Acridine-Labeled Primers as Tools for the Study of Nonenzymatic RNA Oligomerization

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Short, dye-labeled oligonucleotides have been used as primers in template-controlled polymerization reactions of RNA. The synthesis of appropriate acridine derivatives and their attachment to nucleic acids is described. In the nonenzymatic oligomerization of 2-methyl-1*H*-imidazole-activated guanosine 5′-monophosphate, two observations deserve special notice: *1*) reaction rates are almost unchanged by variations of the Na^+ concentration; *2*) the conformational type of the primer-template duplex (A *vs.* B) has considerable influence on the rates and yields of RNA oligomerization. When the incorporation of cytidine was studied in the presence of 1M Na^+ or K^+, the process was almost inhibited by quadruplex formation of the oligo-dG template. However, if these cations were omitted, an efficient primer extension could be observed using template concentrations as high as 100 μM. The chances for nonenzymatic self-replication of RNA thus might be distinctly better than previously assumed.

1. Introduction. – Engaged in the design of artificial phosphodiesterases [1], we have recently reported on the hydrolysis of RNA induced by bis(guanidinium) ions [2]. Similar compounds might as well catalyse the reversal of RNA degradation: bond formation between ribonucleotides. This speculation initiated our interest [3] for the nonenzymatic oligomerization of RNA [4]. Since the occurrence of molecular information carriers capable of reproduction and evolutionary development is often seen as a prerequisite for the origin of life, the search for self-replication phenomena in general[2]) and specifically for nonenzymatic RNA reproduction is considered a major scientific objective.

As shown by *Orgel* [4c] and others [7], the polymerization of activated ribonucleotides on complementary oligonucleotide templates may proceed without the help of enzymes[3]). This process is exclusively controlled by the *Watson-Crick* base-pairing rules and by stacking interactions. The first example of self-replication – the ligation of trinucleotides forming a palindromic hexamer duplex – was given by *von Kiedrowski* [5a]. Replicative oligonucleotide ligation also occurs in the case of pyranosyl-RNA (p-RNA) [9]. A mechanism leading from stereoisomeric starting materials to homochiral polymers by chiroselective ligation processes was recently proposed by *Eschenmoser* and coworkers [10].

For RNA, according to present knowledge, self-replication based on monomers is blocked by serious obstacles. While oligomerization steps of activated guanosine on polycytidylic acid can be highly efficient, templates rich in guanosine tend to form

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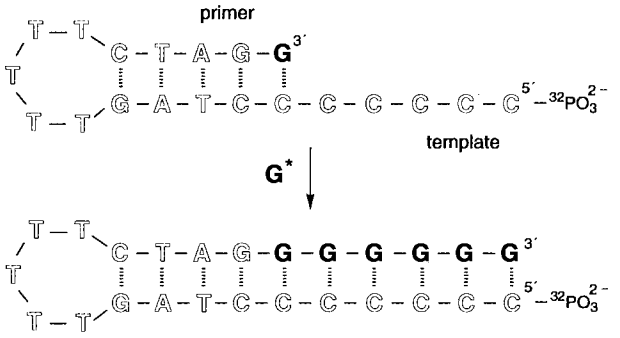
[2]) For self-replicating oligonucleotides, see [5], and for self-replication phenomena observed in non-natural systems, see [6].

[3]) For catalysis of RNA formation by metal ions, peptides, and ribozymes, see [8].

tetrameric aggregates. Because only single-stranded molecules can act as templates for the polymerization of cytidine, strand aggregation prevents the second phase of the replication process[4]). The template-mediated assembly of cytidine [12] has been realized, however, on polymeric 7-deazaguanosine, a synthetic non-aggregating analogue of polyguanylic acid [13]. With naturally occurring ribonucleotides, a special experimental setup was necessary to demonstrate the template controlled incorporation of cytidine [14c]. *Orgel* and coworkers constructed DNA hairpins with a single-stranded, ^{32}P-labeled 5′-region [14]. While the single-stranded parts of these molecules acted as templates, the 3′-ends, formed by ribonucleotides, served as primers (*Scheme 1*). Chain elongation by ribonucleoside 5′-phosphorimidazolides (= ribonucleoside 5′-(imidazolylphosphonates)) was studied by gel electrophoresis combined with autoradiography. Due to the high sensitivity of radioactive-tracer techniques, extremely low hairpin concentrations could be used, thus preventing the aggregation of guanosine rich templates [14c].

Scheme 1. *Hairpin Oligonucleotides Introduced by* Orgel *and Coworkers* [14]. The single-stranded template region directs the incorporation of 5 guanosine units. Due to the radioactive label, these experiments can be performed at nanomolar hairpin concentrations. Quadruplex formation of corresponding oligo-dG templates is thus avoided. Ribonucleotides are symbolized by bold, deoxyribonucleotides by outlined letters.



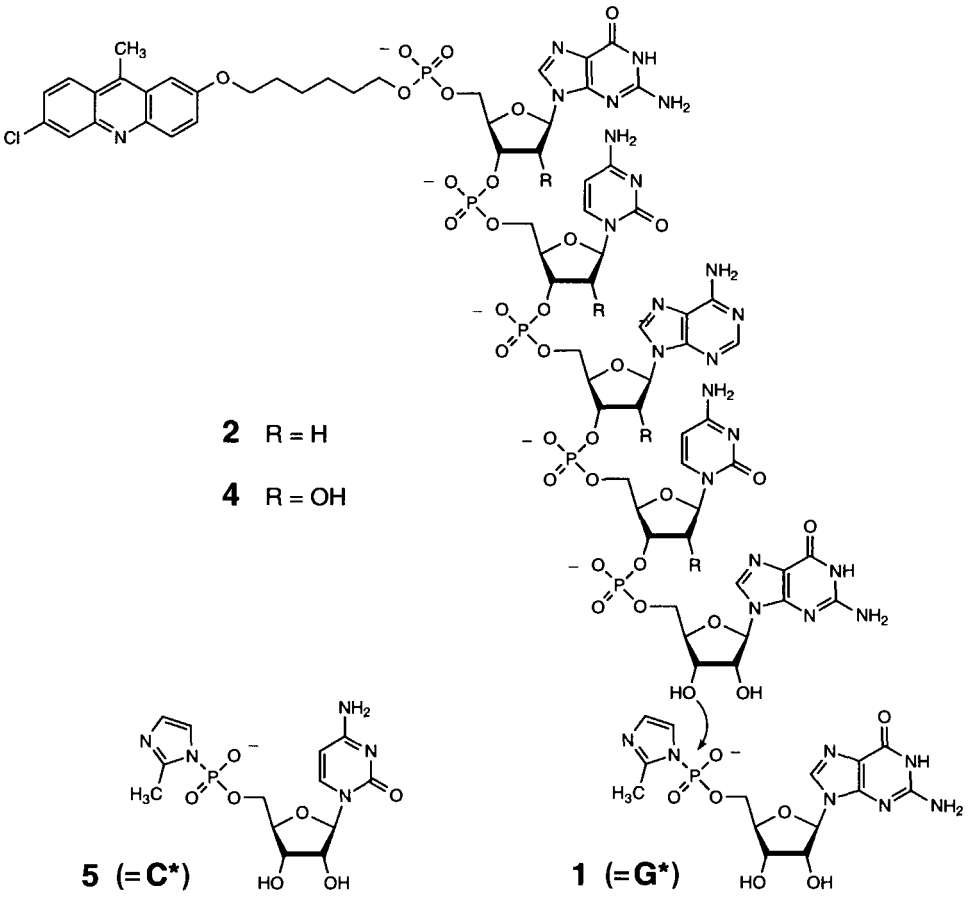
In the present work, we describe an alternative approach to study template-controlled RNA oligomerization based on HPLC analysis of nonradioactive samples. The experimental setup allows the rapid and precise determination of chain-extended products formed by reaction of activated mononucleotides (**1** or **5**) with a short, acridine-labeled primer molecule [15] (*e.g.*, **2** or **4**, see *Scheme 2*) in the presence of hybridized template oligonucleotides[5]). The dissection of primer and template into different molecules has several advantages: *1*) It simplifies HPLC analysis. *2*) By combination with different templates, a large set of experiments is possible using the same labeled primer molecule. *3*) PCR-Like experiments might be realized in the future [16]. Due to the color, fluores-

[4]) According to *Orgel* and coworkers, base pairing between adenosine and uridine is too weak to allow oligomerization of monomeric adenosine derivatives on polyuridylic acid templates with an efficiency comparable to that in the guanosine-cytidine system [11].

[5]) Parts of our results have been published as communications [3a][15].

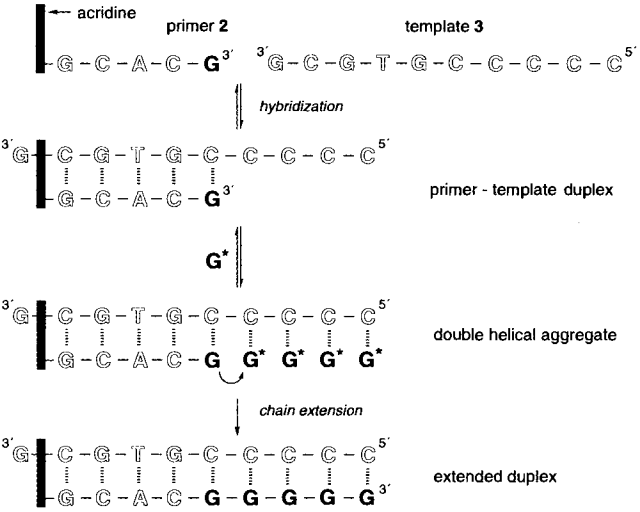
cence, and duplex-stabilizing properties of the acridine dye, high sensitivity was achieved even with short pentanucleotide primers. A typical experiment with primer **2** and template **3** is outlined in *Scheme 3* (*Exper. 1*; a survey is given in *Scheme 4*).

Scheme 2. *Chain Extension of the Acridine-Labeled Primers* **2** *and* **4** *by the Activated Nucleotides* **1** (= **G***) *and* **5** (= **C***)



2. Preparation of Acridine Derivatives. – To achieve a specific detection of the primer and of all chain-extended products, a nonradioactive 5′-end label of the primer by fluorescent dyes was needed. Furthermore, *short* primer sequences should facilitate the separation of oligomerization products by HPLC. A prerequisite for template-controlled reactions, on the other hand, is duplex formation of primer and template. Since duplex stabilities depend on the type and number of base pairs as well as on strand concentrations, the association properties of very short primers are insufficient for oligomerization experiments in the micromolar or submicromolar range. To fulfil these contradicting requirements, *i.e.*, strong primer-template association in spite of a low number of base

Scheme 3. *Template* **3** *Controlling the Chain Extension of Primer* **2** (*Exper. 1*) Ribonucleotides are symbolized by bold, deoxyribonucleotides by outlined letters. The acridine dye is represented by a black rectangle.



pairs, a dye with duplex-stabilizing properties was chosen [17][6]). Intercalating acridin-9-amines are well-known to enhance the melting points of double helices. They can be attached to oligonucleotides as phosphoramidite derivatives [17]. However, their C(9)–N bond is prone to hydrolysis which complicates the preparation and use of such oligonucleotide conjugates (see *Fig. 1*). During hydrolysis, strongly fluorescent acridinones are liberated that interfere with the detection of chain-extended oligomerization products by HPLC. Since no satisfying results could be obtained in preliminary experiments using acridin-9-amines, we decided to develop a novel type of chemically stable acridine dye. In the final molecule, a Me group replaced the labile 9-amino group, and an ether linkage was used to connect dye and oligonucleotide [15] (*Fig. 1*).

Starting from 6,9-dichloro-2-methoxyacridine (**6**), the Me group was introduced by reaction with sodium diethyl malonate followed by acidic ester hydrolysis of **7** and decarboxylation (87% of **8**). In contrast to *Campbell et al.* [20], we used DMSO instead of EtOH in the first step. This allowed complete alkylation of the starting material **6** which is otherwise hydrolyzed to the sparingly soluble acridinone derivative. Acridinone formation not only lowers the yield but severely complicates the purification of product **8**. In the next step, the methyl ether of **8** was cleaved by HBr in AcOH. Alkylation of the crude phenol **9** with tetrahydro-2*H*-pyran-2-yl(THP)-protected 6-bromohexanol or 7-bromoheptanol [22] in the presence of Cs_2CO_3 and DMSO gave *rac*-**10**, or *rac*-**12**, respectively (90–98%). After removal of the THP group, the crystalline acridinols **11** and **13** were obtained in 93–96% yield.

The alcohols **11** and **13** could be transformed to all major types of phosphorylated derivatives used in oligonucleotide synthesis (*Fig. 2*). Treatment of **11** with 2-cyanoethyl tetraisopropyl phosphorodiamidite gave phosphoramidite *rac*-**14**[7]) in 71% yield [23].

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[6]) For acridines linked to DNA, see [18], and for intercalator-DNA conjugates, see [19].

[7]) Samples of the (acridinyloxy)hexyl phosphoramidite *rac*-**14** are available from the authors on request.

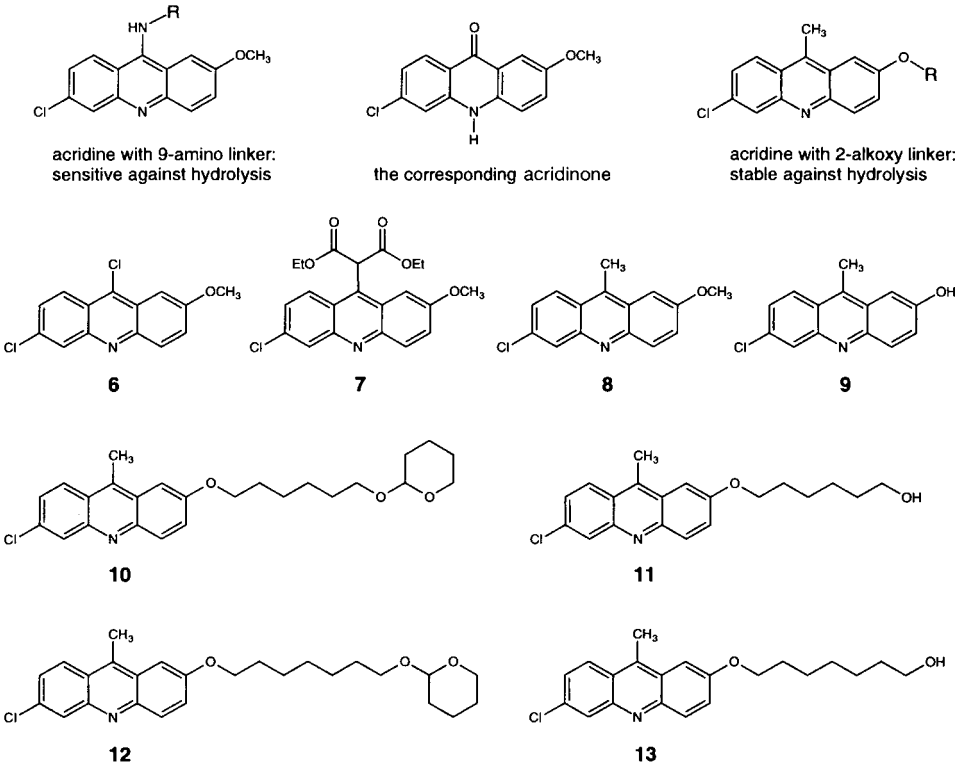


Fig. 1. *Preparation of the acridinols* **11** *and* **13**

The *H*-phosphonates **15** and **16** were obtained by reaction of **11** and **13** with phosphorous trichloride, 1*H*-1,2,4-triazole, and *N*-methylmorpholine in 63–66% yield [25]. In addition, the phosphodiester **17** was prepared from **11** and 2-chlorophenyl phosphorodichloridate for application in the phosphotriester technique [26] (65%). To prevent substitution of the OH group by Cl or pyridine, the temperature in the latter reaction should be kept at $-10°$.

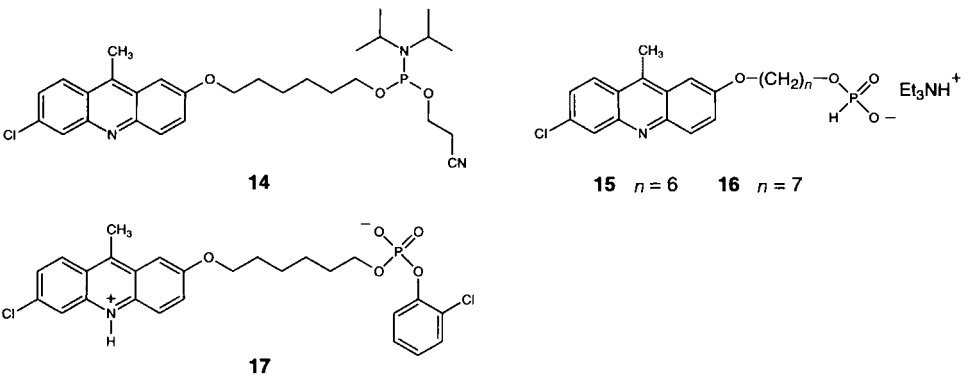
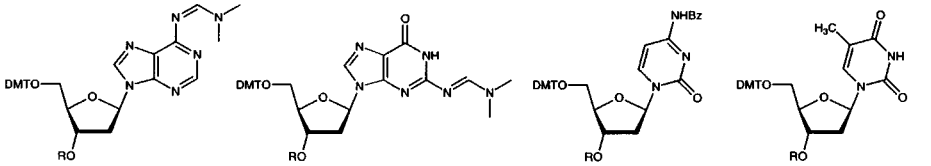


Fig. 2. *Acridine building blocks for different techniques of oligonucleotide synthesis*

3. Synthesis and Physical Properties of Oligonucleotides. – To facilitate the synthesis and to improve chemical stability, deoxyribonucleotides were chosen for the construction of primer **2**. In experiments of *Wu* and *Orgel* according to *Scheme 1*, however, the reaction rates of 3′-terminal ribonucleotides proved to be tenfold higher than those of the corresponding deoxyribonucleotides. This can be explained by pK_a differences of the OH groups [14a]. Furthermore, a riboguanosine in position 3′ would offer maximal stacking interactions with the first mononucleotide to be incorporated. So, for primer **2** the sequence 5′-acridine-d(GCAC)riboG-3′[8]) was selected (*Scheme 2*). Using manual solid-phase synthesis and *H*-phosphonate building blocks [25] (see *Fig. 3*), up to 80 mg of purified **2** could be conveniently obtained in one batch (49% based on solid support). The ^{1}H-NMR spectrum of **2** is shown in *Fig. 4*.



18 R = PO_2H^- Et_3NH^+ **19** R = PO_2H^- Et_3NH^+ **20** R = PO_2H^- Et_3NH^+ **21** R = PO_2H^- Et_3NH^+

Fig. 3. H-*Phosphonates used for the synthesis of primers* **2** *and* **23**

Although only five base pairs are involved, primer **2** formed a stable duplex with template **3**. On addition of **3**, a positive *Cotton* effect around 400 nm was induced in the CD spectrum of **2**. Thermal denaturation studies with **3** and the acridine-free oligonucleotide d(GCACG) demonstrated an increase of T_m by 15° due to the presence of the dye (*Table 1*). So, duplex stabilities with primer **2** would allow template-controlled oligomerization experiments even in the high nanomolar range.

Table 1. *Thermal Stabilities of Oligonucleotide Duplexes*

Primer	Template	T_m[a])	Conditions
30 μM **2**	50 μM **3**	50.2°	[b])
1.2 μM **2**	2 μM **3**	31.3°	[b])
30 μM d(GCACG)	50 μM **3**	35.1°	[b])
1.2 μM d(GCACG)	2 μM **3**	18.8°	[b])
0.8 μM **2**	0.8 μM **3**	19.8°	[c])
0.8 μM **4**	0.8 μM **3**	19.8°	[c])

[a]) Detection at 260 nm. [b]) 0.25M *Tris* · HCl, pH 7.65. [c]) 10 mM phosphate buffer, pH 7.0, 70 mM NaCl.

[8]) The short form 'acridine' is used for the 5′-end label '6-[(6-chloro-9-methylacridin-2-yl)oxy]hexyl'. For convenience, the hyphens representing the diesterified phosphate residues between nucleosides are omitted in the abbreviated names of oligonucleotides, except at the 5′-end in acridine-modified oligonucleotides.

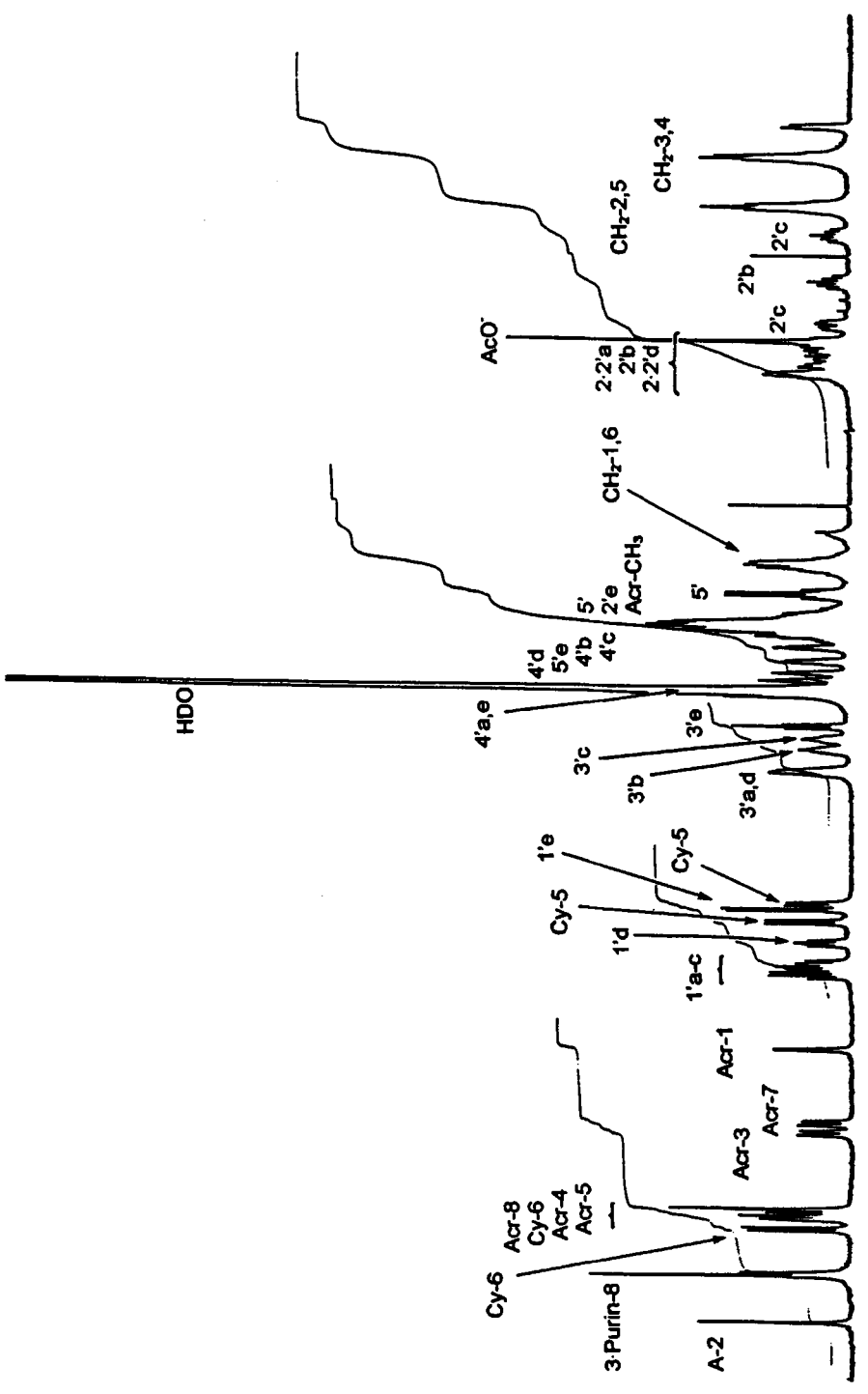


Fig. 4. *^{1}H-NMR Spectrum* (400 MHz, 57°, D_2O) *of primer* **2**. Acr-1 *etc.*: H-atoms at acridine moiety; CH_2-1,6 *etc.*: CH_2 groups of the chain linker at the 5'-end; purin-8: H–C(8) of A and G; Cy-5 and Cy-6: H–C(5) and H–C(6), resp., of C; A-2: H–C(2) of A; primed numbers for pentose H-atoms; a,b,c *etc.*: furanose rings starting from the 5'end.

Compared to standard fluorescent dyes used in DNA sequencing (*e.g.*, fluoresceine, Cy 5, 'Texas Red' *etc.*), the fluorescence of acridines is less pronounced. Since the quantum yields are further reduced in primer **2** by partial quenching due to the 5'-terminal guanosine, we alternatively prepared the primer sequence 5'-acridine-d(CTAC)riboG-3' (**23**) in the same way as before. Indeed, primer **23** exhibited a fourfold stronger fluorescence than compound **2**. This formal advantage, however, was impaired by insufficient duplex stabilities. So, primer **2** and its RNA analogue 5'-acridine-ribo-(GCACG)-3' (**4**) were exclusively used in all experiments discussed below. In the case of **4**, we applied the acridine building block *rac*-**14** together with *Ogilvie*'s 2'-silylated ribonucleotide phosphoramidites [27] following standard synthesizer protocols.

4. Oligomerization Experiments: Incorporation of Guanosine. – 4.1. *Experimental Setup and Product Identification. Scheme 3* outlines a typical experiment: Primer **2** (30 μM) was mixed in aqueous buffer pH 7.7, containing 1M NaCl and 200 mM Mg^{2+}, with oligonucleotide 3'-d(GCGTGCCCCC)-5' (**3**) (100 μM). The high T_m value of the duplex **2** · **3** guarantees complete hybridization of primer **2** at 10°. The single-stranded portion of the complex **2** · **3** serves as the template, associating with up to four monomeric guanosine units **1** (50 mM). The 2-methyl-1*H*-imidazole-activated [28a] nucleoside 5'-monophosphates (*e.g.*, **1**, **5**) have been introduced by *Inoue* and *Orgel*. These compounds and their less efficient [14b] 1*H*-imidazole analogues [28b] have been successfully used in oligomerization experiments by *Orgel* and coworkers [4][12–14] and other groups [3][7][8a][8d–h][29][9]).

Within the double-helical aggregate $(\mathbf{1})_n \cdot \mathbf{2} \cdot \mathbf{3}$, chain extension occurs. This process could be easily analyzed by reversed-phase HPLC. While the unmodified mono- and oligonucleotides were rapidly eluted, **2** was strongly retained on the column due to its lipophilic dye component. Since each chain-extension step increases the polarity of the products, their HPLC peaks are shifted towards shorter retention times. Fluorescence detection led to a further improvement in the detection limit, as shown by the typical chromatogram in *Fig. 5*.

As expected, the consecutive formation of four new peaks was observed. The first peak could be unequivocally identified as compound **24** (= **2** + ***I***) by direct comparison with an authentic sample. Consequently, the peaks were assigned to the four chain-extension products of primer **2**, *i.e.*, **2** + ***I*** to **2** + ***IV***. Enzymatic degradation of these oligonucleotides by ribonuclease T1 followed by alkaline phosphatase led to the unmodified primer **2**, exclusively [14]. Since ribonuclease T1 cleaves only 3',5' bonds after guanosines, leaving 2',5' bonds untouched, the first guanosine must have been incorporated with high constitutional selectivity. According to *Orgel* and coworkers, this 3',5' selectivity is an intrinsic property of 2-methyl-1*H*-imidazole-activated nucleotides [28a]. It is, therefore, reasonable to assume that all further nucleotides are preferentially linked by 3',5' bonds as well. After long reaction times, small quantities of a supplementary product became visible, expected to be the fivefold-extended primer. It might be formed either in a non-template controlled step, or by 'sliding' of the primer on the template [31].

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[9]) For activation of nucleoside 5'-monophosphates by adenine, see [30].

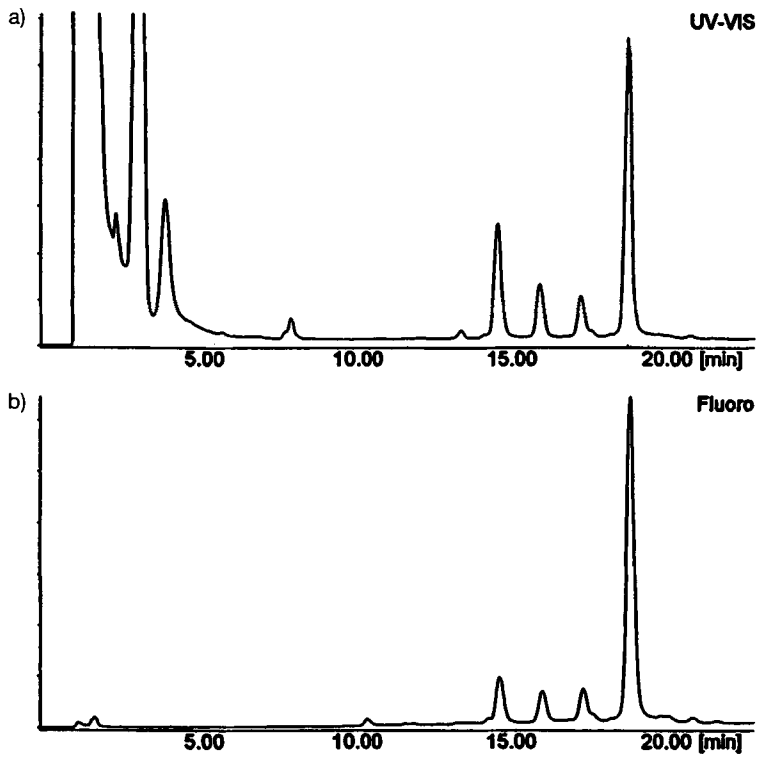


Fig. 5. *Template* **3** *controlling the chain extension of primer* **2**, *typical chromatogram*: a) *UV detection at 260 nm and* b) *fluorescence detection.* From right to left **2**, **2** + ***I***, **2** + ***II***, **2** + ***III***, and **2** + ***IV***. The mixture of unlabeled mono- and oligonucleotides is eluted within the first 5 min and is invisible by fluorescence detection.

Chain elongation is a template-controlled process: when oligonucleotide **3** was omitted, less than 10% of primer **2** reacted within 47 h. In addition to *ca.* 1% of **24** (= **2** + ***I***), a second product with slightly longer retention times was observed (*ca.* 8%), presumably the 2′,5′-linked isomer of **24**. In contrast, the half-life of primer **2** is reduced to *ca.* 4 h in the presence of template **3**. No reaction at all occurred when replacing imidazolide **1** with nonactivated guanosine 5′-monophosphate. Finally, Mg^{2+} ions proved to be indispensable as catalyst for the phosphoryl-transfer step. They could not be substituted by Na^+ or several ammonium and guanidinium derivatives we have checked.

4.2. *Kinetics of Chain Extension.* The calculation of rate constants from the corrected UV integrals of chain-extension products is based on the assumption of four consecutive elongation steps, each being first-order in the oligonucleotide involved (*Eqns. 1–5*).

$$\mathrm{d}[\mathbf{2}] = -k_1[\mathbf{2}]\mathrm{d}t \tag{1}$$

$$\mathrm{d}[\mathbf{2} + \boldsymbol{I}] = (-k_2[\mathbf{2} + \boldsymbol{I}] + k_1[\mathbf{2}])\mathrm{d}t \tag{2}$$

$$\mathrm{d}[\mathbf{2} + \boldsymbol{II}] = (-k_3[\mathbf{2} + \boldsymbol{II}] + k_2[\mathbf{2} + \boldsymbol{I}])\mathrm{d}t \tag{3}$$

$$\mathrm{d}[\mathbf{2} + \boldsymbol{III}] = (-k_4[\mathbf{2} + \boldsymbol{III}] + k_3[\mathbf{2} + \boldsymbol{II}])\mathrm{d}t \tag{4}$$

$$\mathrm{d}[\mathbf{2} + \boldsymbol{IV}] = +k_4[\mathbf{2} + \boldsymbol{III}]\mathrm{d}t \tag{5}$$

Curves of concentration *vs.* time were obtained by numerical integration of *Eqns. 1–5* using a spread-sheet program. By variation of the hypothetical rate constants, beginning with k_1, a good correspondance between calculated and experimental concentrations could be achieved (*Fig. 6*). This set of variables was assumed as experimental rate constants (*Table 2*).

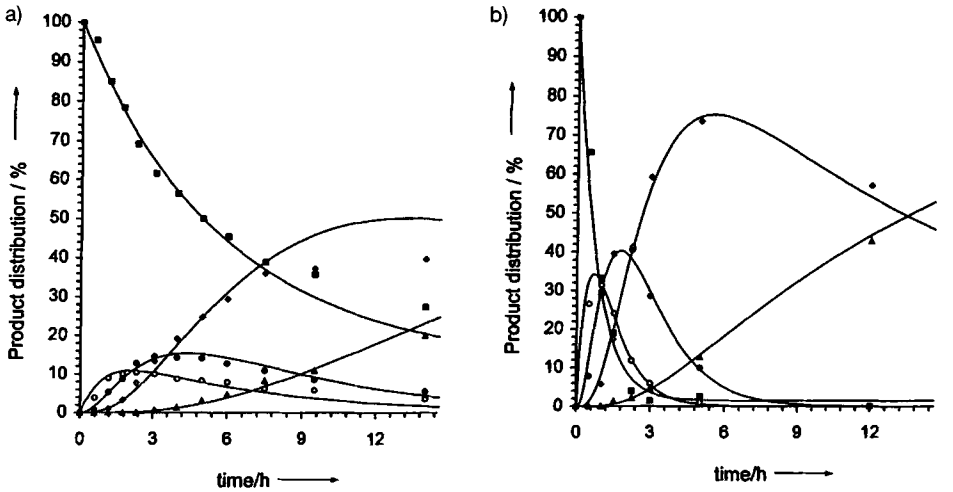


Fig. 6. *Best fit of the experimental and calculated product distribution* a) *in* Exper. 1 (■: primer **2**, ○: **2** + ***I***, ●: **2** + ***II***, ◇: **2** + ***III***, ▲: **2** + ***IV***) *and* b) *in* Exper. 3 *using RNA primer* **4** (■: primer **4**, ○: **4** + **I**, ●: **4** + **II**, ◇: **4** + **III**, ▲: **4** + **IV**)

Table 2. *Pseudo-First-Order Rate Constants* [h^{-1}] *for Primer Extensions by the Guanosine Building Block* **1** (30 μM primer, 100 μM template, 50 mM **1**, 0.25M HEPES · NaOH pH 7.7, 1M NaCl, 0.2M Mg^{2+}, 10°)

Exper.	Primer	Template	Step 1	Step 2	Step 3	Step 4
1	**2** (DNA)	**3** (DNA)	0.17	0.96	0.54	0.05
2	**24** (mixed)	**3** (DNA)		1.20	0.90	0.08
3	**4** (RNA)	**3** (DNA)	1.20	1.50	0.72	0.07
4	**2** (DNA)	**25** (RNA)	1.20	2.20	0.87	0.09
5	**24** (mixed)	**25** (RNA)		2.22	1.20	0.13
6	**4** (RNA)	**25** (RNA)	1.50	1.20		0.48[a])

[a]) The determination of both rate constants was prevented by insufficent peak separation.

The consistency of theoretical and experimental data for the chain elongation of primer **2** in the presence of **1** and template **3** (see *Fig. 6*) verifies of the basic assumptions. After long reaction times (more than 7 h), however, considerable deviations were observed. They are due to side reactions not included into *Eqns. 1–5*. Especially pyrophosphate formation from imidazolide **1** may strongly influence chain-extension rates, as shown by *Kanavarioti* and coworkers [7c].

The oligomerization experiments showed good reproducibility across independent runs. Rate constants rarely deviated more than 15% from the average values. Although we cannot exclude some systematic errors due to the difficult calibration of the extinction

coefficients, this is not relevant to our investigation of catalytical effects (dealing with *relative* rates). When several experiments were performed from identical stock solutions, the consistency of different runs was excellent. Even minor influences of additives could be clearly detected under these conditions.

To our surprise, the concentration of Na^+ in the range from 50 mM to 1.2M hardly affects the kinetics of guanosine oligomerization (*Table 3*). Since Na^+ ions block the reaction of guanosine-rich templates, this observation will be of central importance in the second part of our studies dealing with cytosine incorporation (see below). Guanidinium, a classical denaturating agent for proteins, is tolerated up to high concentrations (*Table 4*). The template-controlled oligomerization of imidazolide **1** proceeded in even 4M solutions of guanidinium hydrochloride. As guanidinium ions are known to interact preferentially with the *Hoogsteen* sites of guanosine, we consider 'selective denaturation' of guanosine quadruplexes as an alternative strategy for template-controlled incorporation of oligocytidine stretches.

Table 3. *Extension of the Duplex* **2** · **3** *by Guanosine: Product Distribution after Constant Time as a Function of Sodium Concentrations* (30 μM **2**, 100 μM **3**, 50 mM **1**, 0.25M *Tris* · HCl pH 7.7 (sodium-free), NaCl, 0.2M Mg^{2+}, 10°). Since **1** was used as a sodium salt, the minimal Na^+ concentration was 0.05M. The data shown in *Tables 3* and *4* were obtained with different templates; thus, they are not fully comparable.

[Na^+]	Primer **2**	**2** + *I*	**2** + *II*	**2** + *III*	**2** + *IV*
0.85M	16.8%	5.1%	9.4%	52.9%	15.7%
0.25M	22.4%	5.8%	9.7%	49.3%	12.8%
0.15M	24.0%	5.6%	9.9%	48.0%	12.4%
0.05M	23.2%	4.8%	8.4%	50.6%	12.9%

Table 4. *Extension of the Duplex* **2** · **28** *by Guanosine: Product Distribution after Constant Time as a Function of Guanidinium Concentrations* (30 μM **2**, 55 μM **28**, 50 mM **1**, 0.25M *Tris* · HCl pH 7.7 (sodium free), 0.2M Mg^{2+}, 10°). The data shown in *Tables 3* and *4* were obtained with different templates; thus, they are not fully comparable.

[Guanidine · HCl]	Primer **2**	**2** + *I*	**2** + *II*	**2** + *III*	**2** + *IV*
0M (after 3 h)	24.7%	4.1%	2.9%	61.6%	6.7%
1M	47.1%	4.4%	4.7%	41.8%	2.0%
2M	67.8%	3.5%	4.2%	24.5%	
4M	84.1%	2.3%	2.7%	10.4%	0.5%
0M (after 20 h)	21.4%	1.5%	2.1%	38.5%	36.4%
1M	17.8%	1.9%	0.5%	53.1%	26.6%
2M	20.6%	2.4%	1.2%	55.4%	20.5%
4M	30.1%	4.1%	3.8%	50.0%	12.0%

Regarding the kinetical data of *Exper. 1* (*Fig. 6* and *Table 2*), three phenomena require special attention:

1) Although template **3** was used in large excess, *ca.* 20% of primer **2** did not react at all. Summarizing the results of different experiments, this residue correlates inversely with the initial rates of primer elongation; high values of k_1 led to complete consumation of the primer, whereas the amount of unreacted primer increased when k_1 declined.

Probably, this inhibition is caused by a template-controlled assembly of the monomer to dimers or oligomers not linked to the primer molecule (and hence invisible).

2) While the central nucleotides were introduced with high and comparable rates, the last nucleotide was incorporated tenfold slower. According to *Wu* and *Orgel* [14a], this effect can be in part explained by reduced stacking interactions of the last mononucleotide in the complex $(\mathbf{1})_n \cdot \mathbf{2} \cdot \mathbf{3}$. Furthermore, there is good evidence that the condensation step of one nucleotide is directly catalyzed by a second phosphoric 2-methyl-1*H*-imidazolide in its neighborhood [14b].

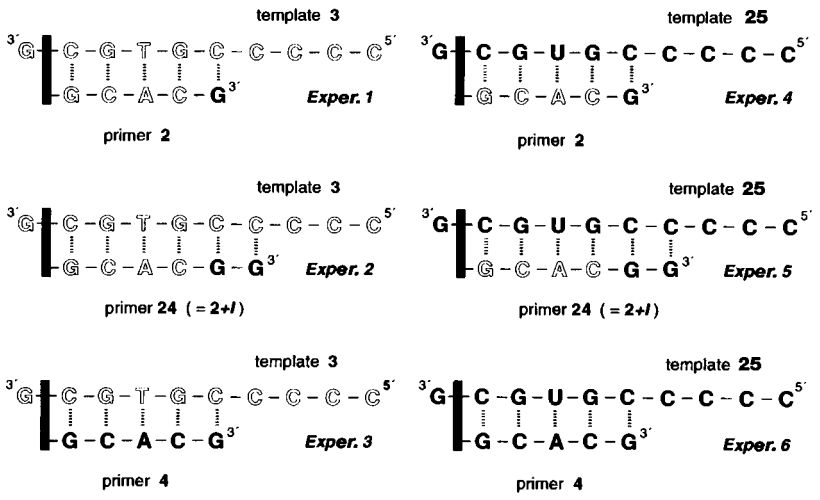
3) Compared with k_2, the rate constant k_1 decreased by a factor of 5. To rationalize this phenomenon, we propose the following hypothesis: chain extension is most efficient if the primer template duplex adopts A-type conformations. The complex $\mathbf{2} \cdot \mathbf{3}$, however, is composed of DNA and thus prefers the B conformation. As a consequence, the first elongation step is slow. RNA-DNA Hybrids are known to be restricted to A-type conformations[10]). When the first bond between ribonucleotides is formed during chain extension, at least a partial change of duplex conformation from B to A can be expected. All further elongation steps are conformationally favored and proceed with higher rates.

4.3. *Correlation between Duplex Conformations and Reactivity*. Two different primers were synthesized to test this hypothesis: **24** 5′-acridine-d(GCAC)riboGriboG-3′ ($\mathbf{24} = \mathbf{2} + \boldsymbol{I}$), the first elongation product of *Exper. 1*, and 5′-acridine-ribo(GCACG)-3′ (**4**), an oligonucleotide with the same sequence as primer **2** but composed entirely of ribonucleotides. Since CD spectroscopy is a suitable method to detect conformational changes of oligonucleotide duplexes [33], hybrids of primers **2**, **4**, and **24** with both, DNA template **3** and RNA template **25** were studied (*Fig. 7*). In spite of the fact that the bands of the acridine moiety were overlapping with those of the nucleotides, the spectrum of $\mathbf{2} \cdot \mathbf{3}$ (DNA/DNA) closely resembled that of B-form duplexes. In contrast, the duplex $\mathbf{4} \cdot \mathbf{3}$ (RNA/DNA) gave a completely different spectrum, bearing the characteristics of the A form. Quite similar spectra were obtained from the duplexes $\mathbf{2} \cdot \mathbf{25}$ (DNA/RNA) and $\mathbf{4} \cdot \mathbf{25}$ (RNA/RNA), respectively. In accordance with our hypothesis, a conformational change was observed when additional ribonucleotides were attached to the duplex $\mathbf{2} \cdot \mathbf{3}$. While the first elongation product formed a duplex $\mathbf{24} \cdot \mathbf{3}$ with spectral properties ranging between those of $\mathbf{2} \cdot \mathbf{3}$ and $\mathbf{4} \cdot \mathbf{3}$, the fully extended duplex $\mathbf{2} + \boldsymbol{IV} \cdot \mathbf{3}$ clearly had the spectrum of an A-type double helix. A survey of primer template duplexes is given in *Scheme 4*.

Can duplex conformations be correlated with kinetic behavior? When the hexamer primer **24** reacted in the presence of **3** and **1**, the first extension step was fast indeed (*Exper. 2*; see *Scheme 4*). As the reactions of *Exper. 2* are part of *Exper. 1* as well, the similarity of corresponding rate constants was no surprise (*Table 2*). The crucial test for our hypothesis is *Exper. 3* in which DNA primer **2** was substituted by its RNA analogue **4**. As predicted, all rate constants $k_1 - k_3$ were comparably fast under these conditions. The same result was obtained when either the DNA primer was combined with the RNA template ($\mathbf{2} \cdot \mathbf{25}$; *Exper. 4*) or when both molecules consisted of ribonucleotides ($\mathbf{4} \cdot \mathbf{25}$; *Exper. 6*). According to CD spectroscopy, the primer template duplexes from *Exper. 1–6* formed two different families bearing the characteristics of either A- or

[10]) For a recent discussion of DNA-RNA hybrid conformation, see [32].

Scheme 4. *Survey of* Exper. 1–6. For symbols, see *Scheme 3*.



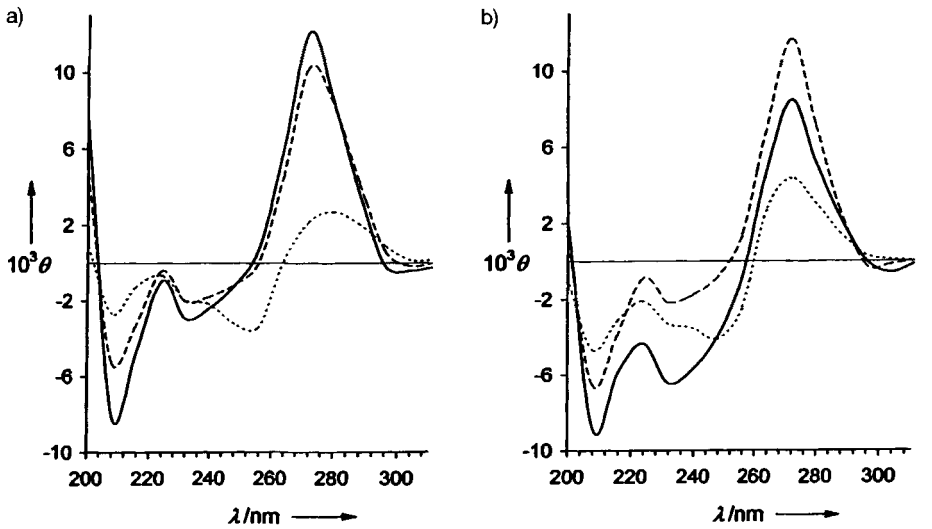
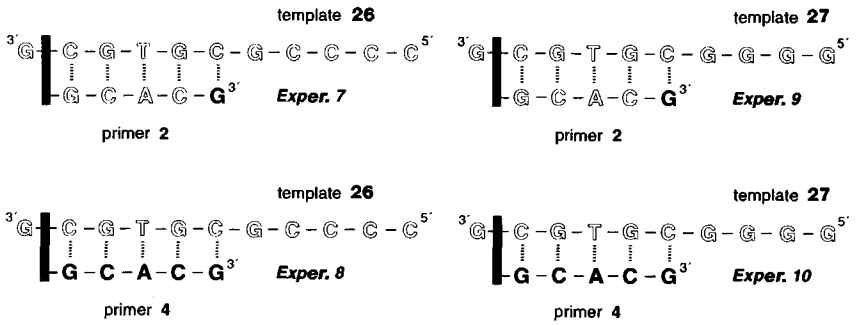


Fig. 7. *CD Spectra of the primer template duplexes* (2 μM primer, 2 μM template, 30 mM phosphate buffer pH 7.0, 70 mM NaCl, 5°, θ in mdeg). *a*) ·····: **2** · **3** (DNA/DNA), ---: **2** · **25** (DNA/RNA), —: **4** · **25** (RNA/RNA). *b*) —: **4** · **3** (RNA/DNA), ·····: **24** · **3** (mixed/DNA), ---: **24** · **25** (mixed/RNA).

B-type conformation. The strict correlation between CD-spectroscopic behavior and reactivity pattern gave strong evidence for the postulation proposed above. Further support came from the conformational change seen in *Exper. 1* on ribonucleotide incorporation. The understanding of these mechanistical details is of major practical importance, since the overall efficiency of template-controlled RNA oligomerization critically depends on high initial rates. In *Exper. 3*, for example, primer consumation is almost quantitative compared to only 80% in *Exper. 1*.

5. Oligomerization Experiments: Incorporation of Cytidine. – When hybridized with primer **2**, the single-stranded part of template **26** should direct the incorporation of one cytidine and four guanosines (*Scheme 5*, *Exper. 7*; general conditions, see *Exper. 1*). The first extension step was slow, because stacking interactions of pyrimidines are weaker than those of purines [14b]. It was, furthermore, hampered by the unfavorable B-type conformation of the primer template helix. Consequently, the overall performance of *Exper. 7* was weak. About 50% of the primer did not react at all, and only 12% of the fully extended chain was produced within 7 d. In the absence of cytidine imidazolide **5**, almost no reaction occurred between primer and guanosine imidazolide **1**. In *Exper. 8*, the DNA primer **2** was substituted by its RNA counterpart **4**. As a result, the reaction became much more efficient. Within 7 d, 94% of primer **4** was consumed ($t_{1/2}$ 8 h), leading to 70% of the two most elongated chains.

Scheme 5. *Survey of* Exper. 7–10. For symbols, see *Scheme 3*.



One of the most challenging problems preventing nonenzymatic self-replication of RNA up to now is the tendency of guanosine-rich oligonucleotides to form quadruplexes [34]. Templates for the polymerization of cytidine are thus inhibited. Certain metal ions, especially K^+ and Na^+, strongly stabilize the so-called G-quartetts [35] which might be regarded as self-assembling crown ethers [36] (*Fig. 8*). In *Exper. 9*, the template **27** should lead to the incorporation of four cytidine residues. We suspected, however, that high alkali-ion concentrations – traditionally present in these experiments – might be sufficient to cause self-aggregation of template **27**. Indeed, CD spectra of **27** revealed drastic conformational changes on addition of 1M K^+. The maximum is shifted from 289 to 263 nm. Similar spectroscopic behavior was associated with the formation of parallel quadruple strands [37]. Those effects are less pronounced in the presence of Na^+ and almost disappeared when K^+ was substituted by Li^+ (*Fig. 9*). In contrast, the spectrum of the oligo-C template **3** remained almost unaffected by the addition of K^+ or Na^+.

In accord with earlier results, the extension of DNA primer **2** in 1M NaCl solution was very inefficient (*Table 5*). Lowering the Na^+ concentration to 50 mM led to a strong rate enhancement (*Exper. 9*). The influence of alkali-metal ions on the reactivity of RNA primer **4** correlated with their potential to induce quadruplex formation: K^+ led to very strong inhibition of chain extension, Na^+ to strong and Li^+ to negligible effects (*Exper. 10*). Our present HPLC system did not allow a complete resolution of the second

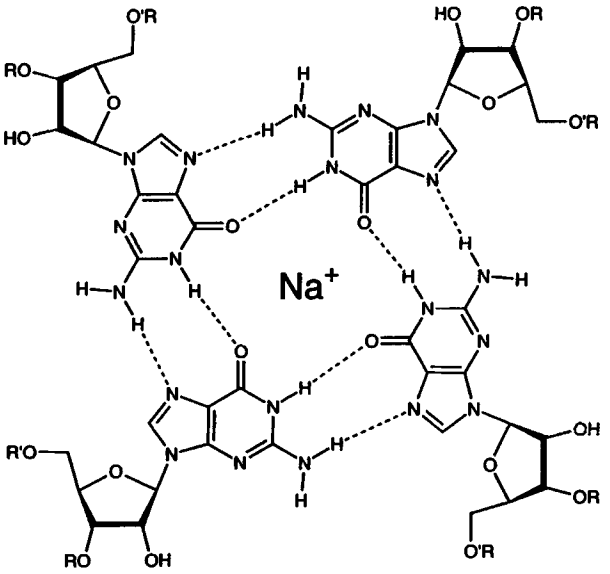


Fig. 8. *Structure of the guanosine quartet*

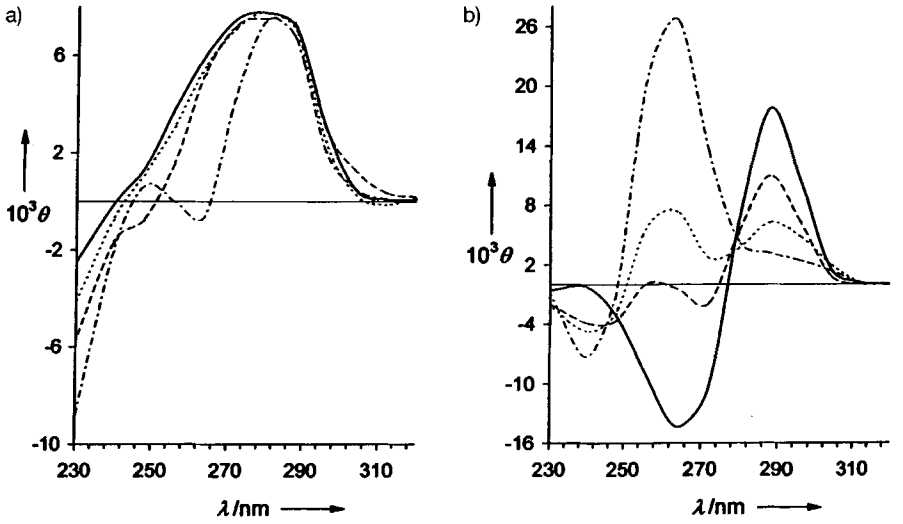


Fig. 9. *CD Spectra of the templates* **3** *and* **27** *as a function of alkali-ion concentration. a*) Oligo-dC template **3**. *b*) Oligo-dG template **27**. Conditions: 20 μM template, 0.25M *Tris* · HCl buffer pH 7.7, 10°, —: 0.25M *Tris* · HCl, ---: 1.0M LiCl, ·····: 1.0M NaCl, ·–·–·: 1.0M KCl θ in mdeg.

and third elongation product in *Exper. 9* and *10*. Although we cannot exclude that a fourth cytidine was properly attached to the primer, we assume that the limitations found in *Orgel*'s system (*Scheme 1*) are valid in our case as well. Using highly diluted hairpins with a single-stranded (guanosine)$_n$ template, only $n - 1$ cytidines were efficiently incor-

porated [14b]. Because of this uncertainty, *Table 5* contains product concentrations instead of rate constants. The data clearly show, however, that the first step in the template-controlled oligomerization of cytidine (*Exper. 10*, 50 mM Na^+) may proceed with comparable rates as guanosine incorporation in *Exper. 1*.

Table 5. *Primer Extension by the Cytidine Building Block* **5**: *Product Distribution after Constant Time in a Typical Experiment* (30 μM primer **2** or **4**, 100 μM **27**, 50 mM **5**, 0.25M *Tris* · HCl pH 7.7 (sodium free), additional salts, 0.2M Mg^{2+}, 10°)

t [h]	DNA Primer **2**, 1M NaCl			DNA Primer **2**, 50 mM NaCl		
	2	**2** + ***I***	**2** + ***II*** to **2** + ***IV***	**2**	**2** + ***I***	**2** + ***II*** to **2** + ***IV***
4	92.7%	6.8%	0.5%	81.5%	15.6%	2.8%
12	82.4%	13.2%	4.4%	58.1%	25.1%	16.8%
24	73.3%	15.4%	11.3%	45.6%	24.6%	29.9%
t [h]	RNA Primer **4**, 1M KCl			RNA Primer **4**, 1M NaCl		
	4	**4** + ***I***	**4** + ***II*** to **4** + ***IV***	**4**	**4** + ***I***	**4** + ***II*** to **4** + ***IV***
4	89.6%	9.2%	1.2%	75.3%	20.2%	4.5%
12	81.4%	14.0%	4.6%	56.5%	27.2%	16.3%
24	75.7%	18.3%	6.0%	42.2%	26.5%	31.3%
t [h]	RNA Primer **4**, 1M LiCl			RNA Primer **4**, 50 mM NaCl		
	4	**4** + ***I***	**4** + ***II*** to **4** + ***IV***	**4**	**4** + ***I***	**4** + ***II*** to **4** + ***IV***
4	51.5%	36.7%	11.8%	52.3%	32.7%	15.0%
12	31.0%	39.7%	30.4%	27.3%	28.4%	44.3%
24	13.8%	25.0%	61.2%	14.8%	20.5%	64.7%[a])

[a]) *Ca.* 87% after 5 days.

6. Comparison with Earlier Work and Conclusions. – In a recent article, it was stated that the '*creation of artificial chemical life*' will be '*a radical challenge for synthetic organic chemistry in the first half of the coming century*' [38]. Based on present knowledge, however, the outlook for RNA as a candidate for nonenzymatic self-replication is rather bad [14c,d]. The tendency of oligo-guanosine sequences to form tetrameric aggregates was identified as a major obstacle[11]). In addition, certain template sequences turned out to be either partial (TT, TG, GT) or total barriers (*e.g.*, AT, TA, AA, GA, AG) for chain extension [14c]. Furthermore, in a prebiotic world, RNA self-replication would suffer from enantiomeric cross inhibition due to the presence of racemic monomers [39].

Our work was motivated by the idea that the incorporation of pyrimidine nucleotides might be promoted by simple catalysts like guanidinium and bis(guanidinium) ions.

[11]) *Wu* and *Orgel* commented on this problem by the following statement [14b]: '*We do not see any way of destabilizing the oligo(G) self-structure without greatly weakening the interaction of oligo(G) with C-containing monomers*'.

Acridine-labeled primers turned out to be a valuable tool for detecting even minor influences on chain extension kinetics. In spite of the fact that such catalysts have not yet been identified, the important result of these experiments is that Na^+ can be omitted from the reaction mixture without negative effects. In contrast, all previous studies used NaCl concentrations higher than 1M.

In the case of guanosine incorporation, *Wu* and *Orgel* reported a half-life of 3 h for the disappearance of the primer (0°) [14a] (*Scheme 1*). Using the RNA template oligo-C, *Kanavarioti* and coworkers observed a half-life of 0.23 h at 23° [7c]. In *Exper. 3* with template **3** and RNA primer **4**, $t_{1/2}$ at 10° was determined to be 0.58 h. These differences can be well explained by the temperature changes. When DNA primers were combined with DNA templates, the B-type conformation of the duplex retarded the first elongation step and reduced the overall performance of RNA oligomerization. This effect was not considered by *Orgel* and coworkers. Nevertheless, it might be in part responsible for some of the negative results obtained in his DNA hairpin system.

For the incorporation of a single cytidine residue within a stretch of guanosines (*Exper. 8*, 10°, $t_{1/2}$ 8 h), again the correspondence of our results with literature is satisfactory ([14b]; 0°, $t_{1/2}$ 16 h).

While many observations of previous studies could be reproduced in our primer-extension system, fundamental differences were seen as far as oligo-dG templates are concerned. Simply by reducing Na^+ concentrations from 1.2M to 50 mM, a rapid oligomerization of cytidine could be achieved using template concentrations as high as 100 μM. Similar results have been obtained in *Orgel*'s hairpin system, but the template was diluted to the nanomolar range in order to avoid quadruplex formation [14b]. This means that, in *Exper. 10*, the problem of guanosine aggregation is virtually eliminated. It might be argued that Na^+-free conditions are not relevant to prebiotic earth. However, the principal aim of our studies is not to 'explain' the historical origin of life but to achieve in the future a laboratory demonstration of nonenzymatic self-replication and evolution. Since quadruplex formation of oligo-G sequences can be modulated to a large extent by choosing appropriate reaction conditions, this obstacle has lost much of its power. When the problem of insufficient A–U association might be solved either by catalysis or by modified base structures [16], the outlook for nonenzymatic self-replication of RNA would be much better than previously assumed.

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Experimental Part

General. Water bath: *Lauda RMT 6*; precision ± 0.2°. TLC: glass plates coated with silica gel *F 254/366* (0.25 mm, *Macherey-Nagel*). Column chromatography (CC): *Silicatech 63-200* (*ICN*). HPLC: *Jasco* high-pressure gradient system consisting of 2 pumps *PU-980*, UV/VIS detector *UV-970* and fluorescence detector *FP-920*; column: *Merck LiChrospher 100 RP-18* (5 μm), 125 × 4 mm; t_R in min. M.p.: *Kofler* hot plate microscope, uncorrected. UV: *Varian Cary 1 Bio* equipped with a *Cary* thermoelectric controller or *Cary 15* (*Applied Physics Corporation*). CD: *Jasco J-715*. FT-IR: *Perkin-Elmer 1600*; in $\tilde{\nu}$ [cm^{-1}]. ^{1}H-NMR: *Varian XL 200*, *Bruker AM 250*, *Bruker WH 270*, or *Bruker AMX 400* spectrometers; chemical shifts (δ) in ppm relativ to Me_4Si (0.00 ppm) or (D_5)DMSO (2.50 ppm) as internal standards, *J* in Hz. ^{31}P-NMR: *Bruker AMX 400* (161.98 MHz); δ rel. to phosphoric acid as external standard (0.00 ppm). ESI-MS: *Fisons VG Platform II* or *Finnigan SSQ 7000*. Elemental analysis: *Heraeus HCN-Rapid*, performed at the Institut für Organische Chemie, J.W. Goethe-Univer-

sität, Frankfurt. *Abbreviations.* acr: acridine; ar: aryl; dam: (dimethylamino)methylidene; DMAP: 4-(dimethylamino)pyridine; EDC: *N*-[(dimethylamino)propyl]-*N'*-ethylcarbodiimide; HEPES: 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; NMM: *N*-methylmorpholine; *Tris*: 1,1,1-tris(hydroxymethyl)methylamine (= 2-amino-2-(hydroxymethyl)propane-1,3-diol.

2. Acridine Derivatives. – 2.1. *6-Chloro-2-methoxy-9-methylacridine* (**8**) [20]. NaH (3.63 g, 91 mmol, 55–65% in oil) was suspended under Ar in dry DMSO (10 ml). After cooling to 0°, a soln. of diethyl malonate (20.7 ml, 136.3 mmol) in DMSO (15 ml) was added dropwise. Then, 6,9-dichloro-2-methoxyacridine (**6**; 5.0 g, 18.2 mmol) was added and dissolved by heating to 120° for 2 h, giving a dark-red mixture and a precipitation of NaCl. The resulting yellow intermediate **7** was precipitated by careful addition of H_2O (10 ml) and fuming aq. HCl soln. (35 ml) at 0°, and decarboxylated by heating to 100° for 1 h. This suspension was cooled to 0° again and poured into a mixture of 6M aq. NaOH (80 ml) and ice (150 g). After dilution with H_2O (200 ml), AcOEt (500 ml) was added at neutral pH. Remaining solids were dissolved by vigorous stirring and heating. Then, the phases were separated, the aq. layer was extracted with hot AcOEt (100 ml), and the combined org. phases were dried (Na_2SO_4). Spontaneous crystallization during the evaporation afforded **8** (4.10 g, 87%) in two portions. Yellow needles. M.p. 173° ([20]: 169–170°). IR (KBr): 3448*m*, 2962*w*, 2934*w*, 2829*w*, 1634*s*, 1567*w*, 1472*s*, 1422*m*, 1272*w*, 1233*m*, 1220*s*, 1150*w*, 1042*m*, 1026*m*, 913*m*, 823*s*, 799*s*. ^{1}H-NMR ((D$_6$)DMSO, 200 MHz): 2.99 (*s*, Me); 3.96 (*s*, MeO); 7.41 (*d*, J = 2.1, H–C(1)); 7.50 (*dd*, ^3J = 9.3, ^4J = 2.6, H–C(3)); 7.52 (*dd*, ^3J = 9.2, ^4J = 2.2, H–C(7)); 7.98 (*d*, J = 9.4, H–C(4)); 8.06 (*d*, J = 2.1, H–C(5)); 8.31 (*d*, J = 9.3, H–C(8)). NOE: 2.99 (Me) → 7.41 (H–C(1)), 8.31 (H–C(8)). ESI-MS: 257 (88, M^+), 214 (100), 179 (41), 151 (24). Anal. calc. for $C_{15}H_{12}ClNO$ (257.72): C 69.91, H 4.69, N 5.43; found: C 69.83, H 4.88, N 5.38.

2.2. *6-Chloro-9-methyl-2-({6-[[(2RS)-tetrahydropyran-2-yl]oxy}hexyloxy)acridine* (*rac*-**10**). A suspension of **8** (2.5 g, 9.7 mmol) in HBr (50 ml, 33% in AcOH) and H_2O (5 ml) was degassed *in vacuo* and refluxed under N_2 for 26 h until TLC (microworkup: $NaHCO_3$, AcOEt) showed completion of the reaction [21]. Evaporation, co-evaporation with i-PrOH in order to remove traces of acid, and drying *in vacuo* at 50° afforded phenol **9** as an orange powder which was resuspended in dry DMF (100 ml) and degassed again. Addition of Cs_2CO_3 (12 g, 36.8 mmol) resulted in a dark-red mixture. After addition of 1-bromo-6-{[(2*RS*)-tetrahydropyran-2-yl]oxy}hexane (3.9 g, 14.6 mmol) [22] and heating to 120° for 4 h, the mixture was cooled down to r.t. and distributed between H_2O (300 ml) and Et_2O (600 ml). The phases were separated, the org. layer was reextracted with Et_2O (200 ml), and the combined org. phases were washed twice with brine (150 ml) and dried (Na_2SO_4). Then, Et_3N (5 ml) and silica gel (5 g) were added, the solvent was evaporated and the residue purified by FC (silica gel, 250 g, AcOEt/hexane 1:3 → 1:2). Crystallization from AcOEt/hexane yielded *rac*-**10** (3.74 g, 90%). Yellow needles. M.p. 96–97°. UV/VIS (EtOH): 263.2 (145600), 319.5 (2670), 334.5 (5100), 352.0 (7570), 382.5 (6640), 402.0 (6300). IR (KBr): 3420*w*, 3062*w*, 2941*s*, 2870*m*, 2786*w*, 1630*s*, 1560*w*, 1518*w*, 1460*s*, 1421*m*, 1388*w*, 1366*w*, 1350*w*, 1269*m*, 1232*s*, 1217*s*, 1171*m*, 1139*m*, 1074*s*, 1033*s*, 917*m*, 819*m*. ^{1}H-NMR ((D$_6$)DMSO, 200 MHz): 1.38–1.58 (*m*, 2 H–C(3'), 2 H–C(4'), 2 H–C(5'), 2 H–C(3''), 2 H–C(4''), 2 H–C(5'')); 1.77–1.81 (*m*, 2 H–C(2')); 3.01 (*s*, Me); 3.32–3.42 (*m*, 2 H, H–C(6'), H–C(6'')); 3,57–3.77 (*m*, H'–C(6'), H'–C(6'')); 4.17 (*t*, J = 6.4, 2 H–C(1')); 4.51–4.52 (*m*, H–C(2'')); 7.43 (*d*, J = 2.6, H–C(1)); 7.50 (*dd*, ^3J = 9.2, ^4J = 2.6, H–C(3)); 7.55 (*dd*, ^3J = 9.3, ^4J = 2.2, H–C(7)); 7.99 (*d*, J = 9.4, H–C(4)); 8.08 (*d*, J = 2.0, H–C(5)); 8.34 (*d*, J = 9.4, H–C(8)). ESI-MS: 427 (4, M^+), 343 (1.5), 256 (5), 245 (11), 243 (30), 85 (100). Anal. calc. for $C_{25}H_{30}ClNO_3$ (427.97): C 70.16, H 7.07, N 3.27; found: C 70.23, H 6.95, N 3.36.

2.3. *6-Chloro-9-methylacridin-2-ol* (**9**) was isolated once for anal. purposes. ^{1}H-NMR ((D$_6$)DMSO, 270 MHz): 2.94 (*s*, Me); 7.42 (*d*, J = 2.5, H–C(1)); 7.49 (*dd*, ^3J = 9.1, ^4J = 2.5, H–C(3)); 7.53 (*dd*, ^3J = 9.2, ^4J = 2.2, H–C(7)); 7.99 (*d*, J = 9.3, H–C(4)); 8.06 (*d*, J = 2.1, H–C(5)); 8.32 (*d*, J = 9.4, H–C(8)).

2.4. *6-Chloro-9-methyl-2-({7-[[(2RS)-tetrahydropyran-2-yl]oxy}heptyloxy)acridine* (*rac*-**12**) was prepared as described in *2.2*: 98% overall yield. Yellow needles. M.p. 99–100°. UV/VIS (EtOH): 263.0 (148600), 319.5 (2750), 335.0 (5190), 351.8 (7580), 382.5 (6650), 402.0 (6330). IR (KBr): 2940*m*, 2868*m*, 1629*s*, 1552*w*, 1461*s*, 1423*m*, 1389*m*, 1269*m*, 1218*s*, 1120*m*, 1026*m*, 917*m*, 821*w*. ^{1}H-NMR ((D$_6$)DMSO, 270 MHz): 1.33–1.75 (*m*, 2 H–C(3'), 2 H–C(4'), 2 H–C(5'), 2 H–C(6'), 2 H–C(3''), 2 H–C(4''), 2 H–C(5'')); 1.82 (*m*, 2 H–C(2')); 3.04 (*s*, Me); 3.25–3.45 (*m*, H–C(7'), H–C(6'')); 3.62 (*dt*, ^2J = 9.6, ^3J = 6.8, 1 H–C(7')); 3.72 (*ddd*, ^2J = 11.3, ^3J = 7.7, 3.4, 1 H–C(6'')); 4.20 (*t*, J = 6.4, 2 H–C(1')); 4.53 (*m*, H–C(2'')); 7.48 (*s*, H–C(1)); 7.55 (*m*, H–C(3), H–C(7)); 8.01 (*d*, J = 9.4, 1 H, H–C(4)); 8.10 (*d*, J = 1.7, H–C(5)); 8.38 (*d*, J = 9.3, H–C(8)). Anal. calc. for $C_{26}H_{32}ClNO_3$ (441.99): C 70.65, H 7.28, N 3.17; found: C 70.55, H 7.27, N 3.11.

2.5. *6-[(6-Chloro-9-methylacridin-2-yl)oxy]hexan-1-ol* (**11**). Compound *rac*-**10** (2.39 g, 5.58 mmol) was dissolved in EtOH (50 ml) at slightly increased temp. Conc. aq. HCl soln. (20 ml) was added, and the resulting suspension refluxed for 1 h. The mixture was cooled down to r.t., and then poured carefully into a mixture of 6M

aq. NaOH (35 ml), ice (100 g), and H_2O (100 ml). After extracting twice with hot AcOEt (2 × 250 ml), the org. phase was dried (Na_2SO_4) and concentrated *in vacuo* until the product started to crystallize. Completion of the crystallization at 4° overnight afforded **11** (1.85 g, 96%). Regular yellow crystals. M.p. 160–162°. UV/VIS (EtOH): 263.2 (142400), 319.0 (2670), 335.0 (5000), 351.5 (7360), 382.3 (6500), 401.5 (6190). IR (KBr): 3299 (br.), 2939*s*, 2863*s*, 1629*s*, 1564*w*, 1518*w*, 1458*s*, 1422*m*, 1389*m*, 1344*w*, 1325*w*, 1271*m*, 1218*s*, 1151*w*, 1074*m*, 1009*m*, 914*m*. ^{1}H-NMR ((D$_6$)DMSO, 200 MHz): 1.38–1.46 (*m*, 2 H–C(3), 2 H–C(4), 2 H–C(5)); 1.76–1.82 (*m*, 2 H–C(2)); 2.97 (*s*, Me); 3.39 (ψt, J = 5.2, 2 H–C(6)); 4.14 (*t*, J = 6.4, 2 H–C(1)); 4.36 (*t*, J = 5.1, OH); 7.38 (*d*, J = 2.5, 1 H, H–C(1′)); 7.48 (*dd*, ^3J = 9.5, ^4J = 2.5, H–C(3′)); 7.52 (*dd*, ^3J = 9.4, ^4J = 2.2, H–C(7′)); 7.97 (*d*, J = 9.4, 1 H, H–C(4′)); 8.05 (*d*, J = 2.0, H–C(5′)); 8.30 (*d*, J = 9.4, H–C(8′)). ESI-MS: 343 (24, M^+), 243 (43), 178 (11), 83 (12), 55 (100). Anal. calc. for $C_{20}H_{22}ClNO_2$ (343.85): C 69.86, H 6.45, N 4.07; found: C 70.12, H 6.44, N 4.07.

2.6. *7-[(6-Chloro-9-methylacridin-2-yl)oxy]heptan-1-ol* (**13**) was prepared as described in 2.5: 93% overall yield. Regular yellow crystals. M.p. 154–155°. UV/VIS (EtOH): 263.2 (133600), 319.0 (1740), 335.0 (4000), 351.5 (6280), 382.3 (5850), 401.5 (5850). IR (KBr): 3441*m*, 3282*m*, 3124*m*, 2942*s*, 2855*m*, 1630*s*, 1566*w*, 1520*w*, 1459*s*, 1423*m*, 1381*m*, 1272*m*, 1220*s*, 1154*m*, 1071*m*, 1004*m*, 921*m*, 884*m*, 829*m*. ^{1}H-NMR ((D$_6$)DMSO, 270 MHz): 1.33–1.53 (*m*, 2 H–C(3), 2 H–C(4), 2 H–C(5), 2 H–C(6)); 1.82 (*m*, 2 H–C(2)); 3.02 (*s*, Me); 3.39 (*m*, J = 5.9, 2 H–C(7)); 4.18 (*t*, J = 6.5, 2 H–C(1)); 4.34 (*t*, J = 5.1, OH); 7.44 (*d*, J = 2.6, H–C(1′)); 7.52 (*dd*, ^3J = 9.5, ^4J = 2.5, H–C(3′)); 7.56 (*dd*, ^3J = 9.2, ^4J = 2.1, H–C(7′)); 7.99 (*d*, J = 9.4, H–C(4′)); 8.09 (*d*, J = 2.1, H–C(5′)); 8.36 (*d*, J = 9.4, H–C(8′)). Anal. calc. for $C_{21}H_{24}ClNO_2$ (357.88): C 70.48, H 6.76, N 3.91; found: C 70.60, H 6.79, N 4.04.

2.7. *6-[(6-Chloro-9-methylacridin-2-yl)oxy]hexyl 2-Cyanoethyl Diisopropylphosphoramidite* (*rac*-**14**) [23]. Diisopropylammonium tetrazolide (75 mg, 0.44 mmol; see *2.8*) [24] and **11** (300 mg, 0.87 mmol) were co-evaporated from dry pyridine (50 ml), dried *in vacuo*, and dissolved in dry CH_2Cl_2 (50 ml) under Ar. Freshly distilled (132–135°/0.6 Torr) 2-cyanoethyl tetraisopropylphosphorodiamidite (0.33 ml, 1.05 mmol) was added and the mixture stirred for 2 h at r.t. After extraction with aq. Na_2CO_3 soln. (50 ml, 5%), the phases were separated, the org. layer was washed with aq. Na_2CO_3 soln. (2 × 20 ml), and the aq. phases were reextracted with CH_2Cl_2 (2 × 20 ml). Then, the combined org. layers were dried (Na_2SO_4), evaporated, and purified by FC (silica gel (15 g), toluene/hexane 1:1 + 3% Et_3N). Crystallization from Et_2O/hexane afforded *rac*-**14** (338 mg, 71%) in two portions. Yellow leaflets. M.p. 80–82°. IR (KBr): 3448*w*, 2965*m*, 2939*s*, 2868*m*, 1630*s*, 1560*w*, 1518*w*, 1459*s*, 1424*w*, 1391*w*, 1364*w*, 1268*w*, 1234*m*, 1220*s*, 1183*m*, 1154*w*, 1125*w*, 1072*m*, 1042*m*, 1013*s*, 974*s*, 917*m*. ^{1}H-NMR (C_6D_6, 400 MHz): 1.14 (*d*, J = 6.8, Me_2CH); 1.16 (*d*, J = 6.8, Me_2CH); 1.42 (*m*, 2 H–C(3), 2 H–C(4)); 1.61 (*t* with fine structure, J = 6.7, CH_2CN); 1.70–1.75 (*m*, 2 H–C(2), 2 H–C(5)); 2.46 (*s*, Me); 3.29 (*dddd*, ^2J = 10.2, J(H,P) = 7.2, ^3J = 6.5, 5.6, H–C(6)); 3.38 (*dddd*, ^2J = 10.2, J(H,P) = 7.2, ^3J = 6.5, 5.6, 1 H–C(6)); 3.57 (2 *sept.*, both ^3J = 6.8, 2 Me_2CH); 3.65 (simplified *dddd*, ^2J = 12.6, ^3J = 7.8, 6.2, 1 H, CH_2CH_2CN); 3.73 (simplified *dddd*, ^2J = 12.6, ^3J = 7.8, 6.2, 1 H, CH_2CH_2CN); 3.78 (*t*, J = 6.4, 2 H–C(1)); 7.07 (*d*, J = 2.5, H–C(1′)); 7.23 (*dd*, ^3J = 9.4, ^4J = 2.2, H–C(7′)); 7.37 (*dd*, ^3J = 9.4, ^4J = 2.6, H–C(3′)); 7.59 (*dd*, ^3J = 9.3, ^4J = 0.5, H–C(8′)); 8.26 (*d*, J = 9.4, H–C(4′)); 8.54 (*d*, J = 1.8, H–C(5′)). ^{31}P-NMR (C_6D_6): 148.48 (*m*, after decoupling *s*). ESI-MS: 543.2 (26, M^+), 390.2 (38), 326.2 (59), 243.1 (87). Anal. calc. for $C_{29}H_{37}ClN_3O_3P \cdot 0.75\ H_2O$ (555.57): C 62.70, H 6.98, N 7.56; found: C 62.56, H 7.10, N 7.55.

2.8. *Diisopropylammonium 1H-Tetrazol-1-ide* [24]. Freshly distilled (i-Pr)$_2$NH (1.20 ml, 8.49 mmol) was added to a soln. of sublimed 1*H*-tetrazole (365 mg, 5.21 mmol) in dry MeCN (8 ml). The resulting suspension was stirred for 20 min at r.t., evaporated, and dried *in vacuo*: 852 mg (95%) of product. Colorless leaflets. M.p. 112–116°. IR (KBr): 3050*m*, 2986*m*, 2874*m*, 2739*s*, 2504*s*, 2143*m*, 1626*m*, 1522*m*, 1473*s*, 1442*m*, 1423*m*, 1400*s*, 1383*m*, 1320*m*, 1278*w*, 1184*s*, 1154*m*, 1126*s*, 1102*m*, 1073*s*, 1021*m*, 997*m*, 942*w*, 885*m*, 805*w*, 703*s*, 522*s*. Anal. calc. for $C_7H_{17}N_5$ (171.25): C 49.10, H 10.01, N 40.90; found: C 48.80, H 9.72, N 40.98.

2.9. *Triethylammonium 6-[(6-Chloro-9-methyl acridin-2-yl)oxy]hexyl* H-*Phosphonate* (**15**) [25]. 1*H*-1,2,4-Triazole (2.70 g, 39.2 mmol) was added to a mixture of PCl_3 (0.45 ml, 5.16 mmol) and NMM (5.67 ml, 51.62 mmol) in dry CH_2Cl_2 (60 ml). After stirring at r.t. for 30 min, a warm soln. of **11** (355 mg, 1.03 mmol) in CH_2Cl_2 (25 ml) was added dropwise at 0°; after 20 min the mixture was washed twice with 1M (Et_3NH)HCO_3 (50 ml) and brine (50 ml), dried (Na_2SO_4), and evaporated. The residue was adsorbed to silica gel (1 g) and purified by FC (silica gel (30 g), CH_2Cl_2/MeOH/Et_3N/hexane 96:2:2:20 → 82:15:2:20). Then, the combined product containing fractions were diluted with CH_2Cl_2 (60 ml), again washed with 1M (Et_3NH) HCO_3 (2 × 50 ml), dried (Na_2SO_4), and evaporated. After further drying *in vacuo*, **15** (344 mg, 66%) was obtained as a yellow solid. A small amount was recrystallized from CH_2Cl_2/Et_2O for anal. purposes. Yellow leaflets. M.p. 73–75°. UV (50 mM phosphate buffer, pH 7.0): 262 (104900), 337 (4600), 353 (7080), 382 (5540); 260 (97400). IR (KBr): 3374*m*, 2940*m*, 2871*w*, 2738*w*, 2676*m*, 2491*w*, 2324*w*, 1630*s*, 1559*w*, 1459*s*, 1424*w*, 1395*w*, 1272*w*, 1215*s*, 1054*s*, 1013*m*, 983*m*. ^{1}H-NMR

((D$_6$)DMSO, 270 MHz): 1.17 (*t*, *J* = 7.3, Et_3NH); 1.37–1.62 (*m*, 2 H–C(3), 2 H–C(4), 2 H–C(5)); 1.82 (*m*, 2 H–C(2)); 2.99 (*q*, *J* = 7.3, Et_3NH); 3.02 (*s*, overlapped by Et_3NH, Me); 3.69 (*m*, 2 H–C(6)); 4.19 (*t*, *J* = 6.4, 2 H–C(1)); 5.76 (*s*, CH_2Cl_2); 6.58 (*d*, *J* = 589, PH); 7.45 (*d*, *J* = 2.3, 1 H, H–C(1')); 7.53 (*dd*, ^3J = 9.3, ^4J = 2.5, H–C(3')); 7.56 (*dd*, ^3J = 9.4, ^4J = 2.2, H–C(7')); 8.00 (*d*, *J* = 9.4, H–C(4')); 8.09 (*d*, *J* = 2.1, H–C(5')); 8.36 (*d*, *J* = 9.4, H–C(8')); 11.33 (br. *s*, exchangeable with D_2O, Et_3NH). ^{31}P-NMR ((D$_6$)DMSO): 2.55 (*dt*, *J*(H,P) = 589.3, *J*(P,H–C(6)) = 8.1, after decoupling *s*). ESI-MS: 406.4 (M^-; calc. 406.8). Anal. calc. for $C_{26}H_{38}ClN_2O_4P \cdot {}^1/_3\ CH_2Cl_2$ (537.34): C 58.86, H 7.25, N 5.21; found: C 58.92, H 7.56, N 5.21.

2.10. *Triethylammonium 7-[(6-Chloro-9-methylacridin-2-yl)oxy]heptyl* H-*Phosphonate* (**16**) was prepared as described in *2.9*: 63% overall yield. Yellow solid. IR (KBr): 3418*m*, 2976*m*, 2941*s*, 2677*m*, 2330*w*, 1629*s*, 1475*s*, 1397*m*, 1220*s*, 1037*s*, 984*m*, 919*w*, 822*m*. ^{1}H-NMR ((D$_6$)DMSO, 270 MHz): 1.19 (*t*, *J* = 7.3, Et_3NH); 1.30–1.42 (*m*, 2 H–C(4), 2 H–C(5)); 1.43–1.57 (*m*, 2 H–C(3), 2 H–C(6)); 1.83 (*m*, 2 H–C(2)); 3.03 (*q*, *J* = 7.3, Et_3NH); 3.06 (*s*, Me); 3.64 (*m*, 2 H–C(7)); 4.21 (*t*, *J* = 6.4, 2 H–C(1)); 6.57 (*d*, *J* = 589.3, PH); 7.49 (*d*, *J* = 2.6, H–C(1')); 7.54 (*dd*, ^3J = 9.4, ^4J = 2.6, H–C(3')); 7.58 (*dd*, ^3J = 9.4, ^4J = 2.2, H–C(7')); 8.01 (*d*, *J* = 9.4, H–C(4')); 8.11 (*d*, *J* = 2.0, H–C(5')); 8.38 (*d*, *J* = 9.3, H–C(8')); 10.49 (br. *s*, exchangeable with D_2O, Et_3NH). ^{31}P-NMR ((D$_6$)DMSO): 2.51 (*dt*, *J*(P,H) = 589.3, *J*(P,H–C(7)) = 8.1, after decoupling *s*). ESI-MS: 420.2 (M^-; calc. 420.9).

2.11. *6-[(6-Chloro-9-methylacridin-2-yl)oxy]hexyl 2-Chlorophenyl Hydrogen Phosphate* (**17**). Alcohol **11** (100 mg, 0.291 mmol) was co-evaporated in dry pyridine and dissolved in dry, warm pyridine (7 ml). Then, the soln. was cooled down to r.t. and added dropwise to a mixture of 2-chlorophenyl phosphorodichloridate (94 µl, 0.581 mmol) in dry pyridine at −10°. The reaction was stopped with H_2O (1 ml) and the solvent removed *in vacuo*. Crystallization with MeOH/pyridine/H_2O and recrystallization with pyridine/acetone afforded **17** (100 mg, 65%). Yellow crystals. M.p. 190°. IR (KBr): 3426*m*, 3071*w*, 2941*m*, 1983*w*, 1629*s*, 1586*m*, 1481*s*, 1452*s*, 1392*m*, 1233*s*, 1093*s*, 1061*s*, 906*m*, 846*m*, 765*m*, 673*w*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz): 1.39–1.53 (*m*, 2 H–C(3), 2 H–C(4)); 1.65 (*m*, 2 H–C(5)); 1.80 (*m*, 2 H–C(2)); 3.03 (*s*, Me); 4.03 (*m*, *J* = 6.7, 2 H–C(6)); 4.16 (*t*, *J* = 6.5, 2 H–C(1)); 7.11 (*t*, *J* = 7.4, 1 arom. H); 7.30 (*dt*, ^3J = 7.6, ^4J = 1.5, 1 arom. H); 7.43 (*d*, *J* = 2.5, H–C(1')); 7.47 (*d*, *J* = 8.2, 1 arom. H); 7.49 (*d*, *J* = 8.4, 1 arom. H); 7.55 (*dd*, ^3J = 9.4, ^4J = 2.5, H–C(3')); 7.59 (*dd*, ^3J = 9.3, ^4J = 2.2, H–C(7')); 8.01 (*d*, *J* = 9.4, H–C(4')); 8.10 (*d*, *J* = 2.1, H–C(5')); 8.36 (*d*, *J* = 9.3, H–C(8')). ^{31}P-NMR ((D$_6$)DMSO): −5.50 (*t*, *J*(P,H–C(6)) = 7.1, after decoupling *s*). Anal. calc. for $C_{26}H_{26}Cl_2NO_5P$ (534.37): C 58.44, H 4.90, N 2.62; found: C 58.11, H 5.11, N 2.33.

3. Preparation of Mononucleotides. – 3.1. *Cytidine 5'-[Sodium (2-Methyl-1*H*-imidazol-1-yl)phosphonate]* (**5** · Na^+) [40]. Cytidine 5'-dihydrogen phosphate (465 mg, 1.44 mmol, dried by co-evaporation with DMF) and 2-methyl-1*H*-imidazole (1.0 g, 12.2 mmol) were dissolved under N_2 in dry, warm DMSO (10 ml). DMF (10 ml), Et_3N (0.5 ml, 3.59 mmol), and Ph_3P (0.7 g, 2.67 mmol) were added, and the mixture was gently heated until a clear soln. was obtained. After addition of 2,2'-bipyridine 1,1'-disulfide (0.8 g, 3.63 mmol), the yellow soln. was stirred for 2 h at r.t. and then poured into a stirred mixture of acetone (400 ml), Et_2O (250 ml), Et_3N (30 ml), and sat. $NaClO_4$ in acetone (2 ml). The precipitate was isolated by filtration, washed with acetone/Et_2O 1:1 and Et_2O, and dried *in vacuo*: 559 mg (95%) of **5** · Na^+. Colorless powder. M.p. > 230°. IR (KBr): 3366*s*, 3226*s*, 2931*m*, 1654*s*, 1609*m*, 1528*w*, 1492*m*, 1405*w*, 1260*m*, 1200*w*, 1102*s*, 1047*m*, 993*w*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz): 2.36 (*s*, Me); 3.63 (*m*, H–C(5')); 3.70 (*m*, H–C(5')); 3.83 (*m*, H–C(3'), H–C(4')); 3.88 (*m*, H–C(2')); 5.15, 5.31 (2 br. *s*, exchangeable with D_2O, OH); 5.68 (*d*, *J* = 7.4, 1 H, H–C(5)); 5.77 (*d*, *J* = 4.4, H–C(1')); 6.59 (*ψs*, 1 H (imidazole)); 7.01 (*ψs*, 1 H (imidazole)); 7.02 (br. *s*, exchangeable with D_2O, NH); 7.22 (br. *s*, exchangeable with D_2O, NH); 7.73 (*d*, *J* = 7.4, H–C(6)). ESI-MS: 410 (100, [**5** · Na^+ + H]$^+$).

3.2. *Guanosine 5'-[Sodium (2-Methyl-1*H*-imidazol-1-yl)phosphonate]* (**1** · Na^+) [40]. As described in *3.1*, with guanosine 5'-(dihydrogen phosphate) (× 14% H_2O; 500 mg, 1.18 mmol, dried by co-evaporation with DMF). Precipitation yielded 513 mg (93%) of **1**. Colorless powder. IR (KBr): 3355*s*, 3320*s*, 2938*m*, 1692*s*, 1602*m*, 1534*w*, 1483*w*, 1404*m*, 1260*m*, 1200*m*, 1101*s*, 1044*m*, 992*w*, 802*w*, 681*w*, 573*m*. ^{1}H-NMR ((D$_6$)DMSO, 270 MHz): 2.37 (*s*, Me); 3.62 (*m*, H–C(5')); 3.73 (*m*, H–C(5')); 3.87 (*m*, H–C(4')); 3.99 (*dd*, *J* = 4.7, 2.9, H–C(3')); 4.48 (*t*, *J* = 5.7, H–C(2')); 5.40 (br. *s*, exchangeable with D_2O, 2 OH); 5.66 (*d*, *J* = 6.4, H–C(1')); 6.59 (*t*, *J* = 1.2, 1 H (imidazole)); 6.76 (br. *s*, exchangeable with D_2O, NH_2); 7.06 (*t*, *J* = 1.2, 1 H (imidazole)); 7.87 (*s*, H–C(8)). ^{31}P-NMR ((D$_6$)DMSO): −8.63 (*t*, *J* = 6.2, after decoupling *s*). ESI-MS: 426.2 (M^-; calc. 426.3). Anal. calc. for $C_{14}H_{17}N_7NaO_7P \cdot 2\,H_2O$ (467.31): C 35.98, H 4.10, N 20.98; found: C 36.41, H 4.79, N 19.93.

4. Preparation of Oligonucleotides. – 4.1. *Guanosine-Functionalized Solid Support with 5'-*O*-(Dimethoxytrityl)-*N^2*-[(dimethylamino)methylidene]guanosine* (**22**) [41]. In addition to classical techniques [42a], the following method was used: Succinic anhydride (1.15 g, 11.5 mmol) and DMAP (28 mg, 0.23 mmol) were added to a

suspension of *Tentagel* [43] (*Rapp Polymere*) (1.00 g, *ca.* 0.23 mmol of RNH_2) in dry pyridine (10 ml). After shaking for 24 h, the derivatized resin was collected, washed with pyridine and CH_2Cl_2, and dried *in vacuo*. Then, dried **22** (177 mg, 0.277 mmol) was taken up in dry pyridine (10 ml), the modified *Tentagel* (400 mg, *ca.* 92 µmol of -COOH), EDC (265 mg, 1.38 mmol), Et_3N (0.1 ml), and DMAP (34 mg, 0.277 mmol) were added, and the mixture was shaken for 2 d. Nonreacted carboxylate groups were deactivated by addition of 4-nitrophenol (193 mg, 1.39 mmol), shaking for 5 h, and reaction with piperidine (2 ml) for 5 min. The resin was collected, washed (MeOH, Et_2O), dried *in vacuo*, and allowed to react with Ac_2O (2 ml) and DMAP (10 mg) in pyridine (6 ml) for 2.5 h. After collection, washing (MeOH, Et_2O) and drying *in vacuo*, the guanosine-functionalized *Tentagel* was obtained: 412 mg. The loading concentration of the monomer, as ascertained by the trityl cation release assay, was typically 75–110 µmol/g.

4.2. *Apparatus.* Syntheses were performed in a glass reaction vessel fitted with a ground glass joint and a stopper at the top, a sintered glass frit and a tap at the bottom. To achieve sufficient agitation of the reaction mixture, the apparatus was mounted on a shaker (*Vibramax*). Resin and reagents were filled in under N_2. After each step, wash and reagent solns. were removed by application of gentle vacuum. Dimethoxytrityl deprotection phases were collected for the determination of coupling yields.

4.3. *Assembly of Oligonucleotides* [25][42a]. *H*-Phosphonate building blocks **18**–**21** (see *Fig. 3*) were prepared according to published procedures [42][44–46]. Using manual solid-phase synthesis, the following steps were carried out: *1*) MeCN/pyridine 1:1 (2 × 5 ml) wash. *2*) CH_2Cl_2 (2 × 10 ml) wash. *3*) 5′-*O*-$(MeO)_2$Tr deprotection: $Cl_2CHCOOH$ in CH_2Cl_2 (2.5%, 5 × 4 ml). Eluates from this step were collected to determine the coupling yields. *4*) MeCN (2 × 5 ml) wash. *5*) MeCN/pyridine 1:1 (2 × 5 ml) wash. *6*) Coupling: 0.12M *H*-phosphonate in MeCN/pyridine 1:1 (5 equiv.) and pivaloyl chloride 0.2M in MeCN/pyridine 1:1 (15 equiv. in several portions, see below). *7*) MeCN/pyridine 1:1 (2 × 5 ml) wash. *8*) MeCN wash (2 × 5 ml). *9*) Repeat of steps *2*–*8* until the chain assembly is finished. *10*) Oxidation I: 0.10M I_2 in pyridine/1-methyl-1*H*-imidazole/H_2O/THF 5:1:5:90 (10 ml, 2.5 min). *11*) Oxidation II [25c]: 0.10M I_2 in Et_3N/H_2O/THF 5:5:90 (10 ml, 2.5 min). *12*) MeCN/pyridine 1:1 (6 × 5 ml) wash. *13*) MeCN (6 × 5 ml) wash. *14*) Cleavage from the support: conc. aq. NH_3 (2 × 10 ml, 2 × 60 min). The reaction solns. were collected into pressure-resistant glass ampoules. *15*) Deprotection: heating to 55° (16 h). *16*) Evaporation and purification.

4.4. *Acridine-d(GCAC)rG*[8] (**2**). Following the general method described in *4.3*, guanosine-functionalized *Tentagel* (692 mg, 60 µmol; loading 87 µmol/g) was reacted with the deoxynucleoside *H*-phosphonates **18**, **19**, and **20**, and the acridine *H*-phosphonate **15** (2.5 ml, 0.3 mmol, 0.12M in MeCN/pyridine 1:1, each) in the presence of pivaloyl chloride (3 × 1.5 ml, 0.3 mmol, 0.2M in MeCN/pyridine 1:1; added in 3 portions within 3 min to the suspension of *H*-phosphonate soln. and resin). After each addition the mixture was thouroughly shaken. After the final addition the reagents were allowed to react for further 2 min and then removed by filtration. Only for the coupling with **15**, the pivaloyl chloride soln. was added in two portions (2 × 2.25 ml, 2 × 1 min). Average condensation yields 93%, as determined by dimethoxytrityl assay. Deprotection, evaporation, purification (*RP-18* HPLC; MeCN (*A*) and 0.05M (Et_3NH)OAc pH 7 (*B*); 10–60% *A* in 30 min; flow rate 4 ml min^{-1}; detection at 280 nm), and desalting (50 g *Sephadex G 10*) afforded **2**. One aliquot was isolated as the triethylammonium salt (45 mg, 31%). A second aliquot, after ion-exchange filtration with *Chelex* (5 g, Na^+ form), gave additional 22 mg of the sodium salt (18%; 49% combined overall yield). Pale-yellow solid. Anal. HPLC (*RP-18*; MeCN/0.05M (Et_3NH)OAc 60:40 (*A*) and MeCN/0.05M (Et_3NH)OAc 10:90 (*B*); 5–36% *A* in 22 min; flow rate 1 ml min^{-1}): t_R 18.6 min. ^{1}H-NMR (D_2O, 400 MHz, 57°, Na^+ salt)[12]): 1.64–1.66 (*m*, 2 H–C(3) (hex), 2 H–C(4) (hex)); 1.94 (*m*, 2 H–C(2) (hex), 2 H–C(5) (hex)); 2.11 (*ddd*, ('*quint.*'), H–C(2′.c)); 2.38 (*ddd*, ('*quint.*'), 1 H–C(2′.b)); 2.63 (*ddd*, ('*dd*'), ^2J = 12.4, ^3J = 6.2, H–C(2′.c)); 2.73–2.88 (*m*, H–C(2′.b), 2 H–C(2′.d)); 2.91–2.98 (*m*, 2 H–C(2′.a)); 4.01–4.08 (*m*, 2 H–C(1) (hex) 2 H–C(6) (hex)); 4.20 (2*dd* ('*q*'), 2 H–C(5′)); 4.28–4.48 (*m*, H–C(2′.e), 7 H–C(5′), Me(acr)); 4.51 (*m*, H–C(4′.c)); 4.58 (*m*, H–C(4′.b)); 4.65 (*m*, H–C(5′.e)); 4.70 (*m*, H–C(4′.d)); 4.76–4.80 (*m*, overlapped, H–C(4′.a), H–C(4′.e)); 4.97 (*dd*, ('*t*'), *J* = 5.2, H–C(3′.e)); 5.05 (*m*, H–C(3′.c)); 5.12 (*m*, H–C(3′.b)); 5.24 (*m*, H–C(3′.a), H–C(3′.d)); 6.01 (*d*, *J* = 7.5, H–C(5.b)); 6.05 (*d*, *J* = 5.1, H–C(1′.e)); 6.12 (*d*, *J* = 7.5, H–C(5.d)); 6.24 (*dd*, *J* = 8.1, 6.1, H–C(1′.d)); 6.36 (*dd*, *J* = 8.0, 5.6, H–C(1′.c)); 6.39–6.45 (*m*, H–C(1′.b), H–C(1′a)); 6.86 (*d*, *J* = 2.4, H–C(1)(acr)); 7.29 (*dd*, ^3J = 9.2, ^4J = 1.8, H–C(7)(acr)); 7.35 (*dd*, ^3J = 9.4, ^4J = 2.5, H–C(3)(acr)); 7.79 (*d*, *s*), H–C(5)(acr)); 7.80 (*d*, overlapped by acr H–C(5), *J* = 9.2, acr H–C(4)); 7.84 (*d*, overlapped by H–C(8)(acr), *J* = 7.3, H–C(6)(Cyt)); 7.85 (*d*, overlapped by H–C(6)(Cyt), *J* = 10.6, H–C(8)(acr)); 7.91 (*d*, *J* = 7.5, H–C(6)(Cyt)); 8.17 (*s*, H–C(8)(purine)); 8.19 (*s*, 2 H, H–C(8)(purine)); 8.46 (*s*, 1 H, H–C(2)(purine)); the signals were assigned by ^{1}H,^{1}H-TOCSY. ^{31}P-NMR (D_2O,

[12]) The furanose and base moieties are labeled a,b,c *etc.*, starting from the 5′-end.

27°): −0.50, −0.42, −0.31 (3*s*, overlapped, 4 P); 1.03 (*s*, 1 P). ESI-MS: 953, 964, 975, 986, 997, 1008, 1019 (charge series −2 with alkali metal ions [anion + xH$^+$ + yNa$^+$ + zK$^+$]$^{2-}$; $x + y + z = 3$), 1294, 1301, 1308, 1316, 1323, 1330, 1338, 1345, 1352 ([2 × anion + xH$^+$ + yNa$^+$ + zK$^+$]$^{3-}$; $x + y + z = 7$).

4.5. *Acridine-d(CTAC)rG*[8]) (**23**). Following the synthesis cycle described in *4.3* guanosine-functionalized *Tentagel* (400 mg, 30 μmol, loading 75 μmol/g) was reacted with the deoxynucleoside *H*-phosphonates **18**, **20**, and **21**, and the acridine *H*-phosphonate **15** (2.2 ml, 0.15 mmol, 0.13M in MeCN/pyridine 1:1, each) in the presence of pivaloyl chloride (2 × 1.1 ml, 0.45 mmol, 0.2M in MeCN/pyridine 1:1; added in 2 portions within 1 min to the suspension of *H*-phosphonate soln. and resin). After each addition, the mixture was thouroughly shaken. After the final addition the reagents were allowed to react for further 2 min and then removed by filtration. Average condensation yields 96%, as determined by dimethoxytrityl assay. Deprotection, evaporation, purification, and desalting as described in *4.4* afforded **23** (13.4 mg, 22%, salt with Na$^+$, after ion-exchange filtration with *Chelex* (4 g, Na$^+$ form)). Yellow solid. Anal. HPLC (*RP-18*; MeCN/0.05M (Et_3NH)OAc 60:40 (*A*) and MeCN/0.05M (Et_3NH)OAc 10:90 (*B*); 5–36% A in 22 min; flow rate 1 ml min^{-1}): t_R 16.8 min. ^{1}H-NMR (D_2O, 400 MHz, 70°)[12]): 1.84–1.94 (*m*, 2 H–C(3) (hex), 2 H–C(4) (hex)); 2.10 (*quint.*, J = 6.2, 2 H–C(2) (hex)); 2.22 (*s*, Me(b)); 2.22–2.36 (*m*, overlapped, 2 H–C(5) (hex), H–C(2′.d)); 2.51–2.58 (*m*, 1 H–C(2′.a), 1 H–C(2′.b)); 2.65–2.71 (*m*, 1 H–C(2′)); 2.91–2.98 (*m*, 1 H–C(2′.a), 1 H–C(2′.b)); 3.06–3.11 (*m*, 2 H–C(2′.c)); 4.33 (*m*, 2 H–C(1) (hex)); 4.39–4.74 (*m*, overlapped, 20 H, all H–C(4′) and H–C(5′), 2 H–C(6) (hex), and Me(acr)); 4.89 (*dd*, (*t*′), J = 3.9, H–C(3′.e)); 5.08 (*m*, H–C(2′.e)); 5.18 (*m*, H–C(3′.d)); 5.25 (*m*, 2 H–C(3′)); 5.37 (*m*, H–C(3′.a) or H–C(3′.b)); 6.17 (*m*, H–C(1′.e)); 6.34 (2*d*, (*t*′)); H–C(5.a), H–C(5.d)); 6.43 (*dd*, (*t*′) = 8.0, H–C(1′.d)); 6.57–6.64 (*m*, 3 H, H–C(1′)); 7.66 (*s*, H–C(1)(acr)); 7.81 (*d*, J = 8.4, H–C(7)(acr)); 7.83 (2*s*, overlapped, H–C(6.b), H–C(8)(purine)); 7.87 (*d*, J = 9.7, H–C(3)(acr)); 8.14 (*d*, J = 7.1, H–C(6.a)); 8.19 (*d*, J = 8.0, H–C(6.d)); 8.30 (*d*, $J \approx 10$, H–C(4)(acr)); 8.31 (*s*, overlapped by H–C(4)(acr), H–C(5)(acr)); 8.37 (*s*, H–C(8)(purine)); 8.47 (*d*, J = 10.2, H–C(8)(acr)); 8.60 (*s*, H–C(2)(purine)); assignments by ^{1}H,^{1}H-COSY. ^{31}P-NMR (D_2O, 25°): −1.00, −0.63, −0.49, −0.41, 0.96 (5*s*, 5 P).

4.6. *Acridine-r(GCACG)*[8]) (**4**). Primer **4** was assembled on a *381A* DNA synthesizer (*Applied Biosystems*) using standard methods (1-μmol scale). Phosphoramidites of damG, BzA, BzC were prepared according to published procedures [27]. To avoid crystallization of (acridinyloxy)hexyl phosphoramidite *rac*-**14** during synthesis, a soln. of *rac*-**14** in MeCN (0.05M) was attached to port X of the synthesizer prior to the coupling step. After the synthesis, the capillary system of the apparatus was immediately washed with MeCN. The oligonucleotide was cleaved from the solid support by treating with a mixture of aq. NH_3 soln./EtOH 3:1. This soln. was heated at 55° for 12 h to remove the base and phosphate protecting groups. The crude product (70 *OD*) was purified in charges of 5 *OD* using HPLC (*RP-18*; MeCN/0.05M (Et_3NH)OAc, pH 6.5, 60:40 (*A*) and MeCN/0.05M (Et_3NH)OAc 10:90 (*B*); 30–90% *A* in 30 min; flow rate 1 ml min^{-1}). Desilylation was accomplished using $Et_3N \cdot 3$ HF for 12 h, followed by desalting on a *SepPak RP-18* (*Waters*) cartridge.

4.7. *Acridine-d(GCAC)rGrG*[8]) (**24**). As described in *4.6*.

4.8. *r(CCCCCGUGCG)*[8]) (**25**). As described in *4.6*. d(CCCCCGTGCG) (**3**), d(CCCCGCGTGCG) (**26**), d(GGGGCGTGCG) (**27**), d(GTCGACCCCCCGTGCG) (**28**), d(GTCGACCCCCCGTAGG) (**29**), and all other DNA oligomers were purchased from *MWG Biotech*.

4.9. *Oligomerization Experiments*. Into a 1.5-ml *Eppendorf* tube were pipetted the following 3 solns.: buffer, primer (**2**, **4** or **23**–**24**), and template (**3** or **25**–**29**). The mixture was heated to 90° for 1 min. After equilibration for 15 min at r.t., it was cooled to 10°. Finally, a freshly prepared aq. soln. of mononucleotides **1** or **5** was added, the soln. mixed (t = 0), and the tube sealed, and maintained at 10 ± 0.2°. The final conc. were: 30 μM primer, 100 μM template, 50 mM mononucleotide, 250 mM buffer (*Tris* · HCl, pH 7.7, Na$^+$ free or 1M in KCl, NaCl or LiCl; or HEPES, pH 7.7, 1M NaCl), 200 mM Mg^{2+}.

4.10. *HPLC Analysis of Oligomerization Experiments*. An aliquot from the reaction mixture (3 μl) was diluted with 8M aq. urea soln. (10 μl), heated to 90° for 1–2 min and then, after cooling, injected onto the column (*RP-18*; MeCN/0.05M (Et_3NH)OAc, pH 6.5, 60:40 (*A*) and MeCN/0.05M (Et_3NH)OAc 10:90 (*B*); linear gradient from 5–36% *A* in 22 min; flow rate 1 ml min^{-1}; UV detection at 260 nm; fluorescence: λ_{ex} 355 nm, λ_{em} 450 nm). The determination of primer and product concentrations from the integrated chromatograms is complicated by the fact that the extinction coefficients of the products increase with the chain length. Therefore, the integrals were multiplied by correction factors obtained by division of the primer coefficient (ε_P) by the corresponding ε_{P+n} of the extended products. To estimate ε_{260} of the primers (ε_P) and the elongation products (ε_{P+I}, ε_{P+II}, ε_{P+III}, and ε_{P+IV}), the sum of the corresponding extinction coefficients of the monomers was multiplied by the usual factor of 0.9 for single stranded DNA (acridine: 97400; G: 11300; C: 7400; A: 15300; T: 9000). **2** (Acr-d(GCAC)rG): ε_{260} 135000; **23** (Acr-d(CTAC)rG): ε_{260} 133000. To obtain the percentage of products formed, each corrected integral was divided by the sum of all corrected areas (primer + products). In some cases, minor signals were

observed as shoulders in the main peaks, which probably correspond to newly formed 2′ → 5′ phosphodiester isomers. These signals were integrated together with the main peak. Assuming a series of consecutive elongation steps, each governed by a first-order rate law, a numerical simulation of the polymerization process led to theoretical curves (concentration *vs.* time) that were fitted to the experimental data. Those variables producing the best fit were then considered as experimental rate constants. In the case of incomplete primer consumation (*e.g.*, *Exper. 1*), a further correction was introduced in the numerical simulations: the residual concentration of primer was subtracted from [primer] when applying *Eqns. 1* and *2*. However, for the display of the theoretical concentration curves this values was added to each calculated point of [primer].

4.11. *UV/VIS Spectroscopy*. Thermal melting profiles of oligomers were carried out by monitoring absorbance at 260 and 355 nm on a *Cary 1-Bio* UV/VIS spectrometer. A heating rate of 0.8°/min was maintained by a *Cary* temp. programmer. Absorbance and temperature were recorded every 0.3°. T_m Values could be deduced by numerical differentiation of the absorbance-temperature curves.

4.12. *CD Spectroscopy*. CD Spectra were recorded on a *Jasco J-715* spectropolarimeter using 1-cm rectangular quartz cells. A temp. of 5 ± 0.3° or 10 ± 0.3° was maintained by an external water bath. All CD data were base line corrected for signals due to the cell and the buffer.

4.13. *Alkaline Hydrolysis of Elongation Products*. A sample of the oligomerization mixture (15 µl) was incubated in 1M aq. NaOH (15 µl) at 60°. After 20 min, the reaction was stopped by addition of AcOH (10 µl). The soln. was desalted on a *Sephadex G 25* cartridge (*PD-10*, *Pharmacia*), lyophylized, and dissolved in H_2O (15 µl). An aliquot (3 µl) was then analyzed by HPLC.

4.14. *Ribonuclease T1 Digestion of Elongation Products*. An aliquot of the oligomerization mixture (10 µl) was lyophylized and dissolved in *Tris*/EDTA buffer (20 µl, *Tris* · HCl, 50 mM, pH 8.8; EDTA, 0.1 mM). T1 Ribonuclease (1 µl, 500 units) was added and the soln. was incubated on a water bath at 37°. At appropriate times (1, 2, 21 h), an aliquot of the soln. (6 µl) was withdrawn and mixed with 8M aq. urea (6 µl). The soln. obtained was then analyzed by HPLC.

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