90. 7-Deazaguanosine: Synthesis of an Oligoribonucleotide Building Block and Disaggregation of the U-G-G-G-G-U G_4 Structure by the Modified Base

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Building blocks derived from 7-deazaguanosine (c^7G, **1**) were prepared for solid-phase oligoribonucleotide synthesis. Compound **1** was converted into the isobutyryl derivative **2b** and the (dimethylamino)methylidene compound **3** (*Scheme 1*). After tritylation (→ **4a, b**), silylation was studied with regard to regioselectivity. It was found that the triisopropylsilyl group in combination with the (dimethylamino)methylidene residue gave the highest 2′-selectivity (→ **5e**). The 2′-*O*-silyl derivative **5e** was reacted with PCl_3 affording the 3′-phosphonate **7** which was used in solid-phase oligoribonucleotide synthesis. Oligonucleotides derived from U-G-G-G-G-U with an increasing number of c^7G residues instead of G were synthesized. Aggregation was studied by polyacrylamide-gel electrophoresis and CD spectroscopy. Disaggregation of the G_4-structure of U-G-G-G-G-U was observed when c^7G replaced G, demonstrating that guanine N(7) participates in the aggregation process.

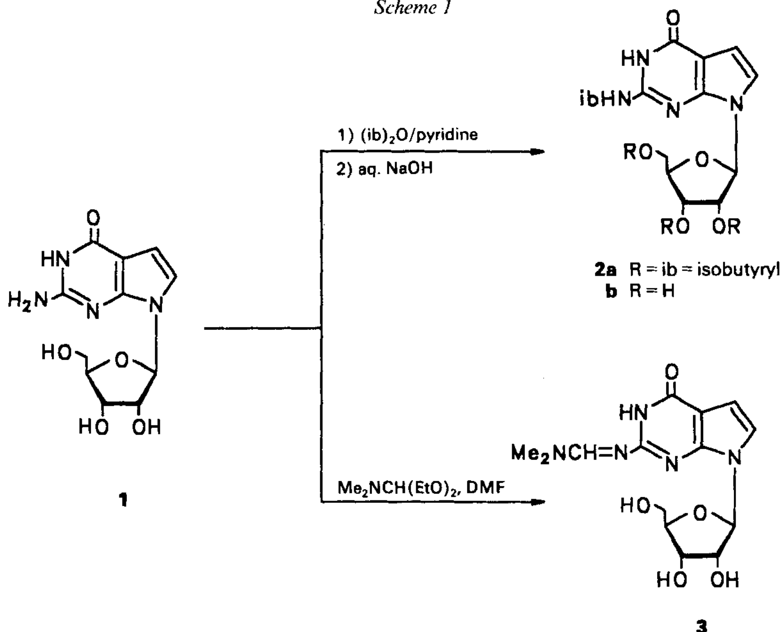
Introduction. – Poly(G) [1] like G itself [2] forms supramolecular structures by self-aggregation. CD Studies on guanosine gels [2] suggested a tetrameric arrangement of guanine residues [3]. Later the four-stranded structure of poly(G) was proposed by X-ray experiments [4]. Tetrahelix formation was also discovered for DNA containing short runs of dG [5] [6]. From methylation studies on DNA, it was suggested that the self-aggregate is hold together by G tetramers which are formed by *Hoogsteen* base pairing in the same manner as in guanosine monomers [5]. Recently, self-aggregation of U-G-G-G-G-U was reported [7]. Aggregate structures were detected by mobility changes in gel electrophoresis.

It was shown that methylation of guanine-containing DNA destroys the aggregate structure [5]. However, methylation of guanine residues within a nucleic acid forms not only an N^7-methyl derivative but generates also a zwitterion with a positively charged N-atom and a negative charge most probably located at O(6). Therefore, methylation experiments do not answer the question of guanine N(7) participation during G aggregation. Earlier, we observed that poly(c^7G) shows favorable properties during enzymic polymerization compared with poly(G) and strong differences in the melting profiles [8]. Now, it has become possible to synthesize oligoribonucleotides by machine-aided methods [9] [10] and to replace G by c^7G at any position of the oligomer. In the following, we describe the synthesis of the 7-deazaguanosine phosphonate **7**. Furthermore, oligonucleotides derived from U-G-G-G-G-U are synthesized, and the replacement of G residues by c^7G is explored with regard to gel-electrophoretic mobility and changes in the CD spectra.

Results and Discussion. – *7-Deazaguanosine Phosphonate* **7**. 7-Deazaguanosine (c^7G, **1**) was already synthesized by nucleobase-anion glycosylation [11] of 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine [12] with 5-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2,3-*O*-(1-methylethylidene)-α-D-ribofuranosyl chloride [13]. For the protection of the exocyclic

amino group of **1**, the isobutyryl residue (ib) was chosen as it was earlier successfully used for G [14] and c^7G_d [15]. *N*-Protection was carried out by peracylation according to *Khorana* and coworkers [16] (→ **2a**), and selective deblocking of the OH groups with 2N NaOH gave **2b** (*Scheme 1*). Base-deprotection experiments on **2b** led to a $t_{1/2}$ of 115 min in conc. NH_3 solution at 40°, which is very similar to that of G^{ib} [14]. Nevertheless, the removal of ib at the end of the oligonucleotide synthesis requires relatively long ammonia treatment, which can lead to phosphodiester hydrolysis [17]. Therefore, the (dimethylamino)methylidene group was introduced into **1**, which was employed earlier on c^7G_d [18] and regular ribonucleoside phosphonates [19]. Reaction of **1** with *N,N*-dimethylformamide diethyl acetal yielded the blocked derivative **3** in 87% yield. With a $t_{1/2}$ of 22 min (25% NH_3 solution, 40°), the amidine residue of **3** is *ca.* 5 times more labile than the isobutyryl group of **2b**, but sufficiently stable for automated oligoribonucleotide synthesis.

Scheme 1



Compounds **2b** or **3** were reacted with (4-methoxytriphenyl)methyl chloride (= 4-methoxytrityl chloride, MeOTrCl) to afford **4a** or **4b**. Next protection of OH–C(2′) was carried out by silylation. The selectivity of silylation towards the 2′-OH function was studied in dependence on the alkylsilyl residue, the catalyst, and the base-protecting group (*Table 1*). At first, compound **4a** was silylated under standard conditions with (*t*-butyl)dimethylsilyl chloride ((*t*-Bu)Me_2SiCl) and 1*H*-imidazole leading to an isomer ratio of 2.5:1 in favor of the 3′-*O*-isomer **6a**. It was shown on regular ribonucleosides that catalysis with $AgNO_3$ instead of 1*H*-imidazole increases the formation of 2′-*O*-isomers [20]. Therefore, **4a** was treated with (*t*-Bu)Me_2SiCl/$AgNO_3$ resulting in the preferred formation of **5a** (*Table 1*). Silylation with (*t*-Bu)Ph_2SiCl led to **5b** and **6b** in a ratio close to 1:1, employing either 1*H*-imidazole or $AgNO_3$. The use of (i-Pr_3)SiCl favored the 2′-*O*-isomer **5c** over **6c**, in particular during catalysis with $AgNO_3$ [21]. As

Table 1. *Influence of the Silylating Reagent and the Catalyst on the Yield of Regioisomeric Silyl Compound Obtained from* **4a** *or* **4b**[a])

Educt (1 mmol)	Silylating reagent [mmol]	Catalyst [mmol]		Product isomers (yield [%])	
		$AgNO_3$	imidazole	2′-*O*-silylation	3′-*O*-silylation
4a	$(t\text{-Bu})Me_2SiCl$ 1.5	–	1.5	**5a** (25)	**6a** (63)
	1.5	1.5	–	**5a** (54)	**6a** (21)
4a	$(t\text{-Bu})Ph_2SiCl$ 1.5	–	2.0	**5b** (38)	**6b** (46)
	2.0	2.0	–	**5b** (42)	**6b** (30)
4a	$(i\text{-Pr})_3SiCl$ 2.5	–	2.5	**5c** (54)	**6c** (29)
	2.5	2.5	–	**5c** (64)	**6c** (16)
4b	$(t\text{-Bu})Me_2SiCl$ 1.5	–	2.5	**5d** (42)	**6d** (31)
	1.3	1.5	–	**5d** (64)	**6d** (21)
4b	$(i\text{-Pr})_3SiCl$ 1.5	2.5	–	**5e** (73)	**6e** (9)

[a]) For conditions, see *Exper. Part.*

Scheme 2

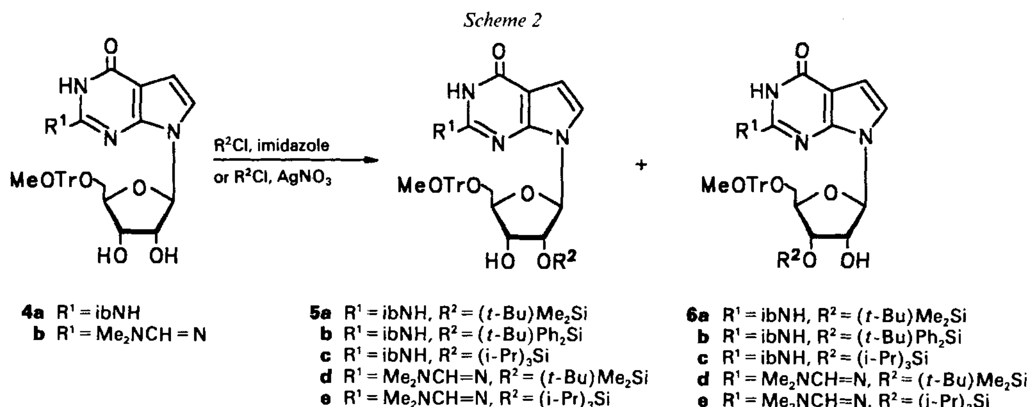


Table 1 indicates, the highest selectivity for 2′-*O*-silylation (**5e**/**6e**, 8:1) was found using formamidine protection of the base in combination with $(i\text{-Pr})_3SiCl/AgNO_3$. The total yield of all reactions was *ca.* 80%. In no case, a 2′,3′-bis-*O*-silylated product was obtained in appreciable quantity.

Because migration of the silyl group may result in problems during chromatographic workup of the 2′-*O*-silyl protected compounds and oligonucleotide synthesis [22] [23], the isomerization of the 2′-*O*-silylated 7-deazaguanosine derivatives **5a–c** was studied in MeOH in the presence of silica gel. The velocity of isomerization decreased in the order **5b** ≈ **5a** > **5c**. The low migration tendency of the $(i\text{-Pr})_3Si$ group is an important aspect for the high yield of 2′-*O*-isomers **5c** and **5e** formed upon silylation of **4a** and **4b**, respectively.

The regioisomers **5** and **6** are characterized by ^1H- and ^{13}C-NMR and NOE difference spectroscopy. ^1H-NMR Signals of the silylated ribonucleosides are assigned by $^1H,^1H$ correlation. According to *Table 2*, the silylation position can be assigned by the disappearence of the particular OH signal. Moreover, the signal of the anomeric proton appears at higher field for the 3′-*O*-isomers **6**. The chemical shift for the (*t*-Bu)Si group is decreased in the case of the 2′-*O*-silylated derivatives **5a**, **b**, **d** compared to the 3′-*O*-isomers **6a**, **b**, **d** (*Table 2*). NOE Difference spectroscopy is used for the unambiguous assignment of the silylation position (*Table 2*). Upon irradiation of

Table 2. *Selected ^{1}H-NMR Data and Chromatographic Mobilities of the 2'-O- and 3'-Silyl Derivatives* **5a–e** *and* **6a–e**

	R_f[a])	δ(OH–C(2')) [ppm]	δ(OH–C(3')) [ppm]	δ(H–C(1')) [ppm]	NOE [%] on irr. of H–C(4')[b])	NOE [%] on irr. of H–C(1')	δ(t-BuSi) [ppm]
4a	–	5.47	5.17	6.03	–	–	–
5a	0.75	–	5.12	6.15	4.9 (OH–C(3'))	[c])	0.72
6a	0.70	5.41	–	5.98	4.2 (MeSi)	1.0 (OH–C(2'))	0.81
5b	0.70	–	5.11	6.16	2.8 (OH–C(3'))	[c])	0.95
6b	0.60	5.45	–	6.03	[c])	1.8 (OH–C(2'))	1.01
5c	0.65	–	5.27	6.27	4.2 (OH–C(3'))	[c])	–
6c	0.55	5.64	–	6.07	[c])	1.0 (OH–C(2'))	–
4b	–	5.44	5.19	6.07	–	–	–
5d	0.50	–	5.06	6.10	2.6 (OH–C(3'))	[c])	0.80
6d	0.40	5.30	–	6.04	3.5 (MeSi)	2.2 (OH–C(2'))	0.87
5e	0.55	–	5.05	6.16	2.2 (OH–C(3'))	[c])	–
6e	0.40	5.32	–	6.08	[c])	2.4 (OH–C(2'))	–

[a]) TLC solvent, see *Exper. Part.*
[b]) H–C(4') was irradiated together with H–C(3').
[c]) No NOE detected.

H–C(4') of the 2'-*O*-silylated compounds **5**, NOE's of 2–5% are observed at OH–C(3'), while irradiation of H–C(1') gives no NOE's. The opposite is true for the 3'-*O*-isomers **6**: NOE's are observed at OH–C(2') upon irradiation of H–C(1'). Moreover, a NOE on the MeSi group is observed upon irradiation of H–C(4') in the case of 3'-*O*-(*t*-Bu)Me_2Si protection (**6a** and **6d**). The silylation position is indicated by the higher TLC mobility of the 2'-*O*-silylated derivatives **5** (*Table 2*) which was also found in other cases [24]. The assignment of the ^{13}C-NMR data (*Table 3*) is accomplished by $^{1}H,^{13}C$-gated decoupled spectra and in analogy to the corresponding deoxy compounds for the base moieties [18] [25]. According to *Pfleiderer* and coworkers, silylation results in an 2–3 ppm upfield shift of the particular C-signal (*Table 3*) [26] [27]. As a result, almost identical chemical shifts are observed for C(2') and C(3') within the series of 3'-*O*-silylated compounds **6**, whereas the signals of the 2'-*O*-silyl derivatives **5** are well separated.

Table 3. *^{13}C-NMR Chemical Shifts of 7-Deazaguanosine Derivatives Measured in Me_2SO at 23°*

	C(2)	C(4)	C(4a)	C(5)	C(6)	C(7a)	CH	Me
1[a])	152.9	159.0	100.2	102.3	117.3	151.3	–	–
2a	147.1	156.6	104.5	104.1	119.4	148.4	34.8–32.9	19–18.5
b[b])	146.9	156.7	104.1	103.0	119.9	148.2	34.8	19.0
3[b])	155.8	159.4	103.3	102.2	118.9	149.8	157.2	34.5
4a	147.0	156.7	104.3	103.1	119.7	148.2	34.8	19.0
b	156.0	159.6	103.4	102.6	118.4	149.9	157.3	34.7
5a[b])	146.9	156.6	104.0	103.4	118.9	148.4	34.7	18.8
b	146.9	156.7	104.3	103.5	119.0	148.4	34.8	18.9
c	147.0	156.7	104.2	103.7	119.0	148.6	34.8	18.9
d	155.9	159.4	103.3	102.5	117.9	149.6	157.1	34.5
e	156.0	159.6	103.5	102.9	118.2	149.7	157.1	34.7
6a[b])	146.9	156.6	104.3	103.2	119.3	148.4	34.7	18.9
b	147.0	156.7	104.4	103.1	119.4	148.5	34.8	19.0
c	147.0	156.7	104.4	103.3	119.3	148.5	34.8	19.0
d	156.1	159.6	103.5	102.7	118.4	150.0	157.4	34.7
e	156.0	159.6	103.5	102.8	118.1	150.1	157.4	34.7
7	156.0	159.5	103.6	102.8	118.1	150.1	157.1	34.7

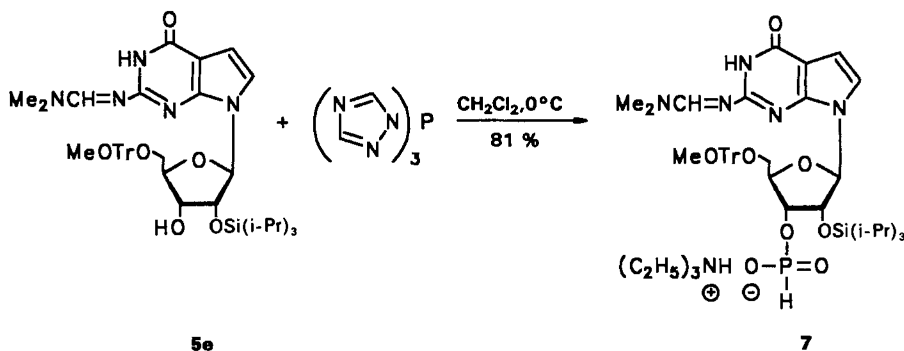
Table 3 (cont.)

	C(1')	C(2')	C(3')	C(4')	C(5')	MeOH	Me_3CSi or Me_2CHSi
1[a])	86.1	73.7	70.7	84.9	61.7	–	–
2a	83.9	72.2	70.4	79.8	63.4	–	–
b[b])	86.0	73.9	70.7	84.9	61.6	–	–
3[b])	86.5	74.0	70.6	84.7	61.7	–	–
4a	86.7	73.7	70.5	82.8	64.2	55.1	–
b	85.8	74.1	70.5	82.4	64.2	55.1	–
5a[b])	85.6	76.0	70.7	83.8	63.9	55.0	17.7
b	86.0	76.4	70.7	84.4	63.9	55.2	18.8
c	86.1	75.9	71.1	84.1	64.0	55.2	11.8
d	86.8	76.4	70.2	82.5	63.8	55.0	17.9
e	87.2	76.4	70.8	83.0	64.0	55.1	11.7
6a[b])	85.9	72.9[c])	72.6[c])	83.4	63.6	55.0	18.0
b	85.9	73.4[c])	73.1[c])	83.8	63.9	55.1	19.0
c	86.1	73.4[c])	72.5[c])	84.0	63.7	55.1	11.8
d	86.9	73.6[c])	72.2[c])	82.6	63.7	55.1	18.1
e	86.4	73.7[c])	72.5[c])	83.1	63.8	55.1	11.9
7	86.7	75.3	72.8	83.0	63.5	55.1	11.8

[a]) [27]. [b]) According to ^1H,^{13}C-gated decoupled spectra. [c]) Tentative assignment.

Phosphoramidites have become the common building blocks in oligodeoxyribonucleotide synthesis. However, this chemistry is less efficient for oligoribo- than for oligodeoxyribonucleotides. The similarity in chromatographic mobility of the ribonucleoside phosphoramidites and the starting material [9] makes the purification more difficult and leads to products of less purity and yield. Coupling times are much longer, and lower coupling yields are obtained compared to oligodeoxynucleotide synthesis [17]. Several groups observed, that ribonucleoside phosphonates are very efficient building blocks in oligoribonucleotide synthesis. They are stable against oxidation and hydrolysis. Purification of valuable triethylammonium salts is very efficient. Therefore, the 2'-*O*-protected nucleoside **5e** was treated with PCl_3/1*H*-1,2,4-triazole in CH_2Cl_2 [28]. Upon hydrolysis, phosphonate **7** was obtained in 81 % yield (*Scheme 3*). Compound **7** was characterized by

Scheme 3



^{1}H-, ^{13}C-, and ^{31}P-NMR spectroscopy as well as by elemental analysis and was used for further experiments.

Oligoribonucleotides **8–14**. Solid-phase oligoribonucleotide synthesis was carried out in 1-µmol scale on a DNA synthesizer. We employed $(MeO)_2Tr$-protected phosphonates of regular nucleosides together with the MeOTr-protected phosphonate **7**. The MeOTr group was chosen in the case of **7** considering the higher stability against acid. As a side effect, the coupling of the base-modified nucleoside can be monitored separately. The synthesis cycle was similar to that of *Stawinsky* and coworkers [10]. Coupling times were slightly increased from 54 to 70 s. A capping step using 0.1M ammonium isopropyl phosphonate (NH_4O_2PH(i-PrO)) in MeCN/pyridine 1:1 was introduced, and a two-step oxidation was carried out. The full cycle is given in the *Exper. Part*.

It is interesting that the coupling times for ribonucleoside phosphonates are much shorter than for ribonucleoside phosphoramidites (70 *vs.* 600 s) [29]. This was already observed for 2′-deoxyxylonucleoside phosphonates [30]. Apparently, the i-Pr groups of the phosphoramidites are shielded sterically by the bulky 2′-*O*-silyl residues. As a result, the attack of the 5′-OH group of the growing chain is hindered. This problem is circumvented in the case of phosphonates for two reasons: *i*) the absence of the cyanoethyl protecting group leads to less steric crowding, and *ii*) the anhydride intermediate may be more reactive than the protonated phosphoramidite. Rearrangement of the anhydride intermediate which can reduce reaction yields [31] is not a problem in view of a coupling time which is not much longer than that for deoxynucleoside phosphonates. The coupling yields for the particular nucleoside phosphonates were 93–95% for C, 95–97% for A, G, and c^{7}G, and 98% for U. The CGP-linked oligomers were detritylated, cleaved from the solid support, and the base-protecting groups were removed. Desilylation was achieved with Bu_4NF/THF. Evaporation of the reaction mixture should be avoided as phosphodiester bonds can be hydrolysed by the F^- ion. In our experiments, F^- was removed before evaporation using anion-exchange chromatography on a *Qiagen* column [32]. The crude oligomers (see *e.g.* **10** in *Fig. 1a* and **11** in *Fig. 1c*) were purified by reversed-phase HPLC. In the cases where separation on HPLC was difficult, polyacrylamide-gel electrophoresis (PAGE) was used under denaturating conditions. The yield of oligonucleotides was determined spectrophotometrically and was 30% at best. The following oligonucleotides (**8**–**14**) were synthesized:

U-G-G-G-G-U **8** U-G-c^{7}G-G-G-U **9** U-G-c^{7}G-G-c^{7}G-U **10**
U-c^{7}G-c^{7}G-c^{7}G-c^{7}G-U **11** G-C-G-C-G-C **12** c^{7}G-C-c^{7}G-C-c^{7}G-C **13**
A-A-A-A-A-U **14**

The nucleoside content of **8–14** was determined after tandem hydrolysis with snake-venom phosphodiesterase followed by alkaline phosphatase. Reversed-phase HPLC of the hydrolysis mixtures separated the nucleosides. Examples are given in *Figs. 1b* and *1d*. Quantification was made by UV spectrophotometry using the extinction coefficients of the monomers.

Disaggregation of the G_4-Structure of U-G-G-G-G-U by Replacement of G by c^{7}G. Guanosine forms viscous gels at low concentrations [2]. X-Ray fibre data on guanosine 3′-phosphate established the tetrameric arrangement of guanine bases [3] (see **A** in *Fig. 2*). Gel formation also takes place with guanine-containing oligoribo- and oligodeoxyri-

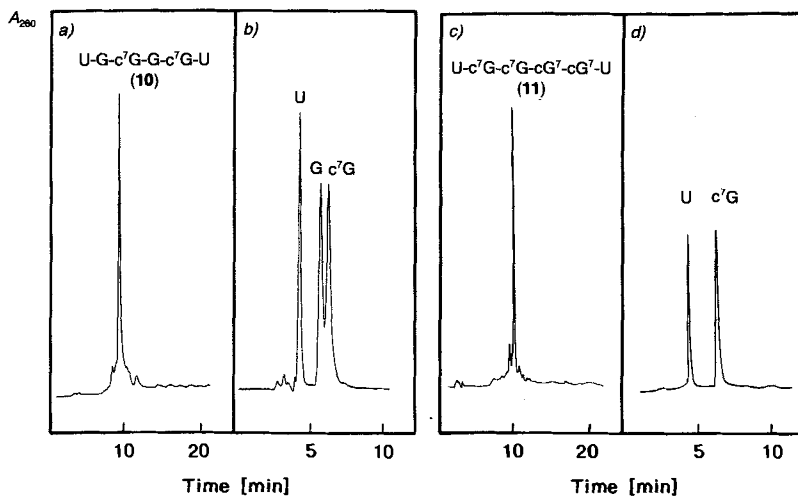


Fig. 1. *HPLC Profiles on a* RP-18 *column of* a) c) *the crude oligomers* **10** *or* **11** *after desilylation and desalting and* b) d) *after enzymatic hydrolysis of* **10** *and* **11** *with snake-venom phosphodiesterase and alkaline phosphatase.* For conditions, see *Exper. Part.*

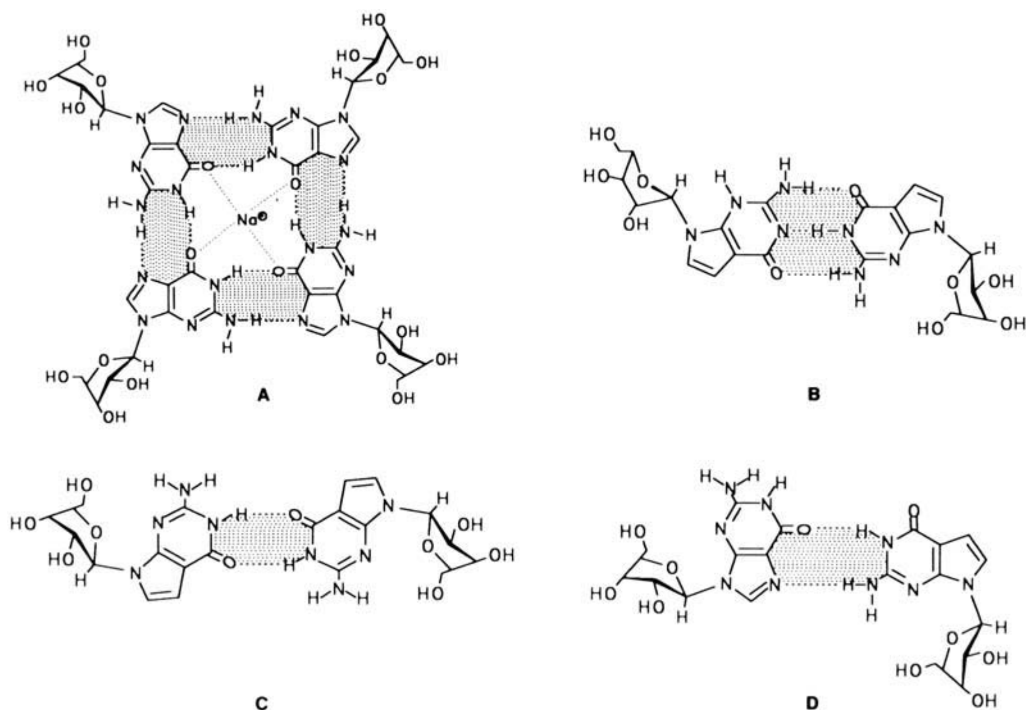


Fig. 2. *Tetrameric structure* **A** *of U-G-G-G-G-U* (**8**) [5], *and proposed dimer structures* **B** *and* **C** *for U-c^7G-c^7G-c^7G-c^7G-U* (**11**), *and dimer structure* **D** *for oligomers containing G and c^7G,* e.g. *U-G-c^7G-G-G-U* (**9**)

bonucleotides [5–7] [33]. Potassium and sodium ions stabilize the gels. Parallel and antiparallel four-stranded structures are possible [5] [33]. It is suggested that the tetrameric structure is formed under participation of N(7) as proton-acceptor site in H-bonding [5]. The use of c^7GTP instead of dGTP during the *Sanger* dideoxy sequencing of DNA is an example how aggregation (band compression) can be avoided and unreadable sequences become accessible [34].

In the series **9–11**, one, two, or all four G residues of the oligomer U-G-G-G-G-U (**8**) are replaced by c^7G. As oligonucleotides containing clusters of guanine show a decreased chromatographic mobility, non-denaturating polyacrylamide gel electrophoresis (PAGE) was used to study the influence of G *vs.* c^7G replacement on the secondary structure [35]. The hexamers **8–11** were incubated overnight in 0.1N NaCl to form the aggregates and then applied to 20% non-denaturating PAGE [36]. Bands were visualized under UV and stained with methylene blue [36]. Staining with ethidium bromide was only successful in the case of G-C-G-C-G-C (**12**), while the oligonucleotides containing c^7G could not be made visible as it was described for c^7G_d [37]. *Fig. 3* shows, that U-G-G-G-G-U (**8**) has a lower electrophoretic mobility than G-C-G-C-G-C (**12**) or c^7G-C-c^7G-C-c^7G-C (**13**) which were used as duplex markers.

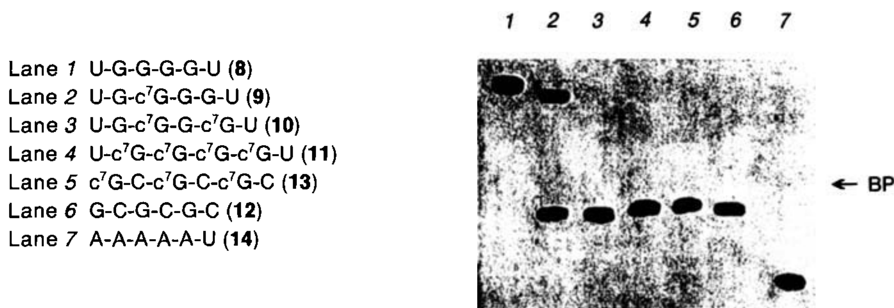


Fig. 3. *Non-denaturating 20% polyacrylamide gel electrophoresis of the oligomers* **8–14** *after incubation of 1* A_{260} *unit with 0.1*M *NaCl.* Conditions, see *Exper. Part.*

In the case of U-G-c^7G-G-G-U (**9**), two bands are observed (*Fig. 3*, lane *2*). The slower migrating one has the same electrophoretic mobility as the parent hexamer **8**, indicating that the replacement of one G residue by c^7G is partly tolerated and tetramer formation takes place (**A** in *Fig. 2*), even when one H-bridge is destroyed. However, this tetramer is in equilibrium with another structure, most probably with a duplex showing the same mobility as **12** or **13**. Replacement of two G residues as in U-G-c^7G-G-c^7G-U (**10**) leads to one band (lane *3*) with the same mobility as the faster zone of **9**, indicating that **10** is not able to build up a four-stranded structure anymore. The electrophoretic mobility of **10** as well as of the fully modified U-c^7G-c^7G-c^7G-c^7G-U (**11**) is the same as that found for **12** and **13**, but slower than that of the single-stranded hexamer A-A-A-A-A-U (**14**), thus strongly supporting duplex formation for **10** and **11**. Due to these results, it is likely that **11** forms a parallel duplex by guanine-guanine base paring either according to structure **B** or **C** (see *Fig. 2*). Homopurine *Hoogsteen* base pairs such as 2D or the reversed base pair were reported in the case of hexose sugar oligonucleotides [38]. Here, the duplex is formed under the participation of N(7).

The disaggregation of U-G-G-G-G-U by the stepwise replacement of G by c^7G observed during electrophoresis is supported by CD data (*Fig. 4*). The CD spectra were measured under neutral and acidic conditions. Hexamer **8** shows a strong positive *Cotton* effect near 260 nm in 0.1N NaCl at pH 7 as it is typical for a G_4 aggregate [39]. The CD spectra of **9** are similar to that of **8**, but the *Cotton* effect is smaller. The CD spectra of **10** show similarity to the A-form of duplex RNA [40]. Acidification (pH 1) which leads to protonation of N(7) changes the CD spectra of **8** and **9** strongly and of **10** to a minor extent. The temperature-dependent CD spectra of **11** (*Fig. 4d*) show a strong change of the CD with increasing temperature indicating a structural change. Further studies are necessary to get a more detailed view of oligoribonucleotide structures containing clusters of G or c^7G.

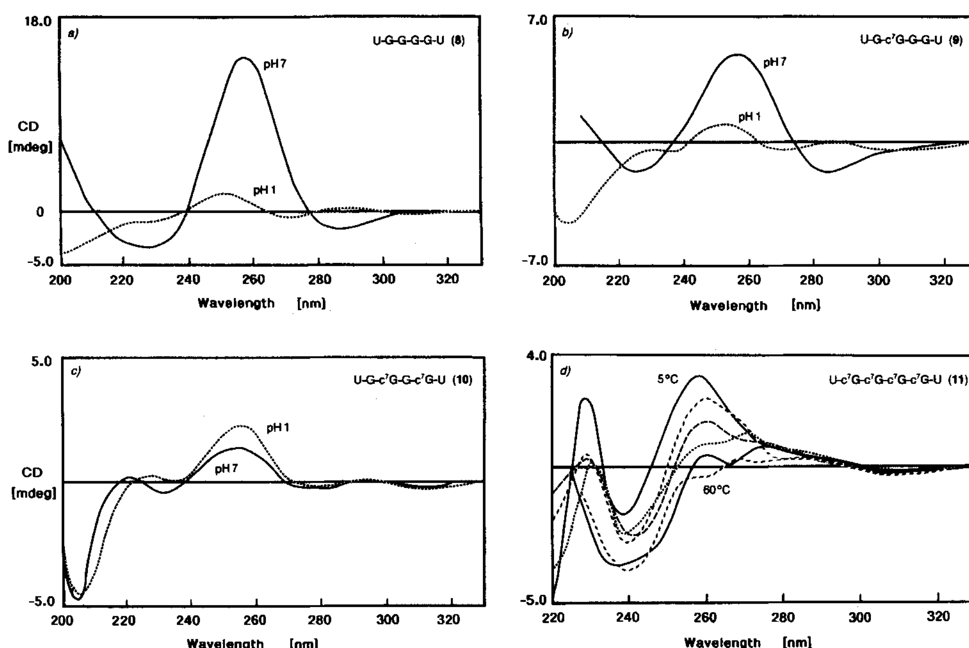


Fig. 4. *CD Spectra of* a) *U-G-G-G-G-U* (**8**), b) *U-G-c^7G-G-G-U* (**9**), c) *U-G-c^7G-G-c^7G-U* (**10**), *and U-c^7G-c^7G-c^7G-c^7G-U* (**11**). 0.5 A_{260} units in 0.1M NaCl at pH 1.0 or pH 7.0 at 5°; temperature-dependent CD spectra at pH 7 of **11** at 5° (——), 10° (----), 20° (– – –), 25° (----), 40° (——), and 60° (– – –).

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Experimental Part

General. Kinetics of the isomerisation of 2'-*O*-silyl protected compounds were measured with a dual-wavelength TLC scanner *CS-930* (*Shimadzu*, Japan). Melting experiments: *Cary-1* UV/VIS spectrophotometer (*Varian*, Australia). TLC: glass plates coated with a 0.25-mm layer of silica gel *Sil G-25 UV*$_{254}$ (*Merck*, FRG); solvent systems $CHCl_3$/MeOH 95:5 (*A*), $CHCl_3$/MeOH 9:1 (*B*), $CHCl_3$/MeOH 4:1 (*C*), CH_2Cl_2/light petroleum ether/AcOEt 1:1:1 (*D*), CH_2Cl_2/light petroleum ether/AcOEt/Et_3N 3:3:3:1 (*E*), CH_2Cl_2/Et_3N 98:2 (*F*), CH_2Cl_2/MeOH/Et_3N 88:10:2 (*G*), CH_2Cl_2/MeOH 4:1 (*H*), CH_2Cl_2/MeOH 9:1 (*I*), AcOEt/MeOH 95:5 (*J*), AcOEt/MeOH 9:1 (*K*). Column chromatography: silica gel *60 H* (*Merck*, Germany) and *Amberlite XAD-4* resin (*Serva*, Germany); flash chromatography (FC) at 0.5 bar using a *Uvicord-II* detector (*LKB Instruments*, Sweden). M.p.: *Büchi-SMP-20* apparatus (*Büchi*, Switzerland); not corrected. UV Spectra: *Hitachi-150-20* spectrometer (*Hitachi*, Japan). CD Spectra: *Jasco-600* spectropolarimeter; thermostatically controlled 1-cm cuvettes connected to a *Lauda-RCS-6* bath (*Lauda*, Germany). NMR Spectra: *Bruker-AC-250* and *-AMX-500* spectrometer; δ values in ppm rel. to tetramethylsilane as internal standard (^1H and ^{13}C) or to external 85% phosphoric acid (^{31}P). Elemental analyses were performed by *Mikroanalytisches Laboratorium Beller*, Göttingen, Germany.

2-[(2-Methylpropanoyl)amino]-7-[2,3,5-tris-O-(2-methylpropanoyl)-β-D-ribofuranosyl]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**2a**). A soln. of anh. **1** (565 mg, 2.0 mmol) in pyridine/isobutyric anhydride 1:1 (40 ml) was heated under reflux for 3 h. After evaporation, the residue was applied to FC (silica gel, column 25 × 5 cm, *A*). From the main zone, **2a** (970 mg, 86%) was obtained. Colorless foam. TLC (*B*): R_f 0.75. UV (MeOH): 270 (13300), 292 (12900). ^1H-NMR ((D_6)DMSO): 1.04–1.15 (*m*, *Me*$_2$CHCO); 2.35–2.80 (*m*, Me_2*CH*CO); 4.31 (*m*, 2 H–C(5')); 4.34 (*m*, H–C(4')); 5.44 (*m*, H–C(3')); 5.69 ('*t*', H–C(2')); 6.21 (*d*, *J* = 7.2, H–C(1')); 6.57 (*d*, *J* = 3.6, H–C(5)); 7.23 (*d*, *J* = 3.6, H–C(6)); 11.51, 11.92 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{27}H_{38}N_4O_9$: C 57.64, H 6.81, N 9.96; found: C 57.76, H 6.92, N 9.91.

2-[(2-Methylpropanoyl)amino]-7-(β-D-ribofuranosyl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**2b**). To a soln. of **2a** (1.0 g, 1.78 mmol) in MeOH (40 ml), ice-cold 2N NaOH was added gradually with stirring until the pH was 12.5. After 15 min, the reaction was stopped by addition of ion exchanger (*Dowex W × 8*, pyridinium form). The neutralized soln. was filtered and the ion-exchange resin washed with MeOH. The filtrate and the washings were combined and evaporated. Crystallisation from MeOH yielded **2b** (465 mg, 74%). Colorless needles. M.p. 197–198°. TLC (*C*): R_f 0.6. UV (MeOH): 270 (12800), 293 (12500). ^1H-NMR ((D_6)DMSO): 1.12 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.76 (*sept.*, *J* = 6.8, Me_2*CH*CO); 3.55 (*m*, H–C(5')); 3.84 (*m*, H–C(4')); 4.07 (*m*, H–C(3')); 4.30 (*m*, H–C(2')); 4.98 (*t*, *J* = 5.3, OH–C(5')); 5.09 (*d*, *J* = 4.3, OH–C(3')); 5.32 (*d*, *J* = 6.0, OH–C(2')); 6.00 (*d*, *J* = 6.4, H–C(1')); 6.50 (*d*, *J* = 3.6, H–C(5)); 7.28 (*d*, *J* = 3.6, H–C(6)); 11.54, 11.84 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{15}H_{20}N_4O_6$: C 51.15, H 5.72, N 15.90; found: C 51.27, H 5.80, N 15.88.

7-[5-O-(4-Methoxytrityl)-β-D-ribofuranosyl]-2-[(2-methylpropanoyl)amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**4a**). Compound **2b** (352 mg, 1.0 mmol) was dried by repeated co-evaporation with anh. pyridine, dissolved in pyridine (5 ml), and MeOTrCl (460 mg, 1.5 mmol) and 4-(dimethylamino)pyridine (20 mg, 0.16 mmol) were added. The soln. was stirred at 55° for 3 h and worked up as described for **4b**. FC (silica gel, column 20 × 5 cm, *B*) afforded a main zone from which colorless, amorphous **4a** (520 mg, 83%) was isolated. TLC (silica gel, *B*): R_f 0.6. UV (MeOH): 270 (15100), 283 (13300), 296 (13600). ^1H-NMR ((D_6)DMSO): 1.12 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.77 (*m*, Me_2*CH*CO); 3.19 (*m*, 2 H–C(5')); 3.73 (*s*, MeO); 3.99 (*m*, H–C(4')); 4.11 (*m*, H–C(3')); 4.36 (*m*, H–C(2')); 5.16 (*d*, *J* = 5.0, OH–C(3')); 5.47 (*d*, *J* = 5.9, OH–C(2')); 6.03 (*d*, *J* = 5.4, H–C(1')); 6.49 (*d*, *J* = 3.6, H–C(5)); 6.87, 7.13–7.40 (2 *m*, arom. H); 7.13 (*d*, *J* = 3.6, H–C(6)); 11.54, 11.87 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{35}H_{36}N_4O_7$: C 67.29, H 5.81, N 8.97; found: C 67.234, H 5.89, N 9.01.

7-{2-O-[(1,1-Dimethylethyl)dimethylsilyl]-5-O-(4-methoxytrityl)-β-D-ribofuranosyl}-2-[(2-methylpropanoyl)amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**5a**). To a soln. of **4a** (240 mg, 0.4 mmol) in dry pyridine (2 ml), $AgNO_3$ (70 mg, 0.4 mmol) was added under stirring at r.t. Stirring was continued for 5 min, and a soln. of (*t*-Bu)Me_2SiCl (60 mg, 0.4 mmol) in THF (5 ml) was introduced under exclusion of light and moisture. A 2nd portion of $AgNO_3$ (35 mg, 0.2 mmol) and (*t*-Bu)Me_2SiCl (30 mg, 0.2 mmol) was added after 10 h. Stirring was continued for another 14 h, AgCl was filtered off, the filtrate treated with 5% aq. $NaHCO_3$ soln. (20 ml), the aq. layer extracted twice with CH_2Cl_2, and the combined org. layer dried (Na_2SO_4), filtered, and evaporated. On FC (silica gel, column 30 × 2 cm, *D*), two zones were separated. From the slower migrating zone, **6a** (62 mg, 21%) was obtained. Evaporation of the faster migrating zone yielded **5a** (160 mg, 54%). Colorless needles. M.p. 224–225° (AcOEt). TLC (*D*): R_f 0.75. UV (MeOH): 269 (15400), 282 (13800), 295 (sh). ^1H-NMR ((D_6)DMSO): –0.15, 0.00 (2*s*, Me_2Si); 0.77 (*s*, *t*-BuSi); 1.16 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.81 (*m*, Me_2*CH*CO); 3.28 (*m*, 2 H–C(5')); 3.79 (*s*, MeO); 4.10 (*m*, H–C(4'), H–C(3')); 4.50 (*m*, H–C(2')); 5.12 (*d*, *J* = 4.3, OH–C(3')); 6.15 (*d*, *J* = 6.9, H–C(1'));

6.59 (*d*, *J* = 3.6, H–C(5)); 6.94, 7.29–7.48 (2 *m*, arom. H); 7.18 (*d*, *J* = 3.6, H–C(6)); 11.63, 11.91 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{41}H_{50}N_4O_7Si$: C 66.64, H 6.82, N 7.58; found: C 66.65, H 6.94, N 7.60.

7-{3-O-[(1,1-Dimethylethyl)dimethylsilyl]-5-O-(4-methoxytrityl)-β-D-ribofuranosyl}-2-[(-methylpropanoyl)-amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**6a**). A soln. of **4a** (240 mg, 0.4 mmol), (*t*-Bu)Me_2SiCl (105 mg, 0.6 mmol), and 1*H*-imidazole (54 mg, 0.8 mmol) in DMF (5 ml) was stirred for 19 h at r.t. Sat. aq. $NaHCO_3$ soln. was added and the soln. extracted twice with CH_2Cl_2. The combined org. extracts were dried (Na_2SO_4), filtered, and evaporated. The residue was applied to FC (silica gel, column 30 × 2 cm, *D*) resulting in two zones. The faster migrating zone afforded **5a** (73 mg, 25%). From the slower migrating zone, colorless amorphous **6a** (200 mg, 68%) was isolated. TLC (*D*): R_f 0.7. UV (MeOH): 269 (15200), 282 (13900), 295 (sh). ^{1}H-NMR ((D_6)DMSO): –0.05, 0.01 (2*s*, Me_2Si); 0.82 (*s*, *t*-BuSi); 1.16 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.78 (*m*, Me_2*CH*CO); 3.10 (*m*, 2 H–C(5')); 3.73 (*s*, MeO); 3.91 (*m*, H–C(4')); 4.18 (*m*, H–C(3')); 4.40 (*m*, H–C(2')); 5.41 (*d*, *J* = 6.6, OH–C(2')); 5.98 (*d*, *J* = 6.5, H–C(1')); 6.52 (*d*, *J* = 3.6, H–C(5)); 6.88, 7.22–7.40 (2 *m*, arom. H); 7.17 (*d*, *J* = 3.6, H–C(6)); 11.89, 12.12 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{41}H_{50}N_4O_7Si$: C 66.64, H 6.82, N 7.58; found: C 66.71, H 6.99, N 7.45.

7-{2-O-[(1,1-Dimethylethyl)diphenylsilyl]-5-O-(4-methoxytrityl)-β-D-ribofuranosyl}-2-[(2-methylpropanoyl)amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**5b**). As described for **5a**, with **4a** (240 mg, 0.4 mmol), pyridine (2 ml), $AgNO_3$ (70 mg, 0.4 mmol), (*t*-Bu)Ph_2SiCl (103 µl, 0.4 mmol), and THF (5 ml), and after 10 h, $AgNO_3$ (70 mg, 0.4 mmol) and (*t*-Bu)Ph_2SiCl (103 µl, 0.4 mmol; stirring continued for 20 h). Chromatographic separation (column 30 × 2 cm, *D*) afforded **6b** (103 mg, 30%) from the slower migrating zone. Evaporation of the fast migrating zone gave **5b** (145 mg, 42%). Colorless foam. TLC (*D*): R_f 0.65. UV (MeOH): 271 (15100), 282 (sh), 296 (sh). ^{1}H-NMR ((D_6)DMSO): 0.95 (*s*, *t*-BuSi); 1.13 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.80 (*m*, Me_2*CH*CO); 2.99, 3.11 (2 *m*, 2 H–C(5')); 3.73 (*s*, MeO); 4.05 (*m*, H–C(4')); 4.17 (*m*, H–C(3')); 4.59 (*m*, H–C(2')); 5.27 (*d*, *J* = 4.8, OH–C(3')); 6.27 (*d*, *J* = 6.9, H–C(1')); 6.44 (*d*, *J* = 3.6, H–C(5)); 6.76, 7.08–7.64 (2 *m*, arom. H); 6.85 (*d*, *J* = 3.6, H–C(6)); 11.55, 11.87 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{51}H_{54}N_4O_7Si$: C 70.97, H 6.31, N 6.49; found: C 70.95, H 6.39, N 6.41.

7-{3-O-[(1,1-Dimethylethyl)diphenylsilyl]-5-O-(4-methoxytrityl)-β-D-ribofuranosyl}-2-[(2-methylpropanoyl)amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**6b**). As described for **6a**, with **4a** (240 mg, 0.4 mmol), (*t*-Bu)Ph_2SiCl (155 µl, 0.6 mmol), 1*H*-imidazole (54 mg, 0.8 mmol), and anh. DMF (5 ml; stirring for 30 h). Two zones were separated by FC (column 30 × 2 cm, *D*), **5b** (130 mg, 38%) being isolated from the faster migrating zone. The slower migrating zone gave colorless amorphous **6b** (160 mg, 46%). TLC (*D*): R_f 0.55. UV (MeOH): 271 (15300), 282 (sh), 295 (sh). ^{1}H-NMR ((D_6)DMSO): 1.01 (*s*, (*t*-Bu)Si); 1.09 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.81–2.73 (*m*, 2 H–C(5'), Me_2*CH*CO); 3.69 (*s*, MeO); 3.95 (*m*, H–C(4')); 4.18 (*m*, H–C(3')); 4.39 (*m*, H–C(2')); 5.64 (*d*, *J* = 6.7, OH–C(2')); 6.07 (*d*, *J* = 6.8, H–C(1')); 6.43 (*d*, *J* = 3.6, H–C(5)); 6.74, 6.99–7.66 (*m*, arom. H); 6.88 (*d*, *J* = 3.6, H–C(6)); 11.46, 11.87 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{51}H_{54}N_4O_7Si$: C 70.97, H 6.31, N 6.49; found: C 70.93, H 6.37, N 6.49.

7-[5-O-(4-Methoxytrityl)-2-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl]-2-[(2-methylpropanoyl)amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**5c**). As described for **5a**, with **4a** (240 mg, 0.4 mmol), dry pyridine (2 ml), $AgNO_3$ (110 mg, 0.6 mmol), (i-Pr)$_3$SiCl (115 µl, 0.6 mmol), and THF (5 ml), and after 24 h, $AgNO_3$ (70 mg, 0.4 mmol) and (i-Pr)$_3$SiCl (76 µl, 0.4 mmol; stirring continued for 24 h). FC (column 30 × 2 cm, *E*) yielded **6c** (50 mg, 16%) from the slower migrating zone and colorless amorphous **5c** (200 mg, 64%) form the faster zone. TLC (*D*): R_f 0.7. UV (MeOH): 270 (15400), 283 (sh), 296 (sh). ^{1}H-NMR ((D_6)DMSO): 0.94–0.84 (*m*, i-PrSi); 1.13 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.78 (*m*, Me_2*CH*CO); 3.16, 3.26 (2*m*, 2H–C(5')); 3.76 (*s*, MeO); 4.08 (*m*, H–C(4')); 4.14 (*m*, H–C(3')); 4.60 (*m*, H–C(2')); 5.11 (*d*, *J* = 4.8, OH–C(3')); 6.16 (*d*, *J* = 6.8, H–C(1')); 6.55 (*d*, *J* = 3.6, H–C(5)); 6.90, 7.25–7.44 (2*m*, arom. H); 7.16 (*d*, *J* = 3.6, H–C(6)); 11.58, 11.87 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{44}H_{56}N_4O_7Si$: C 67.66, H 7.23, N 7.17; found: C 67.76, H 7.27, N 7.10.

7-[5-O-(4-Methoxytrityl)-3-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl]-2-[(2-methylpropanoyl)amino]-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**6c**). As described for **6a**, with **4a** (240 mg, 0.4 mmol), (i-Pr)$_3$SiCl (152 µl, 0.8 mmol), 1*H*-imidazole (82 mg, 1.2 mmol), and anh. DMF (5 ml, stirring for 48 h). FC (column 30 × 2 cm, *E*) afforded **5c** (170 mg, 54%) from the faster migrating zone and **6c** (90 mg, 29%) from the slower migrating zone. Colorless foam. TLC (*D*): R_f 0.6. UV (MeOH): 270 (15700), 282 (sh), 296 (sh). ^{1}H-NMR ((D_6)DMSO): 0.87–1.02 (*m*, i-PrSi); 1.15 (*d*, *J* = 6.8, *Me*$_2$CHCO); 2.81 (*m*, Me_2*CH*CO); 3.14 (*m*, 2 H–C(5')); 3.76 (*s*, MeO); 4.02 (*m*, H–C(4')); 4.30 (*m*, H–C(3')); 4.46 (*m*, H–C(2')); 5.45 (*d*, *J* = 6.6, OH–C(2')); 6.02 (*d*, *J* = 6.3, H–C(1')); 6.56 (*d*, *J* = 3.6, H–C(5)); 6.89, 7.14–7.41 (2*m*, arom. H); 7.15 (*d*, *J* = 3.6, H–C(6)); 11.50, 11.90 (2*s*, NH–C(2), NHCO). Anal. calc. for $C_{44}H_{56}N_4O_7Si$: C 67.67, H 7.23, N 7.17; found: C 67.58, H 7.10, N 7.16.

2-{[(Dimethylamino)methylidene]amino}-7-(β-D-ribofuranosyl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**3**). To a suspension of **1** (564 mg, 2 mmol) in dry DMF (5 ml) *N,N*-dimethylformamide diethyl acetal (2 ml) was added

under Ar while stirring. Stirring was continued for 1 h at 50° and the mixture evaporated and co-evaporated with MeOH (10 ml). Pale-yellow crystals of **3** (590 mg, 87%) were obtained from MeOH. M.p. 234°. TLC (*H*): R_f 0.55. UV (MeOH): 239 (18500), 285 (sh), 311 (17700). ^{1}H-NMR ((D_6)DMSO): 3.03, 3.17 (2*s*, Me_2N); 3.55 (*m*, 2 H–C(5')); 3.87 (*m*, H–C(4')); 4.10 (*m*, H–C(3')); 4.33 (*m*, H–C(2')); 4.98 (*t*, *J* = 5.3, OH–C(5')); 5.12 (*d*, *J* = 4.3, OH–C(3')); 5.28 (*d*, *J* = 6.1, OH–C(2')); 6.00 (*d*, *J* = 6.0, H–C(1')); 6.37 (*d*, *J* = 3.5, H–C(5)); 7.09 (*d*, *J* = 3.5, H–C(6)); 8.55 (*s*, N=CH); 11.05 (*s*, NH(3)). Anal. calc. for $C_{14}H_{19}N_5O_5$: C 49.85, H 5.68, N 20.76; found: C 49.68, H 5.82, N 20.59.

2-{[(Dimethylamino)methylidene]amino}-7-[5-O-(4-methoxytrityl)-β-D-ribofuranosyl]-3H-pyrrolo[2,3-d]-pyrimidin-4(7H)-one (**4b**). Compound **3** (337 mg, 1 mmol) was dissolved in pyridine (10 ml) under warming, and (i-Pr)$_2$EtN (0.65 ml, 4 mmol) and MeOTrCl (463 mg, 1.5 mmol) were added. The mixture was stirred for 2 h at 50° and the reaction stopped by addition of MeOH (10 ml) and 5% aq. $NaHCO_3$ soln. (20 ml). The aq. layer was extracted with CH_2Cl_2 and the org. extract dried (Na_2SO_4), filtered, and evaporated. FC (silica gel, column 30 × 3 cm, *I*) afforded **4b** (580 mg, 95%). Colorless foam. TLC (*I*): R_f 0.5. UV (MeOH): 234 (36800), 286 (sh), 312 (17900). ^{1}H-NMR ((D_6)DMSO): 3.04, 3.15 (2*s*, Me_2N); 3.21 (*m*, 2 H–C(5')); 3.75 (*s*, MeO); 4.01 (*m*, H–C(4')); 4.16 (*m*, H–C(3')); 4.33 (*m*, H–C(2')); 5.12 (*d*, *J* = 5.5, OH–C(3')); 5.44 (*d*, *J* = 5.8, OH–C(2')); 6.07 (*d*, *J* = 4.9, H–C(1')); 6.36 (*d*, *J* = 3.5, H–C(5)); 6.97 (*d*, *J* = 3.5, H–C(6)); 6.37, 7.43–6.88 (2*m*, arom. H); 8.57 (*s*, N=CH); 11.09 (*s*, NH(3)). Anal. calc. for $C_{34}H_{35}N_5O_6$: C 66.99, H 5.79, N 11.49; found: C 66.92, H 5.92, N 11.42.

2-{[(Dimethylamino)methylidene]amino}-7-{2-O-[(1,1-dimethylethyl)dimethylsilyl]-5-O-(4-methoxytrityl)-β-D-ribofuranosyl}-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**5d**). As described for **5a**, with **4b** (152 mg, 0.25 mmol), pyridine (5 ml), $AgNO_3$ (64 mg, 0.38 mmol), (*t*-Bu)Me_2SiCl (38 mg, 0.25 mmol) and THF (5 ml), and after 10 h, (*t*-Bu)Me_2SiCl (11 mg, 0.3 equiv.; stirring continued for 20 h). FC (column 30 × 2 cm, *J*) yielded **6d** (40 mg, 21%) from the slower migrating zone. Compound **5d** (115 mg, 64%) was isolated from the faster migrating zone. Colorless foam. TLC (*K*): R_f 0.5. UV (MeOH): 234 (38000), 285 (sh), 312 (18300). ^{1}H-NMR ((D_6)DMSO): –0.09, 0.00 (2*s*, Me_2Si); 0.80 (*s*, *t*-BuSi); 3.04, 3.13 (2*s*, Me_2N); 3.28 (*m*, 2 H–C(5')); 3.76 (*s*, MeO); 4.05 (*m*, H–C(4')); 4.13 (*m*, H–C(3')); 4.37 (*m*, H–C(2')); 5.06 (*d*, *J* = 5.5, OH–C(3')); 6.10 (*d*, *J* = 4.8, H–C(1')); 6.36 (*d*, *J* = 3.6, H–C(5)); 7.01 (*d*, *J* = 3.6, H–C(6)); 6.70–6.93, 7.46–7.27 (2*m*, arom. H); 8.54 (*s*, N=CH); 11.09 (*s*, NH(3)). Anal. calc. for $C_{40}H_{49}N_5O_6Si$: C 66.36, H 6.82, N 9.67; found: C 66.29, H 6.99, N 9.50.

2-{[(Dimethylamino)methylidene]amino}-7-{3-O-[(1,1-dimethylethyl)dimethylsilyl]-5-O-(4-methoxytrityl)-β-D-ribofuranosyl}-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**6d**). As described for **6a**, with **4b** (152 mg, 0.25 mmol), pyridine (5 ml), 1*H*-imidazole (44 mg, 0.63 mmol), and (*t*-Bu)Me_2SiCl (56 mg, 0.38 mmol; stirring for 24 h). FC (column 30 × 2 cm, *J*) yielded **5d** (76 mg, 42%) from the faster migrating zone. From the slower migrating zone, **6d** (56 mg, 31%) was obtained. Colorless foam. TLC (*K*): R_f 0.4. UV (MeOH): 235 (37200), 285 (sh), 312 (17800). ^{1}H-NMR ((D_6)DMSO): –0.03, 0.03 (2*s*, Me_2Si); 0.87 (*s*, *t*-BuSi); 3.01, 3.10 (2*s*, Me_2N); 3.72 (*s*, MeO); 3.92 (*m*, H–C(4')); 4.22 (*m*, H–C(3')); 4.30 (*m*, H–C(2')); 5.30 (*d*, *J* = 6.3, OH–C(2')); 6.02 (*d*, *J* = 5.0, H–C(1')); 6.35 (*d*, *J* = 3.4, H–C(5)); 6.98 (*d*, *J* = 3.5, H–C(6)); 6.82, 7.4–7.2 (2*m*, arom. H); 8.51 (*s*, N=CH); 11.11 (*s*, NH(3)). Anal. calc. for $C_{40}H_{49}N_5O_6Si$: C 66.36, H 6.82, N 9.67; found: C 66.28, H 6.88, N 9.59.

2-{[(Dimethylamino)methylidene]amino}-7-{5-O-(4-methoxytrityl)-2-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl}-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**5e**). As described for **5a**, with **4b** (152 mg, 0.25 mmol), dry pyridine (5 ml), $AgNO_3$ (106 mg, 0.63 mmol), (i-Pr)$_3$SiCl (75 µl, 0.38 mmol), and THF (5 ml), and after 10 h, (i-Pr)$_3$SiCl (50 µl, 0.25 mmol; stirring continued for 26 h). FC (column 30 × 2 cm, *J*) gave two zones. From the faster migrating zone, **5e** (140 mg, 73%) was obtained. Colorless foam. TLC (*K*): R_f 0.55. UV (MeOH): 234 (38500), 284 (sh), 312 (18300). ^{1}H-NMR ((D_6)DMSO): 0.87–0.95 (*m*, i-PrSi); 3.03, 3.10 (2*s*, Me_2N); 3.2 (*m*, 2 H–C(5')); 3.74 (*s*, MeO); 4.05 (*m*, H–C(4')); 4.16 (*m*, H–C(3')); 4.53 (*m*, H–C(2')); 5.05 (*d*, *J* = 5.9, OH–C(3')); 6.13 (*d*, *J* = 5.1, H–C(1')); 6.34 (*d*, *J* = 3.6, H–C(5)); 6.98 (*d*, *J* = 3.6, H–C(6)); 6.88, 7.24–7.43 (2*m*, arom. H); 8.50 (*s*, N=CH); 11.05 (*s*, NH(3)). Anal. calc. for $C_{43}H_{55}N_5O_6Si$: C 67.42, H 7.24, N 9.14; found: C 67.55, H 7.36, N 9.20.

2-{[(Dimethylamino)methylidene]amino}-7-{5-O-(4-methoxytrityl)-3-O-[tris(1-methylethyl)silyl]-β-D-ribofuranosyl}-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**6e**; 18 mg, 9%) was obtained as a colorless foam from the slower migrating zone of the above FC. TLC (*K*): R_f 0.4. UV (MeOH): 234 (37900), 285 (sh), 312 (17900). ^{1}H-NMR ((D_6)DMSO): 0.86–0.99 (*m*, i-PrSi); 3.01, 3.08 (2*s*, Me_2N); 3.72 (*s*, MeO); 3.99 (*m*, H–C(4')); 4.32 (*m*, H–C(3'), H–C(2')); 5.32 (*d*, *J* = 5.4, OH–C(2')); 6.08 (*d*, *J* = 5.1, H–C(1')); 6.36 (*d*, *J* = 3.4, H–C(5)); 6.94 (*d*, *J* = 3.4, H–C(6)); 6.86, 7.22–7.39 (2*m*, arom. H); 8.50 (*s*, N=CH); 11.07 (*s*, NH(3)). Anal. calc. for $C_{43}H_{55}N_5O_6Si$: C 67.42, H 7.24, N 9.14; found: C 67.28, H 7.32, N 9.15.

2-{[(Dimethylamino)methylidene]amino}-7-}5-O-(4-methoxytrityl)-2-O-[tris-(1-methylethyl)silyl]-β-D-ribofuranosyl}-3H-pyrrolo[2,3-d]pyrimidin-4-(7H)-one 3-(Triethylammonium Phosphonate) (**7**). To a soln. of PCl_3 (43 µl, 0.5 mmol) and *N*-methylmorpholine (543 µl, 5.0 mmol) in dry CH_2Cl_2 (5 ml), 1*H*-1,2,4-triazole (264 mg,

1.67 mmol) was added under stirring at r.t. After 30 min stirring at r.t., the mixture was cooled to 0°, and a soln. of **5e** (77 mg, 0.1 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise within 10 min. After stirring for another 20 min at 0°, the mixture was poured into 1M aq. $(Et_3NH)HCO_3$ (TBK; 10 ml, pH 8.0), shaken, and extracted with CH_2Cl_2 (2 × 5 ml). The combined org. extract was dried (Na_2SO_4) and evaporated and the residue submitted to FC (silica gel, column 30 × 2 cm, *F* (150 ml), then *G* (250 ml)). The residue of the main zone was dissolved in CH_2Cl_2 (10 ml), extracted with 0.1M TBK (6 × 5 ml, pH 7.0), dried (Na_2SO_4), and co-evaporated with acetone: **7** (75 mg, 81%). Colorless foam. TLC (*G*): R_f 0.75. UV (MeOH): 234 (37700), 284 (sh), 312 (18100). ^{1}H-NMR ((D_6)DMSO): 0.82–0.91 (*m*, i-PrSi); 1.14 (*t*, CH_3CH_2); 2.97 (*m*, CH_3CH_2); 3.00, 3.11 (2*s*, Me_2N); 3.25 (*m*, 2 H–C(5')); 3.73 (*s*, MeO); 4.25 (*m* H–C(4')); 4.7 (*m*, H–C(3'), H–C(2')); 6.09 (*d*, *J* = 6.0, H–C(1')); 6.31 (*d*, *J* = 3.3, H–C(5)); 6.51 (*d*, *J* = 589, H–P); 6.95 (*d*, *J* = 3.3, H–C(6)); 6.86, 7.22–7.40 (2*m*, arom. H); 8.48 (*s*, N=CH); 11.01 (*s*, NH(3)). Anal. calc. for $C_{49}H_{71}N_6O_8PSi$: C 63.20, H 7.68, N 9.02; found: C 63.20, H 7.69, N 8.82.

Solid-Phase Synthesis of the Oligoribonucleotides **8–14**. Oligoribonucleotide synthesis was carried out on an automated DNA synthesizer, model *380B* (*Applied Biosystems*, Germany), on a 1-μmol scale using the cycle shown in *Table 4*. Phosphonates of the regular ribonucleosides and **7** were employed. The $[(MeO)_2Tr](m_2fa)^2G(tbds)^{2'}$, $[(MeO)_2Tr](m_2fa)A(tbds)^{2'}$ were synthesized as described by [19]. $[(MeO)_2Tr]bz^4C(tbds)^{2'}$ and 2'-$[(MeO)_2Tr]U(tbds)^{2'}$ [41] were obtained from *Chem Genes* (USA). The phosphonates of the regular ribonucleosides were synthesized as described for **7**. CPG-Linked ribonucleosides were commercial materials (*Milligene*, Germany). Base-deprotection was carried out with 25% NH_3 soln./EtOH 3:1 at 40° for 4 h. The solvent was evaporated and the residue dried by evaporation with abs. EtOH. Silyl deprotection occurred at r.t. with 1M Bu_4NF/THF (1 ml; *Aldrich*, USA) within 16 h. This soln. was treated with 0.1N aq. $(Et_3NH)HCO_3$ (TBK; 10 ml, pH 7.5) and applied to a *Qiagen*-tip-*500* anion exchange cartridge (*Diagen*, Düsseldorf, Germany) prewashed with 0.1N TBK (5 ml, pH 7.0). After washing with 0.1N TBK (5 ml, pH 7.0), the oligonucleotides were eluated with 1N TBK (10 ml, pH 8.0) and the solns. evaporated. Further purification was done either by PAGE or HPLC. Aq. buffers used in this experiments were autoclaved (120°, 2 h) or filtered through a *PV-050/3-Vacuflo* filtration apparatus (*Schleicher & Schüll*, Germany). The glass- and plasticware was autoclaved.

Table 4. *Reaction Cycle for the Solid-Phase Synthesis of the Oligoribonucleotides* **8–14**

Step	Reagents and solvents	Time [s]
A. Condensation Procedure		
1. Detritylation	2.5% Cl_2CH_2COOH in CH_2Cl_2	5 × 20
2. Wash	MeCN	50
	MeCN/pyridine 1:1, MeCN	2 × 20
3. Condensation	monomer (0.04M) and pivaloyl chloride (0.2M) in MeCN/pyridine 1:1, together	25
	wait	45
4. Wash	MeCN/pyridine 1:1, MeCN	2 × 20
5. Capping	ammonium isopropyl phosphonate (0.1M) in MeCN/pyridine 1:1	
	wait	20
6. Wash	MeCN/pyridine 1:1, MeCN	2 × 20
B. Ending procedure		
7. Detritylation	2.5% Cl_2CH_2COOH in CH_2Cl_2	5 × 20
8. Wash	MeCN	50
	MeCN/pyridine 1:1, MeCN	2 × 20
9. Oxidation	I_2 (0.2M) in pyridine/H_2O/Et_3N 1:1:8 (Ox. 1)	30
	hold	300
	Ox. 1 and H_2O/Et_3N/THF 1:2:17	30
	hold	240
10. Wash	MeCN	100
C. Cleavage from the support	NH_3/EtOH 3:1	3 × 1800

HPLC Purification of the Oligoribonucleotides **8–14**. The oligomers **8–14** were applied to a *LiChrosorb* HPLC column (4 × 250 *RP-18*, *Merck*, Germany) and a *Merck Hitachi* HPLC apparatus with one pump (model *655 A-12*), a variable-wavelength monitor (model *655 A*), and a controller (model *L-5000*) connected with an

integrator (model *D-2000*). Solvent systems: 0.1M $(Et_3NH)OAc$ (sterile, pH 7.5)/MeCN 95:5 (I); MeCN (II); gradient: 20 min 0–20% II in I. The main fraction was collected, evaporated to 5 ml, and desalted.

Preparative Gel Electrophoresis of the Oligonucleotides **8**–**14**. Prep. denaturating polyacrylamide-gel electrophoresis [36] was carried out using a 20% polyacrylamide gel (ratio mono- *vs.* bisacrylamide 29:1, 15 × 30 × 0.15 cm, 7M urea), run at 10 W in *Tris*-borate/EDTA buffer (90 mM *Tris*-borate, 2 mM EDTA, 0.1% SDS, pH 8.2). The hexamers **8**–**14** were dissolved in loading buffer (0.25% xylenexyanol, 0.25% bromophenol blue in H_2O, 10 µl per A_{260} unit) and treated with deionized formamide/H_2O 1:1 (10 µl per A_{260} unit). After a pre-run of 1 h, the samples were heated to 95° for 2 min, quickly cooled down, and loaded into a 1-cm wide line. Gels were run at r.t. and 10 W (450 V) until the bromophenol blue dye had migrated *ca.* 15 cm. The gel was wrapped in a polyethylene foil and the bands visualized over a fluorescent TLC glas plate (silica gel UV_{254}, 15 × 15 cm, *Merck*, Germany) by UV light (254 nm). The desired bands were sliced out and the gel pieces extracted with 0.1M NH_4OAc (3 × 2 ml) [36] and desalted.

Desalting of the Oligoribonucleotides **8**–**14**. After HPLC or PAGE purification, the oligomer-containing soln. was applied to an *Oligopak* cartridge (*Millipore*, Germany). It was autoclaved and prewashed with MeCN (5 ml), 0.05M $(Et_3NH)OAc$ (pH 7.0)/MeCN 1:1 (5 ml), and 0.05M $(Et_3NH)OAc$ (5 ml). After washing with 0.05M $(Et_3NH)OAc$ (5 ml), the oligoribonucleotides were eluated with MeOH/MeCN/H_2O 1:1:1 (5 ml) and evaporated on a *SpeedVac* concentrator yielding white powders.

Generation of G_4-Structures and Analytical Polyacrylamide-Gel Electrophoresis under Non-denaturating Conditions. The purified hexamers **8**–**14** (1.0 A_{260} units, each) were dissolved in 0.1M NaCl containing 10 mM *Tris*-HCl (10 µl, pH 8.2) and the mixture heated to 95° for 2 min and incubated overnight at 5° [7]. The samples were treated with loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 15% *Ficoll* in H_2O; 5 µl) and applied to anal. polyacrylamide electrophoresis. Electrophoresis was carried out using a non-denaturating 20% polyacrylamide gel (15 × 30 × 0.1 cm) in *Tris*-borate/EDTA buffer (see prep. PAGE). The gels were run at 5° and 10 W until the bromophenol blue dye had migrated *ca.* 20 cm. The bands were stained with methylene blue [36] and photographed (see *Fig. 3*).

Enzymatic Hydrolysis of the Oligonucleotides **8**–**14** *and Determination of the Nucleoside Content.* The oligomers **8**–**14** (0.3 A_{260} units) were dissolved in 0.1M *Tris*-HCl buffer, (pH 8.3, 200 µl) and treated with snake-venom phosphodiesterase (*EC 3.1.4.1*, *Crotalus durissus*; *Boehringer Mannheim*, Germany; 6 µg) at 37° for 45 min and alkaline phosphatase (*EC 3.1.3.1*, calf intestine; *Boehringer Mannheim*, Germany; 2 µg) for 30 min at 37°. The mixture was analysed by reversed-phase HPLC (see HPLC purification, solvent I). Quantification was made at 260 nm on the basis of the peak areas which were divided by the extinction coefficients of the nucleoside constituent (ε_{260}: A 15300, C 7600, G 12200, U 10200, c^7G 13100. See *Figs. 1b* and *1d*).

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