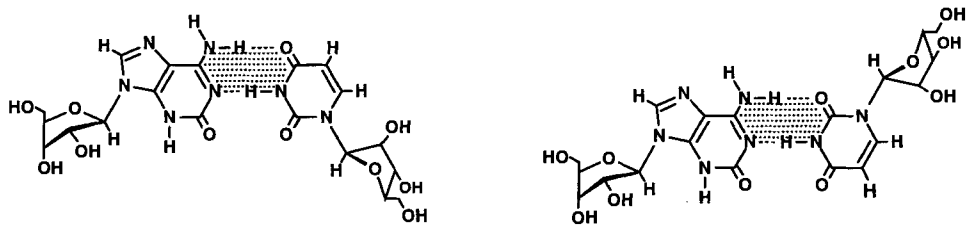


Fig. 3. *Proposed base pairs of isoG-U*

We acknowledge a generous gift of 2-aminoadenosine by Prof. Dr. *H. Vorbrüggen, Schering AG*, Berlin.

Experimental Part

General. See [25]. Elemental analyses were performed by *Mikroanalytisches Laboratorium Beller*, Göttingen, Germany. The kinetics of the isomerization of the 2′-*O*-silylated nucleoside **7** was monitored by TLC using a *CS-930* TLC scanner (*Shimadzu*, Kyoto, Japan).

*6-{[(Dimethylamino)methylidene]amino}-1,9-dihydro-9-(β-*D*-ribofuranosyl)-2H-purin-2-one* (**4**). A soln. of **1** (1.0 g, 3.53 mmol) in abs. DMF (50 ml) was stirred in the presence of *N,N*-dimethylformamide diethyl acetal (10 ml, 58.7 mmol) at r. t. for 12 h. The solvent was evaporated and co-evaporated first with toluene then with acetone. The residue was crystallized from MeOH. Colourless crystals (1.06 g, 88%) which decompose over 230°. TLC (CH_2Cl_2/MeOH, 3:2): R_f 0.45. UV (H_2O): 340 (25300), 258 (14900), 223 (22300). ^{1}H-NMR ((D_6)DMSO): 3.09, 3.20 (2s, Me_2N); 3.61 (*m*, 2H–C(5′)); 3.91 (*m*, H–C(4′)); 4.08 (*m*, H–C(3′)); 4.53 (*m*, H–C(2′)); 5.11 (*m*, OH–C(3′)); 5.41 (*m*, OH–C(2′)); 5.59 (*m*, OH–C(5′)); 5.65 (*d*, J = 5.9, H–C(1′)); 8.05 (*s*, H–C(8)); 9.16 (*s*, N=CH); 11.12 (br. *s*, NH). Anal. calc. for $C_{13}H_{18}N_6O_5$ (338.33): C 46.15, H 5.36, N 24.84; found: C 46.42, H 5.47, N 24.75.

*6-{[(Dimethylamino)methylidene]amino}-1,9-dihydro-9-[5′-*O*-(4-methoxytriphenylmethyl)-β-*D*-ribofuranosyl]-2H-purin-2-one* (**5**). Compound **4** (500 mg, 1.48 mmol) was dried by repeated co-evaporation from anh. pyridine, dissolved in anh. pyridine (100 ml), and reacted with MeOTrCl (690 mg, 2.23 mmol) and $(i\text{-}Pr)_2$ EtN (757 mg, 5.87 mmol). The soln. was stirred at 60° for 12 h and then treated with MeOH (50 ml) and 5% aq. $NaHCO_3$ soln. (20 ml). The resulting mixture was extracted with CH_2Cl_2, the combined org. layer dried (Na_2SO_4), filtered, and evaporated. The residue was dissolved in CH_2Cl_2 and submitted to FC (column 15 × 3 cm, CH_2Cl_2/MeOH 9:1). The main zone yielded a colourless powder (662 mg, 73.4%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.35. UV (MeOH): 346 (17100), 261 (13400), 223 (27800). ^{1}H-NMR ((D_6)DMSO): 3.08, 3.16 (2*s*, Me_2N); 3.72 (*s*, MeO); 4.00 (*m*, H–C(4′)); 4.16 (*m*, H–C(3′)); 4.49 (*m*, H–C(2′)); 5.18 (*d*, J = 5.8, OH–C(3′)); 5.56 (*d*, J = 5.4, OH–C(2′)); 5.73 (*d*, J = 4.1, H–C(1′)); 6.64–7.37 (*m*, arom. H); 7.96 (*s*, H–C(8)); 9.13 (*s*, N=CH); 11.11 (br. *s*, NH). Anal. calc. for $C_{33}H_{34}N_6O_6$ (610.65): C 64.90, H 5.61, N 13.76; found: C 65.01, H 5.71, N 13.71.

*9-[5′-O-(4,4′-Dimethoxytriphenylmethyl)-β-D-ribofuranosyl]-6-{[(dimethylamino)methylidene]amino}-1,9-dihydro-2*H*-purin-2-one* (**6**). Compound **4** (100 mg, 0.30 mmol) was dried by repeated co-evaporation from anh. pyridine, dissolved in anh. pyridine (15 ml), and then reacted with $(MeO)_2TrCl$ (150 mg, 0.44 mmol) at 60° for 12 h. Then a 2nd portion of $(MeO)_2TrCl$ (50 mg, 0.15 mmol) was added. The mixture was treated with 5% aq. $NaHCO_3$ soln. (5 ml) and extracted with CH_2Cl_2, the combined org. layer dried (Na_2SO_4) and evaporated, and the residue dissolved in CH_2Cl_2 and submitted to FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 9:1). The main zone gave a colourless powder (132 mg, 69.9%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.4. UV (MeOH): 346 (13800), 261 (11100), 227 (28400). ^{1}H-NMR ((D$_6$)DMSO): 3.09, 3.19 (2*s*, Me_2N); 3.71 (*s*, 2 MeO); 4.00 (*m*, H–C(4′)); 4.19 (*m*, H–C(3′)); 4.48 (*m*, H–C(2′)); 5.13 (*d*, *J* = 5.9, OH–C(3′)); 5.53 (*d*, *J* = 5.3, OH–C(2′)); 5.72 (*d*, *J* = 4.1, H–C(1′)); 6.64–7.37 (*m*, arom. H); 7.95 (*s*, H–C(8)); 9.14 (*s*, N=CH); 11.11 (br. *s*, NH). Anal. calc. for $C_{34}H_{36}N_6O_7$ (640.68): C 63.74, H 5.66, N 13.12; found: C 63.72, H 5.69, N 13.12.

*6-{[(Dimethylamino)methylidene]amino}-1,9-dihydro-9-{5′-O-(4-methoxytriphenylmethyl)-2′-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl}-2*H*-purin-2-one* (**7**) *and 6-{[(Dimethylamino)methylidene]amino-1,9-dihydro-9-{5′-O-(4-methoxytriphenylmethyl)-3′-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl}-2*H*-purin-2-one* (**8**). *Method A:* To a soln. of **5** (150 mg, 0.24 mmol) in anh. pyridine (2 ml), $AgNO_3$ (72 mg, 0.48 mmol) was added under stirring at r.t. After dissolution of $AgNO_3$, a soln. of $(i\text{-}Pr)_3SiCl$ (50 µl, 0.25 mmol) in anh. THF (5 ml) was introduced under exclusion of light and moisture. After 24 h, a 2nd portion of $(i\text{-}Pr)_3SiCl$ (25 µl, 0.125 mmol) and $AgNO_3$ (20 mg, 0.12 mmol) were added. The mixture was stirred another 24 h, AgCl filtered off, the filtrate treated with 5% aq. $NaHCO_3$ soln. (10 ml) and extracted with CH_2Cl_2 (4 ×), and the combined org. phase dried (Na_2SO_4) and evaporated. FC (silica gel, 30 × 2 cm, AcOEt/MeOH 4:1) gave **7** (133 mg, 70.6%); faster migrating) and **8** (11.3 mg, 6%; slower migrating) as colourless powders.

Method B: A soln. of **5** (120 mg, 0.24 mmol) in abs. pyridine (2 ml) was cooled to 0°, treated with $(i\text{-}Pr)_3SiCl$ (58 µl, 0.29 mmol) and 1*H*-imidazole (33 mg, 0.48 mmol), stirred under exclusion of light and moisture for 1 h at 0° and then for 23 h at r.t. Then a 2nd portion of 1*H*-imidazole (16.5 mg, 0.24 mmol) and $(i\text{-}Pr)_3SiCl$ (48 µl, 0.24 mmol) were added. The mixture was stirred for 24 h at r.t. Then 5% $NaHCO_3$ soln. (10 ml) was added, the soln. extracted with CH_2Cl_2, the combined org. extract dried (Na_2SO_4) and evaporated, and the residue applied to FC (silica gel, column 30 × 2 cm, AcOEt/MeOH 4:1). From the faster migrating main zone, **7** (77 mg, 59.0%) was isolated. Colourless powder. TLC (AcOEt/MeOH 4:1): R_f 0.55. UV (MeOH): 346 (20800), 259 (17700), 228 (34100). ^{1}H-NMR ((D$_6$)DMSO): 0.91–0.96 (*m*, $(i\text{-}Pr)_3Si$); 3.09, 3.19 (2*s*, Me_2N); 3.72 (*s*, MeO): 4.03 (*m*, H–C(4′)); 4.21 (*m*, H–C(3′)); 4.77 (*m*, H–C(2′)); 5.06 (*d*, *J* = 6.8, OH–C(3′)); 5.79 (*d*, *J* = 4.9, H–C(1′)); 6.84–7.66 (*m*, arom. H); 7.99 (*s*, H–C(8)); 9.11 (*s*, N=CH); 11.05 (br. *s*, NH). Anal. calc. for $C_{42}H_{54}N_6O_6Si$ (666.99): C 65.77, H 7.10, N 10.96; found: C 65.65, H 7.08, N 10.80.

The slower migrating zone yielded **8** (30.1 mg, 22.9%). Colourless solid. TLC (AcOEt/MeOH 4:1): R_f 0.45. UV (MeOH): 346 (20800), 259 (17700), 228 (34000). ^{1}H-NMR ((D$_6$)DMSO): 0.91–0.96 (*m*, $(i\text{-}Pr)_3Si$); 3.09, 3.19 (2*s*, Me_2N); 3.72 (*s*, MeO); 4.00 (*m*, H–C(4′)); 4.36 (*m*, H–C(3′)); 4.61 (*m*, H–C(2′)); 5.49 (*m*, OH–C(2′)); 5.79 (*d*, *J* = 5.1, H–C(1′)); 6.84–7.66 (*m*, arom. H); 7.99 (*s*, H–C(8)); 9.11 (*s*, N=CH); 11.05 (br. *s*, NH). Anal. calc. for $C_{42}H_{54}N_6O_6Si$ (666.99): C 65.77, H 7.10, N 10.96; found: C 65.65, H 7.08, N 10.80.

*6-{[(Dimethylamino)methylidene]amino}-1,9-dihydro-9-{5′-O-(4-methoxytriphenylmethyl)-2′-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl}-2*H*-purin-2-one 3′-(Triethylammonium Phosphonate)* (**2**). To a soln. of PCl_3 (120.5 µl, 1.38 mmol) and *N*-methylmorpholine (1.52 ml, 13.8 mmol) in dry CH_2Cl_2 (10 ml), 1,2,4-1*H*-triazole (729 mg, 10.5 mmol) was added. After 30 min stirring at r.t., the soln. was cooled to 0° and a soln. of **7** (212 mg, 0.28 mmol) in dry CH_2Cl_2 (2.5 ml) added dropwise over 10 min. The mixture was stirred for another 20 min at 0° and then hydrolyzed with 1M $(Et_3NH)HCO_3$ buffer (30 ml, pH 8.0). The aq. layer was extracted with CH_2Cl_2 (2 × 30 ml) and the org. layer dried (Na_2SO_4) and evaporated. Chromatography (silica gel, column 30 × 2 cm, CH_2Cl_2/Et_3N 98:2 (150 ml), then CH_2Cl_2/MeOH/Et_3N 85:13:2 (250 ml)) yielded a colourless foam (207 mg, 80.3%) after washing with 0.1M $(Et_3NH)HCO_3$ (6 × 5 ml), drying (Na_2SO_4), and co-evaporation with acetone. TLC (CH_2Cl_2/MeOH/Et_3N 85:13:2): R_f 0.63. UV (MeOH): 346 (17000), 257 (15700), 229 (27000). ^{1}H-NMR ((D$_6$)DMSO): 0.91–0.96 (*m*, $(i\text{-}Pr)_3Si$); 1.13, 2.93 (2*m*, Et_3N); 3.09, 3.19 (2*s*, Me_2N); 3.73 (*s*, MeO); 4.29 (*m*, H–C(4′)); 4.59 (*m*, H–C(3′)); 4.88 (*m*, H–C(2′)); 5.51, 7.87 (*d*, *J* = 590.2, H–P); 5.83 (*d*, *J* = 6.6, H–C(1′)); 6.64–7.66 (*m*, arom. H); 7.93 (*s*, H–C(8)); 9.10 (*s*, N=CH); 11.0 (br. *s*, NH). ^{31}P-NMR ((D$_6$)DMSO): 2.91. Anal. calc. for $C_{48}H_{69}N_7O_8PSi$ (931.15): C 61.91, H 7.47, N 10.53; found: C 61.90, H 7.61, N 10.26.

*6-{[(Dimethylamino)methylidene]amino}-1,9-dihydro-9-{5′-O-(4-methoxytriphenylmethyl)-2′-O-succinyl-3′-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl}-2*H*-purin-2-one* (**9**). Compound **8** (190 mg, 0.25 mmol) was dissolved in pyridine (5 ml) and treated with 4-(dimethylamino)pyridine (15 mg, 0.125 mmol), Et_3N (208 µl, 1.5

mmol), and succinic anhydride (75 mg, 0.75 mmol). The mixture was stirred at 40° for 3 d, evaporated, co-evaporated with toluene, and dissolved in CH_2Cl_2 (30 ml). The org. layer was extracted with 5% aq. $NaHCO_3$ soln. (10 ml), dried (Na_2SO_4), and applied to FC (silica gel, column 20 × 2 cm, MeCN/MeOH/H_2O 8.5:0.5:1). The main zone yielded a colourless solid (127 mg, 59%). TLC (MeCN/MeOH/H_2O 2:1:17): R_f 0.7. ^{1}H-NMR ((D_6)DMSO): 0.91–0.96 (*m*, (i-Pr)$_3$Si); 2.26, 2.38 (2*s*, CH_2CH_2); 3.08, 3.17 (2*s*, Me_2N); 3.36 (*m*, 2 H–C(5′)); 3.73 (*s*, Me_3O); 3.55 (*m*, H–C(4′)); 4.00 (*m*, H–C(3′)); 4.96 (*m*, H–C(2′)); 5.78 (*s*, COOH); 5.95 (*m*, H–C(1′)); 6.64–7.66 (*m*, arom. H); 8.04 (*s*, H–C(8)); 9.15 (*s*, N=CH). Anal. calc. for $C_{46}H_{58}N_6O_9Si$ (867.06): C 63.72, H 6.74, N 9.69; found: C 63.83, H 6.66, N 9.63.

Fractosil-*Linked Isoguanosine* **3**. A soln. of **9** (87 mg, 0.1 mmol) in 1,4-dioxane containing 5% pyridine (2 ml) was treated with 4-nitrophenol (21 mg, 0.15 mmol) and dicyclohexylcarbodiimide (41 mg, 0.2 mmol). The mixture was stirred overnight and dicyclohexylurea filtered off. The filtrate was added to a suspension of amino-linked silica gel (*Fractosil 200*/450 μmol NH_2/g; *Merck*) in dry DMF (2 ml). After shaking for 4 h, Ac_2O (60 μl) was added and shaking was continued for another 30 min. Silica gel was filtered off, washed with DMF, EtOH, and Et_2O, and dried *in vacuo*. The amount of covalently linked **9** was determined after the release of monomethoxytrityl cation from the support (5 mg). Upon treatment with 0.1M TsOH in MeCN (1 ml), the loading was found to be 55 μmol/g modified *Fractosil*.

Solid-Phase Synthesis of the Oligoribonucleotides **10** *and* **11**. See [25]. Compounds **10** and **11** were synthesized using the 3′-phosphonates of [$(MeO)_2$Tr]bz^{6}A(tbds)$^{2'}$, [$(MeO)_2$Tr]fa^{2}G(tbds)$^{2'}$, and (MeOTr)U(tbds)$^{2'}$(tbds = (*t*-Bu)Me_2Si) which were commercial products of *Chem. Genes*, USA, and **2**. CPG supports of the unmodified ribonucleosides were obtained from *Milligene* (Eschborn, Germany). Oligoribonucleotide synthesis was carried out on an automated DNA synthesizer, model *380B* (*Applied Biosystems*, Weiterstadt, Germany), on a 1-μmol scale using a protocol described earlier [25]. The oligoribonucleotides were obtained after detritylation and were cleaved from the solid support with 25% NH_3 soln./EtOH 3:1. Deprotection of the bases was carried out by treatment with 25% NH_3 soln./EtOH 3:1 at 55° for 16 h. The soln. was evaporated and co-evaporated with abs. EtOH. Desilylation was accomplished with 1 ml of 1M Bu_4NF/THF (*Aldrich*, USA) for 16 h at r.t. The H_2O used for purification was sterilized by autoclaving (120°, 2 h) or filtration through a *PV 050/3 Vacuflo* filtration apparatus (*Schleicher & Schüll*, Germany). All glass- and plasticware used for the deprotected oligoribonucleotides was autoclaved. Desilylation was stopped by addition of 0.1M $(Et_3NH)HCO_3$ (10 ml, pH 7.0) followed by desalting using a *Diagen-tip-500* anion-exchange cartridge (*Diagen*, Düsseldorf, Germany). The oligonucleotides were eluted with 2M $(Et_3NH)HCO_3$ buffer (pH 8.0), the solns. evaporated, and the residues dissolved in 500 μl of sterile H_2O. Further purification was performed by HPLC. To avoid the formation of secondary structures, the oligoribonucleotides were heated to 95°, quickly cooled down to 0°, and injected. The main peak was collected, evaporated to 5 ml, and applied to an *Oligo-Pak* cartridge (*Millipore*, Germany) which was autoclaved before and prewashed with MeCN, 0.5M (Et_3NH)AcO (pH 7.0)/MeCN 1:1 and 0.05M $(Et_3NH)HCO_3$ (5 ml each). After washing with 0.05M $(Et_3NH)HCO_3$ (5 ml), the oligoribonucleotides were eluted with MeOH/MeCN/H_2O 1:1:1 (5 ml) and dried on a *Speed-Vac* concentrator to a white powder. Total yields: 13% of **10** and 6% of **11**, resp. UV (H_2O): 259 (**10**); 255, 296 (**11**).

Composition Analysis of the Oligoribonucleotides. The oligomers (0.3 A_{260} units) were dissolved in 0.1M *Tris*-HCl buffer (pH 8.3; 200 μl) and treated with snake-venom phosphodiesterase (*Crotallus durissus*; *Boehringer Mannheim*, Germany; 6 μg) at 37° for 45 min and alkaline phosphatase (calf intestine; *Boehringer Mannheim*, Germany; 2 μg) for 30 min at 37°. The mixture was analyzed by reversed phase HPLC (*RP-18*): t_R of U 6.04, of isoG 6.94, of G 9.46, and of A 16.06 min. Quantification was made on the basis of the peak areas which were devided by the extinction coefficient of the nucleosides (ε_{260}: A 15300, G 12200, U 10200, and isoG 4300; ε_{280}: A 2400, G 8100, isoG 7300, and U 4200).

Enzymatic Hydrolysis of the Oligoribonucleotides with RNAse T_1. Oligomer **11** (0.4 A_{260} units) was dissolved in 10 mM *Tris*-HCl buffer (100 μl) containing 1 mM (ethylenedinitrilo)tetraacetic acid (EDTA) and treated with RNAse T_1 (*Aspergillus oryzae*; *Boehringer Mannheim*, Germany). The enzyme substrate ratio was 1:1000. The soln. was incubated at 37° for 15 min. After 0, 7.5, and 15 min, samples (20 μl) were taken, treated with alkaline phosphatase, and incubated for 10 min at 37°. The mixture was analyzed by reversed-phase HPLC (*RP-18*): t_R for U-A-G 5.2, for U-U-isoG-U-U-A-G 13.6, and for U-A-G-U-U-isoG-U-U-A-G 16.1 min.

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