62. A Method for Preparing Oligodeoxynucleotides Containing an Apurinic Site

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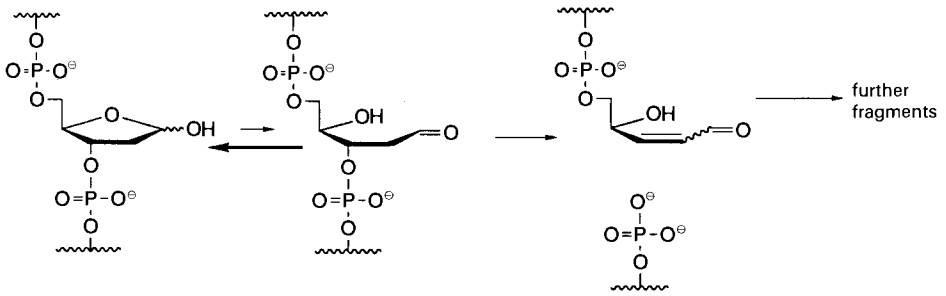
In three steps, 2-deoxy-D-ribose has been converted into a phosphoramidite building block bearing a (*t*-Bu)Me_2Si protecting group at the OH function of the anomeric centre of the furanose ring. This building block was subsequently incorporated into DNA oligomers of various base sequences using the standard phosphoramidite protocol for automated DNA synthesis. The resulting silyl-oligomers have been purified by HPLC and selectively desilylated to the corresponding free apurinic DNA sequences. The hexamer d(A-A-A-A-X-A) (X representing the apurinic site) which was prepared in this way was characterized by ^{1}H- and ^{31}P-NMR spectroscopy. The other sequences as well as their fragments, which formed upon treatment with alkali base, were analyzed by polyacrylamide gel electrophoresis.

1. Introduction. – An apyrimidinic or apurinic(AP) site in DNA arises from hydrolytic cleavage of the glycosylic bond between a nucleobase and the corresponding deoxyribose moiety. Such a process, which is referred to as depurination or depyrimidination, occurs spontaneously under physiological conditions at a rate of *ca.* 10^{-11} depurinations/nucleotide/second, whereby purine nucleotides hydrolyze faster by *ca.* 2 orders of magnitude over pyrimidine nucleotides [1]. Protonation of the nucleobases as well as modification with electrophiles, especially alkylating agents, greatly increase the lability of the glycosylic bond, thus facilitating the depurination process [1] [2]. AP sites also occur as intermediates during the enzymatic repair of damaged DNA by the action of DNA glycosylases [2] [3]. These enzymes recognize and catalyze the hydrolysis of chemically altered or damaged nucleobases. As a result of the lack of coding information during DNA replication, AP sites are thought to be mutagenic and have, therefore, recently been the subject of extensive research [1].

In this context, there is emerging a growing need for short oligodeoxynucleotides containing AP sites at preselected positions for biochemical, chemical, and biophysical experiments. An obstacle to the synthesis of short oligodeoxynucleotides containing AP sites represents the inherent lability of such DNA strands towards base. It is well established that the deoxyribose fragment in its open-chain aldehydic form readily undergoes a base-catalyzed β-elimination leading to scission of the phosphodiester backbone at the 3′-end of the AP site [4] [5] *(Scheme 1)*.

Several chemically stable models of AP sites mimicking both the cyclic as well as the open-chain form of the deoxyribose residue have, therefore, been synthesized and incorporated into oligodeoxynucleotides [6]. Although most of the structural aspects [6] [7] of such mimicks seem to be relevant to the natural system, they certainly do not reflect the chemical reactivity of apurinic DNA.

Scheme 1



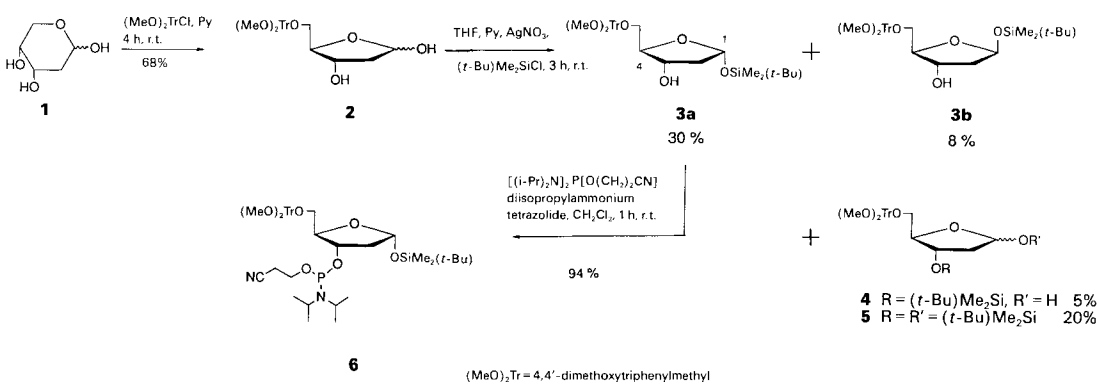
Only one way of selectively generating defined AP sites has been reported so far. The process involves the enzymatic hydrolysis of uracil with uracil-DNA glycosylase in synthetic oligodeoxynucleotides containing deoxyuridine [8]. The only hitherto known chemical synthesis of short apurinic DNA sequences was reported by *Vasseur et al.* [9]. This method, however, is limited to the synthesis of oligodeoxypyrimidines containing apurinic sites.

We present here a versatile *nonenzymatic* method of synthesizing oligodeoxynucleotides of any sequence with predefined AP sites by taking advantage of the solid-support phosphoramidite chemistry using a DNA synthesizer.

2. Results. – 2.1. *Synthesis of the AP Site Precursor.* Recent progress in the field of oligoribonucleotide chemistry has shown the (*tert*-butyl)dimethylsilyl ((*t*-Bu)Me_2Si) group to be a suitable protecting group for the 2′-OH function of the ribonucleosides [10]: it is almost inert under the chemical conditions used in the chain elongation and oxidation steps according to the standard phosphoramidite or H-phosphonate methodology. Furthermore, the (*t*-Bu)Me_2Si group is also stable under the conditions used in the deprotection of the nucleobases and the phosphates after the assembly of the oligomers [10b]. We, therefore, decided to use the (*t*-Bu)Me_2Si group to protect the hemiacetal OH group of 2-deoxy-D-ribose (**1**). The synthesis is outlined in *Scheme 2*.

Deoxy-D-ribose **1** has been converted regioselectively to the anomeric mixture of the 4,4′-dimethoxytriphenylmethyl ($(MeO)_2$Tr) ether **2** in analogy with the procedure of

Scheme 2



Bredereck et al. [11]. The inherent preference of the bulky $(MeO)_2Tr$ group for primary OH functions resulted in the selective formation of the deoxyribose skeleton in the desired furanose form. Silylation of the anomeric mixture **2** with $(t\text{-Bu})Me_2SiCl$ in the presence of $AgNO_3$ led to a mixture of mono- and bis-silylated products which could be separated by column chromatography. The main component (30%) was found to be the α-D-anomer **3a** of the desired 1-*O*-silylated furanose. The corresponding β-D-anomer **3b** was isolated in 8% yield together with 5% of the 3-*O*-silylated furanose **4** (as a mixture of C(1)-epimers) and 20% of the bis-silyl derivative **5** (as a mixture of C(1)-epimers).

The configuration of **3a** and **3b** was established unambiguously by ^{1}H-NMR spectroscopy: because of the absence of coupling between an exchangeable OH proton with the anomeric H–C(1) and the presence of coupling between H–C(3) and an exchangeable OH proton in both structures **3a** and **3b**, one has to assign the silyloxy group to the anomeric centre. The relative configuration at C(1) was determined by NOE difference spectroscopy (see *Exper. Part*), a method which already proved to be very useful in the determination of the anomeric configuration of *C*-furanosides [12]. The presence of an NOE between H–C(4) and H–C(1) and *vice versa* in the case of **3b** as well as the absence of the analogous effect in **3a** allows the assignment of the β-D- and α-D-configuration to **3b** and **3a**, respectively.

The regioselectivity of this silylation reaction as reflected by the mono-silyl compounds **3a**, **3b**, and **4** (7:1 in favor of 1-*O*-silylation) can most likely be attributed to the higher acidity of the hemiacetal OH group [13]. Efforts to reduce the amount of the bis-silylated derivatives **5** have been unsuccessful[1]). Transformation of **3a** to the diastereoisomeric mixture of the phosphoramidites **6** was accomplished by phosphinylation with (2-cyanoethoxy)bis(diisopropylamino)phosphine using diisopropylammonium tetrazolide as the activator.

The rather low yield (30%) of a pure representative of the desired 1-*O*-silyl compounds was compensated by the ease of access in gram quantities of the corresponding phosphoramidite building block **6**.

2.2. *Solid-Phase Synthesis of Oligomers Containing an Apurinic Site.* The phosphoramidite **6** can be used like each of the four natural deoxynucleoside cyanoethyl phosphor-

7 d (A-A-A-A-X^{Si}-A)
8 d (G-G-T-A-X^{Si}-A-C-C)
9 d (C-G-C-G-A-A-T-X^{Si}-C-G-C-G)
10 d (C-G-C-G-X^{Si}-G-C-G)

11 d (A-A-A-A-X-A)
12 d (G-G-T-A-X-A-C-C)
13 d (C-G-C-G-A-A-T-X-C-G-C-G)
14 d (C-G-C-G-X-G-C-G)

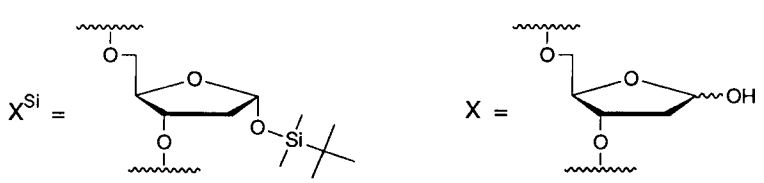


Fig. 1. *Synthesized oligomers containing an apurinic site*

[1]) In a later series of experiments, we found that the use of $(t\text{-Bu})Me_2Si(CF_3SO_3)$ as silylating agent gave more reproducible results.

amidites for the automated solid-phase synthesis of oligodeoxynucleotides. In this way, we have prepared the silyl-oligomers **7–10** and the corresponding apurinic DNA sequences **11–14** *(Fig. 1)*. With the exception of a slightly longer reaction time in the coupling steps, standard cycles could be used throughout the synthesis. The coupling efficiency for the AP building block **6** as well as that for the subsequent nucleosides in the growing chain, was generally higher than 95% as judged by photometric trityl assay. The assembly of each oligonucleotide was completed by removal of the 5′-protecting group followed by detachement of the oligonucleotide from the solid support as the last step in the synthesis cycle. Removal of the base- and phosphate-protecting groups was achieved in 24–36 h at room temperature using 25% aqueous ammonia/EtOH 3:1. The silyl-protected oligomers **7–10** were subsequently purified by reverse-phase and/or DEAE ion-exchange HPLC. *Fig. 2a* shows the HPLC profile of the crude reaction mixture of octamer **8** as an example.

Efficient removal of the silyl-protecting group was achieved by reacting the purified oligomers in phosphate buffer pH 2.0 at room temperature for 1 h as can be seen from the HPLC of crude octamer **12** *(Fig. 2b)*. In the case of hexamer **7**, the course of desilylation was monitored by reverse-phase HPLC over a period of 10 h *(Fig. 2c)*. The reaction was

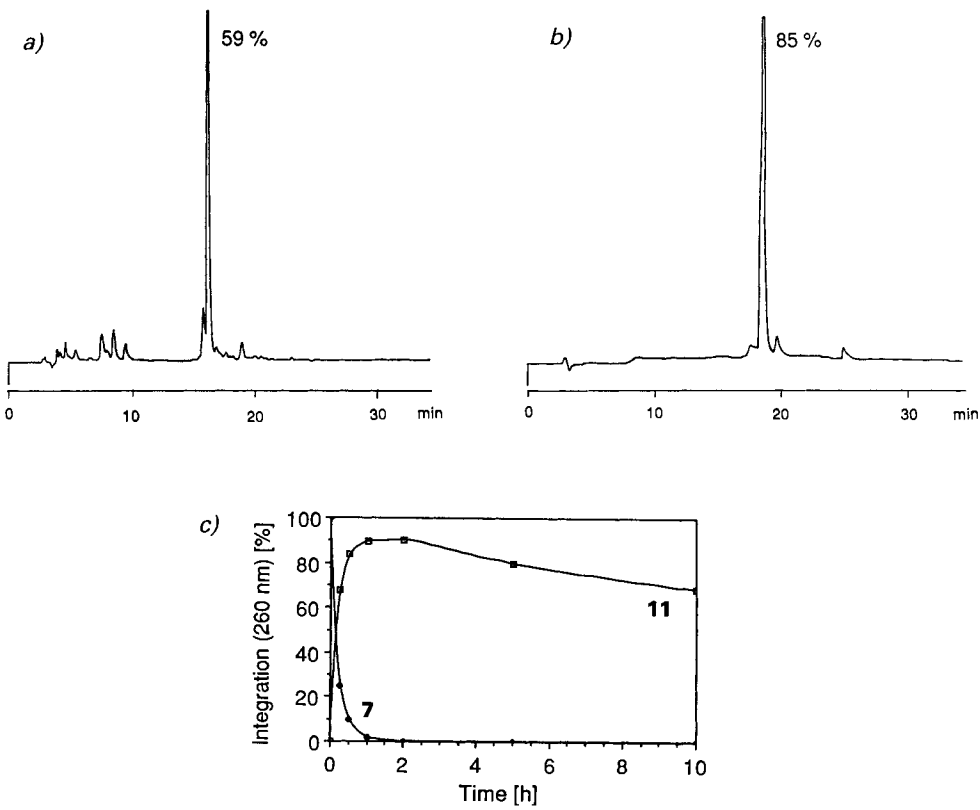


Fig. 2. *HPLC* (detection at 260 nm) *of* a) *crude silyl-octamer* **8** *and* b) *crude apurinic octamer* **12**. c) *Conversion of hexamer* **7** *to* **11** *at pH 2.0 as a function of time*

virtually complete after 1 h. Prolonged treatment under the applied acidic conditions resulted in increasing decomposition of the oligomer. While after 2 h still more than 90% could be attributed to the deprotected hexamer **7**, it decreased to 80% after 5 and to 68% after 10 h.

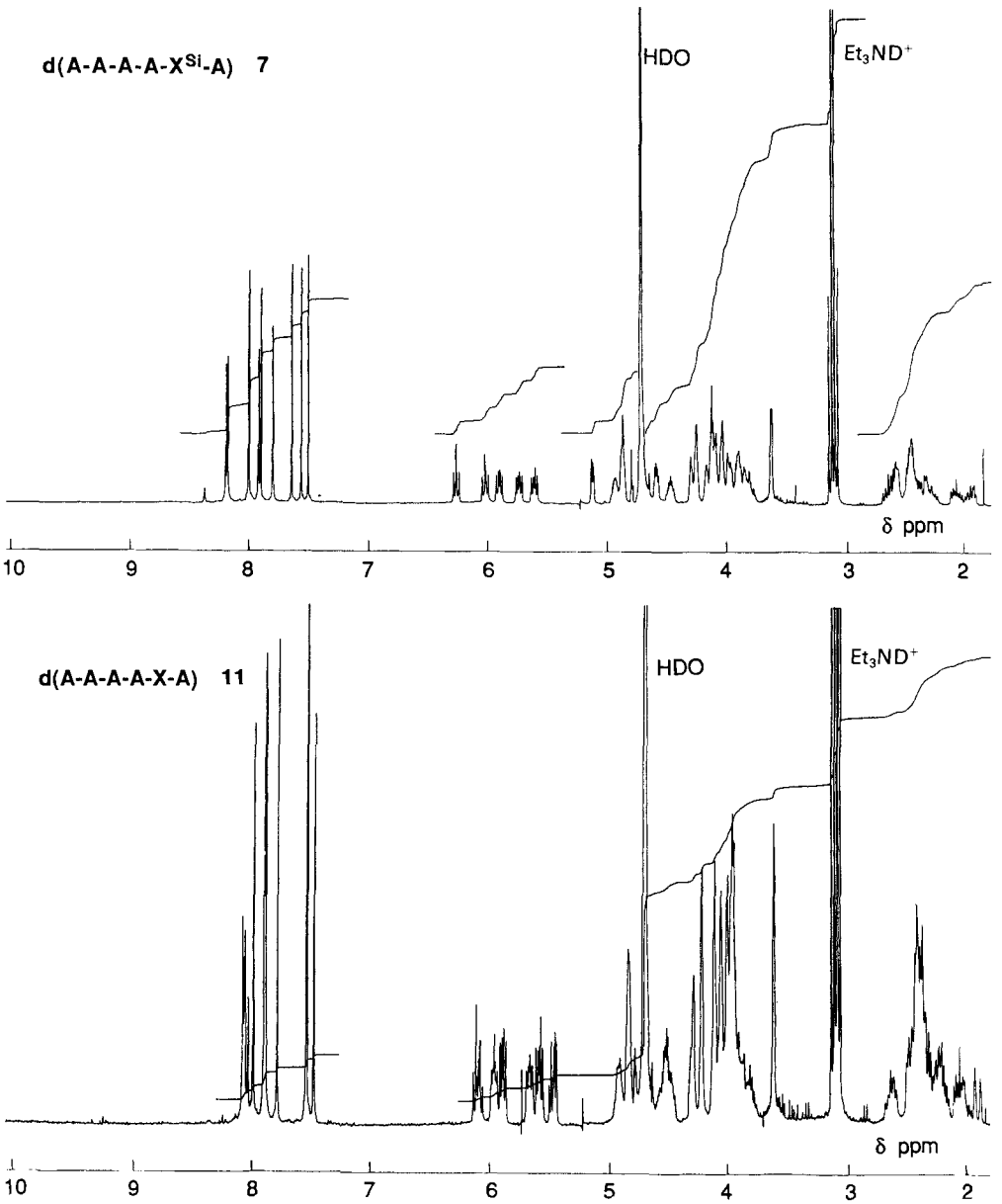


Fig. 3. *^{1}H-NMR spectra of hexamer* **7** (1 Et_3NH^+ 4 K^+ salt; c = 6.7 mM in D_2O) *and of* **11** (Et_3NH^+ salt; c = 3.5 mM in 20 mM potassium phosphate pH 6.0 in D_2O at 25°)

In attempts to deprotect the silyl-nucleotides with Bu_4NF according to standard procedures used in ribonucleotide chemistry [10], we always observed (by chromatography) a considerable amount of strand cleavage at the apurinic site. This is probably due to the F^- ion acting as a base, thus catalyzing the β-elimination depicted in *Scheme 1*. As expected, almost no acid-catalyzed hydrolysis of the glycosylic bond of the nucleotides occurred within the required time for the silyl deprotection as can be seen from the HPLC *(Fig. 2b)*.

We have prepared the apurinic hexamer **11** in milligram quantities in order to verify its structure by ^{1}H-NMR spectroscopy *(Fig. 3)*. The spectrum of the silyl-protected precursor **7** shows well-resolved resonances of H–C(1) of the silyl-deoxyribose residue (5.11 ppm) and of the five H–C(1′) of the adenosine nucleotides (5.6–6.2 ppm) as well as of the aromatic-base protons H–C(2) and H–C(8) (7.4–8.2 ppm). Upon deprotection of **7**, the spectrum becomes more complex, due to the presence of the two anomeric forms of **11**. The signal at 5.1 ppm is replaced by two new signals at 5.44 and 5.48 ppm attributable to the protons of the anomeric hemiacetal centre. Integration of these signals indicated a ratio of 3:2 for the two anomers of **11**. By comparison with the analogous signals of 2-deoxy-D-ribose 3,5-diphosphate [14], one can observe almost no difference in chemical shift, coupling constants, and relative proportion of the anomeric forms. This shows, that the short ordered oligo dA single strand on the 5′-side of the Ap residue in **11** does not affect the constitution and conformation of the deoxyribose residue. There is also no distinct signal for an aldehydic proton detectable, indicating that the open-chain form of the AP residue plays *structurally* a negligible role in single-stranded oligomers of deoxyadenosine. Both effects, the almost equal distribution of the two anomeric forms and the absence of an open-chain aldehydic form, have already been observed in double-stranded oligodeoxynucleotides containing an apurinic site [15].

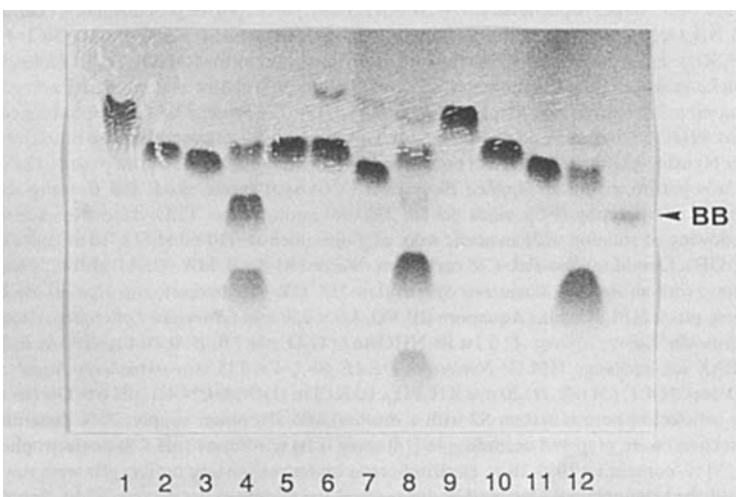


Fig. 4. *UV shadowing of a 20% denaturing polyacrylamide gel showing the selfcomplementary DNA sequences d(C-G-C-G-A-A-T-T-C-G-C-G), d(G-G-T-A-T-A-C-C), and d(C-G-C-G-C-G-C-G)* (lanes 1, 5, 9), *the corresponding purified silyl-oligomers* **9**, **8**, *and* **10** (lanes 2, 6, 10), *the crude apurinic oligomers* **13**, **12**, *and* **14** (lanes 3, 7, 11), *and the crude oligomers* **13**, **12**, *and* **14** *after treatment with 1* M *NaOH for 14 h at r.t.* (lanes 4, 8, 12)

The desilylation of the octamers **8** and **10** and the dodecamer **9** as well as the fragmentation of the resulting apurinic oligomers **12**, **14**, and **13**, respectively, has been monitored by 20% denaturing polyacrylamid gel electrophoresis *(Fig. 4)*. As can be judged from lanes 1–3, 5–7, and 9–11, the desilylation leads to pure products which have almost the same mobility as the corresponding native and silyl-protected oligomers. In order to effect strand cleavage, the apurinic sequences **13**, **12**, and **14** have been treated with 1M NaOH solution for 14 h at room temperature (lanes 4, 8, and 12). As expected, β-elimination occurred, leading to distinct oligomers of shorter size.

3. Conclusion. – We have shown that the easily accessible silylated phosphoramidite **6** can be used as a precursor for an apurinic site in the automated synthesis of oligodeoxynucleotides according to the phosphoramidite procedure. The silyl protecting group at the anomeric centre of **6** proved to be stable during the steps of chain elongation, oxidation, detachment from the solid support, and base deprotection. The silyl-oligomers could be purified by HPLC and were subsequently converted into the corresponding apurinic sequences in high yield by an easy protocol. This synthetic procedure has no restrictions concerning the base sequence of an oligomer and allows also the synthesis of apurinic DNA oligomers in large quantities, as required for biophysical experiments, without the use of any enzymes.

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Experimental Part

1. *General.* AcOEt, hexane: technical grade, distilled before use. CH_2Cl_2: technical grade, distilled over CaH_2. Et_2O: technical grade, distilled over NaH. MeCN, pyridine: *Fluka puriss. p.a.*, distilled over CaH_2. THF: *Fluka puriss. p.a.*, distilled over Na/benzophenone. EtOH, MeOH: *Fluka puriss. p.a.* Orthophosphoric acid: *Fluka puriss. p.a.* Crystallized NH_4OH: *Merck p.a.*, 25%. Sodium cacodylate: *Fluka purum p.a.* (*t*-Bu)Me_2SiCl: *Fluka purum.* (*t*-Bu)$Me_2Si(CF_3SO_3)$: *Fluka purum.* 4,4′-Dimethoxytriphenylmethyl chloride ($(MeO)_2TrCl$): *Fluka purum.* 2-Deoxy-D-ribose: *Fluka purum.* (2-Cyanoethoxy)bis(diisopropylamino)phosphine was prepared according to [16]. Diisopropylammonium tetrazolide was prepared according to [17]. Tetrazole: *Fluka purum*, sublimed before use. $AgNO_3$: *Siegfried*, PHHV, powdered and dried (r.t./0.01 Torr, over night). Unless otherwise stated, reactions were carried out under N_2 using glassware which had previously been dried for at least 2 h in an oven at 120°. Automated DNA synthesis was performed on an *Applied Biosystems* DNA synthesizer, mod. *380 B*, using standard ABI chemicals. Flash chromatography (FC): silica gel *60*, 230–400 mesh, *Fluka*. TLC: *Macherey-Nagel, SIL G-25, UV254*; UV shadowing or staining with an acidic soln. of *p*-anisaldehyde (10 ml H_2SO_4/10 ml anisaldehyde/2 ml AcOH/180 ml EtOH). Desalting: *Sep-Pak-C18* cartridges, *Waters*, Milford, MA (USA). HPLC: *Pharmacia LKB 2249* gradient pump with an *Applied Biosystems Spectroflow 757*, UV/VIS detector, and *Hewlett-Packard-3396-A* integrator. Reverse-phase HPLC: anal.: Aquapore RP 300, 4.6 × 220 mm *(Brownlee Labs)* prep.: *Aquapore octyl*, 10 × 250 mm *(Brownlee Labs)*; solvent *A*: 0.1M Et_3NHOAc in H_2O, pH 7.0; *B*: 0.1M Et_3NHOAc in MeCN/H_2O 4:1, pH 7.0. DEAE ion-exchange HPLC: *Nucleogen DEAE 60-7*, 4 × 125 mm *(Macherey-Nagel)*; *C*: 20 mM KH_2PO_4, in H_2O/MeCN 4:1, pH 6.0; *D*: 20 mM KH_2PO_4, 1M KCl in H_2O/MeCN 4:1, pH 6.0. Gel electrophoresis: *BRL* sequencing gel electrophoresis system *S2* with a *BioRad 3000/300* power supply; 20% denaturing gels (7M urea, 0,8 mm thickness) were prepared according to [18] using 0.1M tris-borate (pH 8.3) as electrophoresis buffer and formamide (90%, containing 10% 10 × electrophoresis buffer) as loading buffer; gels were run at constant power (30 W) until the bromophenol blue marker dye reached the middle of the gel (*ca.* 2.5 h). Specific rotation: *Perkin-Elmer-241* polarimeter, d = 10 cm (c = g/100 ml). IR: selected bands in cm^{-1}. ^{1}H-NMR: 300 MHz; δ in ppm *vs.* TMS (for D_2O, δ *vs.* HDO = 4.70 ppm), J in Hz. Difference NOE: δ of irradiated H→δ of H's with NOE. ^{13}C-NMR: 75 MHz; δ in ppm *vs.* TMS, assignments based on DEPT spectra. ^{31}P-NMR: 121 MHz; δ in ppm *vs.* 85% H_3PO_4 as external standard. EI-MS: selected peaks; m/z (%).

2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-D-ribofuranose (**2**). To a stirred soln. of 2-deoxy-D-ribose (**1**; 2.5 g, 18.8 mmol) in pyridine (75 ml) were added, at r.t., 4,4'-dimethoxytriphenylmethyl chloride (8.21 g, 24.2 mmol). After 4 h, the mixture was cooled to 0° and quenched with H_2O (100 ml). The resulting soln. was extracted with CH_2Cl_2 (1 × 300 ml and 2 × 200 ml) and the combined org. extract filtered through cotton and evaporated. FC (4 × 40-cm column, CH_2Cl_2/MeOH 50:1, then CH_2Cl_2/MeOH 15:1) of the crude material yielded 5.65 g (68%) of **2**, 7:3 mixture of anomers. Slightly orange foam. TLC ($CHCl_3$/MeOH 15:1): R_f 0.58. IR ($CHCl_3$): 3590*w* (br.), 3530*w* (br.), 3400*w* (br.), 3050*w*, 3030*w*, 3005*m*, 2960*w*, 2940*w*, 2910*w*, 2870*w*, 1735*w*, 1730*w*, 1720*w*, 1610*m*, 1580*w*, 1575*w*, 1510*s*, 1465*m*, 1445*m*, 1440*m*, 1415*w*, 1375*w*, 1305*m*, 1300*m*, 1250*s*, 1245*s*, 1180*s*, 1155*w*, 1135*w*, 1115*w*, 1080*m*, 1040*m*, 955*w*, 915*w*, 905*w*, 860*w*, 830*m*, 705*m*, 660*w*, 640*w*. ^{1}H-NMR ($CDCl_3$): 1.95 (*d*, *J* = 4.5, 0.3 H, OH–C(3)); 2.04 (*d*, *J* = 13.4, 0.7 H, H–C(2)); 2.11 (*ddd*, *J* = 2.1, 6.1, 13.6, 0.3 H, H–C(2)); 2.19 (*ddd*, *J* = 4.5, 5.7, 13.4, 0.7 H; H–C(2)); 2.27 (*ddd*, *J* = 5.4, 6.1, 13.6, 0.3 H, H–C(2)); 2.74 (*d*, *J* = 7.8, 0.7 H, OH–C(3)); 3.08 (*dd*, *J* = 5.2, 10.0, 0.7 H, H–C(5)); 3.13 (*dd*, *J* = 4.5, 10.0, 0.7 H, H–C(5)); 3.22 (*d*, *J* = 6.0, 0.3 H, OH–C(1)); 3.28 (*dd*, *J* = 5.4, 9.7, 0.3 H, H–C(5)); 3.37 (*dd*, *J* = 4.2, 9.7, 0.3 H, H–C(5)); 3.38 (*d*, *J* = 4.5, 0.7 H, OH–C(1)); 3.77 (*s*, 6 H, MeO); 3.99 (br. *q*, 0.3 H, H–C(4)); 4.27 (br. *t*, 0.7 H, H–C(3)); 4.35 (*dt*, *J* = 1.0, 4.5, 0.7 H, H–C(4)); 4.52 (*m*, 0.3 H, H–C(3)); 5.55 (br. *dt*, 0.3 H, H–C(1)); 5.61 (br. *t*, 0.7 H, H–C(1)); 6.79–7.44 (*m*, 13 arom. H). ^{13}C-NMR ($CDCl_3$): 41.44, 43.02 (2 *t*, C(2)); 55.21 (*q*, MeO); 64.09, 64.50 (2 *t*, C(5)); 73.22, 73.74 (2 *d*, C(3)); 85.10, 86.84 (2 *d*, C(4)); 86.20 (*s*, *C*–O–C(5)); 98.90, 99.51 (*d* C(1)); 113.15, 113.23, 126.80, 126.96, 127.83, 127.93, 128.16, 130.03 (8*d*, arom C); 135.73, 135.92, 135.99, 144.76, 158.52, 158.61 (6*s*, arom. C). EI-MS: 436 (9, M^+), 303 (100), 243 (13), 213 (10), 151 (10), 135 (17), 105 (11), 75 (9).

Silylation of **2** *with (*tert*-Butyl)chlorodimethylsilane.* To a stirred soln. of 2-deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-D-ribofuranose (**2**; 100 mg, 0.23 mmol) in THF (1 ml) was added at r.t. pyridine (70 µl, 0.87 mmol), $AgNO_3$ (47 mg, 0.28 mmol), and (*t*-Bu)Me_2SiCl (49 mg, 0.33 mmol). After stirring the resulting white suspension for 3 h, the reaction was quenched by addition of CH_2Cl_2 (10 ml) and H_2O (10 ml). The two phases were separated and the aq. layer extracted with CH_2Cl_2 (2 × 10 ml). The combined org. phases were washed with sat. NaCl soln., filtered and evaporated. The crude mixture was separated by FC (1-cm column, 10 g of silica gel) with hexane/Et_2O 2:1. Fractions showing a single spot by TLC were combined and evaporated: 30 mg (20%) of *1,3-bis-*O*-[(*tert*-butyl)dimethylsilyl]-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-α/β-*D*-ribofuranose* (**5**; 1:1 mixture of anomers by ^{1}H-NMR), 38 mg (30%) of *1-*O*-[(*tert*-butyl)dimethylsilyl]-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-α-*D*-ribofuranose* (**3a**), 10 mg (8%) of *1-*O*-[(*tert*-butyl)dimethylsilyl]-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-β-*D*-ribofuranose* (**3b**), and 7 mg (5%) of *3-*O*-[(*tert*-butyl)dimethylsilyl]-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-α/β-*D*-ribofuranose* (**4**; 3:7 mixture of anomers by ^{1}H-NMR), all as clear colorless oils.

Data for **3a**: TLC (hexane/Et_2O 2:1): R_f 0.38. $[\alpha]_D$ = +43.6 (*c* = 1.03, $CHCl_3$). IR ($CHCl_3$): 3530*w*, 3025*m*, 3015*m*, 3010*m*, 2950*m*, 2930*m*, 2860*m*, 2840*m*, 1610*m*, 1580*w*, 1510*s*, 1460*m*, 1445*m*, 1440*m*, 1410*w*, 1360*w*, 1250*s*, 1175*s*, 1110*m*, 1070*s*, 1035*m*, 985*m*, 955*m*, 935*w*, 835*m*, 830*m*. ^{1}H-NMR ($CDCl_3$): 0,14, 0.15 (2*s*, Me_2Si); 0.89 (*s*, *t*-BuSi); 1.99 (*d*, *J* = 13.2, 1 H–C(2)); 2.23 (*ddd*, *J* = 4.2, 5.6, 13.2, 1 H–C(2)); 3.05 (*dd*, *J* = 5.5, 9.7, 1 H–C(5)); 3.13 (*d*, *J* = 11.4, OH–C(3)); 3.14 (*dd*, *J* = 5.5, 9.7, 1 H–C(5)); 3.78 (*s*, 2 MeO); 4.22 (*dd*, *J* = 5.6, 11.4, H–C(3)); 4.32 (*t*, *J* = 5.5, H–C(4)); 5.61 (*d*, *J* = 4.2, H–C(1)); 6.82–7.41 (*m*, 13 arom. H). Difference NOE: 4.32 (H–C(4)) → 3.14 (H–C(5)), 6.82–7.41 (arom. H); 5.61 (H–C(1))→0.14, 0.15 (Me_2Si), 2.23 (H–C(2)), 3.05 (H–C(5)), 6.82–7.41 (arom. H). ^{13}C-NMR ($CDCl_3$): −5.34, −4.40 (2*q*, Me_2Si); 17.84 (*s*, $(CH_3)_3CSi$); 25.70 (*q*, $(CH_3)_3$CSi); 42.77 (*t*, C(2)); 55.21 (*q*, MeO); 64.12 (*t*, C(5)); 73.97 (*d*, C(3)); 86.07 (*s*, *C*–O–C(5)); 87.00 (*d*, C(4)); 99.64 (*d*, C(1)); 113.10, 126.74, 127.79, 128.19, 130.10 (5*d*, arom. C); 136.03, 136.12, 144.89, 158.46 (4*s*, arom. C). EI-MS: 550 (0.5, M^+), 303 (100), 135 (8), 131 (3), 115 (2), 105 (4), 101 (3), 75 (16), 73 (16), 59 (4), 57 (2).

Data for **3b**: TLC (hexane/Et_2O 2:1): R_f 0.12. $[\alpha]_D$ = −37.8 (*c* = 1.09, $CHCl_3$). IR ($CHCl_3$): 3590*w* (br.), 3080*w*, 3060*w*, 3030*w*, 3005*m*, 2960*m*, 2935*m*, 2905*m*, 2890*m*, 2860*m*, 2840*m*, 1610*m*, 1580*w*, 1570*w*, 1510*s*, 1470*m*, 1465*m*, 1455*m*, 1440*m*, 1410*m*, 1390*w*, 1360*w*, 1300*m*, 1255*s*, 1250*s*, 1180*s*, 1175*s*, 1155*m*, 1120*m*, 1090*m*, 1035*s*, 1005*m*, 990*w*, 960*m*, 945*m*, 940*m*, 915*w*, 905*w*, 835*s*, 815*w*, 810*w*, 805*w*, 705*w*, 660*w*, 640*w*, 620*w*. ^{1}H-NMR ($CDCl_3$): −0.06, 0.02 (2*s*, Me_2Si); 0.78 (*s*, *t*-BuSi); 1.92 (*d*, *J* = 3.6, OH–C(3)); 2.01 (*ddd*, *J* = 4.8, 6.6, 12.8, H–C(2)); 2.11 (*ddd*, *J* = 2.3, 6.6, 12.8, 1 H–C(2)); 3.08 (*dd*, *J* = 8.1, 9.1, 1 H–C(5)); 3.87 (*dd*, *J* = 4.9, 9.1, 1 H–C(5)); 3.78 (*s*, 2 MeO); 3.94 (*dt*, *J* = 4.9, 8.1, H–C(4)); 4.42 (br. *quint.*, H–C(3)); 5.49 (*dd*, *J* = 2.3, 4.8, H–C(1)); 6.80–7.45 (*m*, 13 arom. H). Difference NOE: (3.94 (H–C(4)) → 1.92 (OH–C(3)), 2.01 (H–C(2)); 3.08 (H–C(5)); 3.37 (H–C(5)), 4.42 (H–C(3)); 5.49 (H–C(1)); 6.80–7.45 (arom. H); 5.49 (H–C(1)) → −0.06, 0.02, 0.78 ((*t*-Bu)Me_2Si), 2.01 (H–C(2)); 3.94 (H–C(4)); 6.80–7.45 (arom. H). ^{13}C-NMR ($CDCl_3$): −5.43, −4.24, (2*q*, Me_2Si); 17.74 (*s*, $(CH_3)_3CSi$); 25.64 (*q*, $(CH_3)_3$CSi); 43.44 (*t*, C(2)); 55.19 (*q*, MeO); 65.65 (*t*, C(5)); 74.26 (*d*, C(3)); 84.06 (*d*, C(4)); 86.29 (*s*, *C*–O–C(5)); 98.64 (*d*, C(1)); 113.17, 126.78, 127.84, 128.12, 130.03 (5*d*, arom. C); 135.99, 136.18, 144.89, 158.52 (4*s*, arom. C). EI-MS: 550 (3, M^+), 303 (100), 243 (13), 173 (11), 135 (17), 117 (18), 106 (16), 101 (11), 81 (12), 80 (17), 74 (29), 72 (27).

Data for **4**: TLC (hexane/Et_2O 2:1): R_f 0.18. ^{1}H-NMR ($CDCl_3$): −0.63, −0.01, 0.01, 0.07, 0.09 (4*s*, 6 H, Me_2Si); 0.81 (*s*, 2.7 H, *t*-BuSi); 0.88 (*s*, 6.3 H, *t*-BuSi); 1.89–2.23 (*m*, 2 H, H–C(2)); 2.90–3.37 (*m*, 2 H, H–C(5)); 3.03 (*d*, *J* = 6.1, 0.3 H, OH–C(1)); 3.79 (*s*, 6 H, MeO); 3.98 (*d*, *J* = 12.0, 0.7 H, OH–C(1)); 3.98, 4.31, 4.52 (*m*, 2 H, H–C(3), H–C(4)); 5.42 (*dd*, *J* = 5.0, 12.0, 0.7 H, H–C(1)); 5.56 (*m*, 0.3 H, H–C(1)); 6.79–7.45 (*m*, 13 arom. H).

Data for **5**: TLC (hexane/Et_2O 2:1): R_f 0.70. ^{1}H-NMR ($CDCl_3$): −0.12, −0.05, −0.03, 0.01, 0.02, 0.08, 0.16, 0.18 (8*s*, 12 H, 2 Me_2Si); 0.79, 0.83, 0.84, 0.94 (4*s*, 18 H, 2 *t*-BuSi); 1.83 (*ddd*, *J* = 2.9, 5.3, 13.0, 0.5 H, H–C(2)); 1.99 (*dd*, *J* = 4.0, 5.8, 1 H, H–C(2)); 2.34 (*ddd*, *J* = 5.4, 7.3, 13.0, 0.5 H, H–C(2)); 3.01 (*dd*, *J* = 4.7, 10.0, 0.5 H, H–C(5)); 3.13 (*d*, *J* = 5.3, 1 H, H–C(5)); 3.14 (*d*, *J* = 5.3, 0.5 H, H–C(5)); 3.20 (*dd*, *J* = 4.7, 10.0, 0.5 H, H–C(5)); 3.79 (*s*, 6 H, MeO); 3.95 (*ddd*, *J* = 4.3, 4.7, 5.3, 0.5 H, H–C(4)); 4.10 (*m*, 1 H, H–C(3), H–C(4)); 4.37 (*dt*, *J* = 5.8, 4.4, 0.5 H, H–C(3)); 5.55 (*t*, *J* = 4.0, 0.5 H, H–C(1)); 5.56 (*dd*, *J* = 5.4, 2.9, 0.5 H, H–C(1)); 6.82–7.50 (*m*, 13 arom. H).

Silylation of **2** *with (*tert*-Butyl)dimethylsilyl Trifluoromethanesulfonate.* To a soln. of **2** (836 mg, 1.9 mmol) in pyridine (8 ml) was added (*t*-Bu)$Me_2Si(CF_3CO_3)$ (484 µl, 2.1 mmol) at 0° with a syringe. After stirring for 1 h at 0°, a mixture analogous to that reported above had formed as checked by TLC (hexane/Et_2O 2:1). Workup and FC of the crude material under the conditions reported above afforded 320 mg (30%) of **3a** and 98 mg (9%) of **3b**.

*1-O-[(*tert*-Butyl)dimethylsilyl]-3-O-[(2-cyanoethoxy)*(N,N-*diisopropylamino)phosphino]-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)*-α-D-*ribofuranose* (**6**). A soln. of **3a** (825 mg, 1.49 mmol) in CH_2Cl_2 (5 ml) was evaporated and the residue subsequently dried at 0.05 Torr for 2 h. Under Ar, 127 mg (0.74 mmol) of diisopropylammonium tetrazolide was added and the flask evacuated (0.05 Torr) for another 2 h. The residue was then suspended in dry CH_2Cl_2, and (2-cyanoethoxy)bis(diisopropylamino)phosphine (809 mg, 2.68 mmol) was added dropwise within 2 min with a syringe at r.t. After stirring for 1 h, the mixture was diluted with AcOEt (100 ml) and extracted with sat. $NaHCO_3$ soln. (100 ml) at 0°. The org. layer was washed with sat. NaCl soln. (100 ml) and evaporated. The crude material was purified by FC (3 cm column, 90 g of silica gel) with hexane/Et_2O 2:1 containing 2% of Et_3N. The product-containing fractions were combined and evaporated. After drying at 0.05 Torr overnight, 1.00 g (94%) of **6** (1:1 mixture of diastereoisomers) was isolated as a clear colorless oil. TLC (hexane/Et_2O 2:1): R_f 0.35. ^{1}H-NMR ($CDCl_3$): 0.14, 0.15, 0.16, 0.17 (4*s*, 6 H, Me_2Si); 0.97 (*d*, *J* = 7.0, 3 H, $(CH_3)_2CH$); 1.12 (*d*, *J* = 7.0, 6 H, $(CH_3)_2CH$); 1.14 (*d*, *J* = 7.0, $(CH_3)_2CH$); 2.00 (*m*, 1 H, H–C(2)); 2.37 (*dt*, *J* = 3.7, 6.5, 1 H, CH_2CN); 2.57 (*dt*, *J* = 2.3, 6.4, 1 H, CH_2CN); 3.06 (*dd*, *J* = 4.3, 9.8, 0.5 H, H–C(5)); 3.08 (*dd*, *J* = 4.6, 9.8, 0.5 H, H–C(5)); 3.22 (*dd*, *J* = 3.1, 10.0, 0.5 H, H–C(5)); 3.27 (*dd*, *J* = 3.2, 10.0, 0.5 H, H–C(5)); 3.42–3.67 (*m*, 4 H, $(CH_3)_2CH$, CH_2CH_2CN); 3.78, 3.79 (*s*, 6 H, MeO); 4.20–4.38 (*m*, 2 H, H–C(3), H–C(4)); 5.61 (*m*, 1 H, H–C(1)); 6.80–7.45 (*m*, 13 arom. H). ^{31}P-NMR ($CDCl_3$): 148.4, 149.1.

Automated Solid-Phase Synthesis of Oligomers **7–10**. The oligonucleotide syntheses were accomplished on 1-µmol scales using 0.1M solns. of the standard cyanoethyl phosphoramidites of [$(MeO)_2$Tr]bz^{6}A$_d$, [$(MeO)_2$Tr]ib^{2}G$_d$, [$(MeO)_2$Tr]bz^{4}C$_d$, [$(MeO)_2$Tr]T$_d$, and 0.2M **6** in MeCN. The syntheses were carried out following the standard-cycle protocol of *Applied Biosystems* but with the coupling time extended to 3 min. The ending procedure included the removal of the last $(MeO)_2$Tr group and the detachement from the solid support with 25% aq. NH_3 soln. The resulting solns. (*ca.* 1 ml) were diluted with 1 ml of EtOH and 2 ml of 25% aq. NH_3 soln. and left for 24–36 h at r.t.

Purification of the Silylated Oligomers. The oligomers **8–10** were purified by prep. reverse-phase HPLC using a linear gradient of 10–30% *B* within 30 min in the case of **9** and **10** and 10–40% *B* within 30 min in the case of **8**. The oligomer **7** was purified by ion-exchange HPLC using a linear gradient of 0–40% *D* within 30 min. All silylated oligomers were desalted by filtration over *Sep-Pak-C18* cartridges (the buffer salts were eluted first with H_2O, while the oligomers were eluted with H_2O/MeOH 7:3) and lyophilyzed. For the ^{1}H-NMR of **7**, purified material from six 1-µmol runs and that from six anal. control injections (reverse-phase HPLC, 0–40% *B* in 30 min) were combined, desalted, and lyophilized: 7 mg of **7** (1 Et_3NH^+ 4 K^+ salt). ^{1}H-NMR (D_2O)/Et_3N: −0.42, −0.41 (2*s*, Me_2Si); 0.36 (*s*, *t*-BuSi); 1.18 (*t*, *J* = 7.4, $(CH_3CH_2)_3NH^+$); 1.64–2.68 (*m*, H–C(2′)); 3.10 (*q*, *J* = 7.4, $(CH_3CH_2)_3NH^+$); 3.62 (*d*, *J* = 3.1, H–C(5′) of A^1); 3.75–4.94 (*m*, H–C(3′), H–C(4′), H–C(5′) of A$^{1-4}$, A^6, X^{Si}); 5.11 (*dd*, *J* = 1.7, 5.1, H–C(1′) of X^{Si}); 5.60 (*dd*, *J* = 5.4, 9.5), 5.72 (*dd*, *J* = 5.4, 9.6), 5.89 (*dd*, *J* = 5.5, 9.3), 6.01 (*t*, *J* = 7.1), 6.25 (*t*, *J* = 6.9, 5 H–C(1′) of A$^{1-4}$, A^6); 7.49, 7.55, 7.63, 7.79, 7.89, 7.91, 7.98, 7.99, 8.16, 8.18 (10*s*, H–C(2), H–C(8) of A$^{1-4}$, A^6).

Desilylation to Apurinic Oligonucleotides **12–14**. The purified and lyophilyzed oligonucleotides **8–10** (1.2 OD_{260} units) were dissolved in potassium-phosphate buffer (0.4 ml, 10 mM, pH 2.0). After 1 h, the reaction was quenched by addition of potassium-phosphate buffer (1 ml, 0.1M, pH 7.0) and the resulting soln. desalted over a *Sep-Pak-C18* cartridge (elution of inorg. salt with H_2O and of oligomer with H_2O/MeOH 7:3). The purity of the resulting apurinic oligomers **12–14** was judged by integration in the HPLC (anal. reverse phase, 0–30% *B* within 30 min 260 nm) and was 85% for **12**, 95% for **13**, and 82% for **14**.

Desilylation of **7**. The purified oligomer **7** (7 mg) from above was dissolved in potassium-phosphate buffer (10 ml; 10 mM, pH 2.0). After 1 h, hexamer **11** had formed in 94% yield (HPLC). The reaction was subsequently quenched by addition of potassium-phosphate buffer (10 ml; 0.1M, pH 7.0) and the resulting soln. purified by prep. reverse-phase HPLC (0–40% *B* within 30 min). After desalting over *Sep-Pak-C18* as described above and lyophilization, 3.8 mg of pure **11** was obtained in its Et_3NH^+ form. ^{1}H-NMR (D_2O, 20 mM potassium phosphate (pH 6.0)): 1.17 (*t*, *J* = 7.3, $(CH_3CH_2)_3NH^+$); 1.86–2.68 (*m*, H–C(2′)); 3.10 (*q*, *J* = 7.3, $(CH_3CH_2)_3NH$); 3.61 (*d*, *J* = 2.9, H–C(5′) of A^1); 3.75–4.95 (*m*, H–C(3′), H–C(4′), H–C(5′) of A^{2-4}, A^6, X); 5.44 (*dd*, *J* = 1.5, 5.5, H–C(1′) of X); 5.48 (*t*, *J* = 4.9, H–C(1′) of X); 5.57 (*dd*, *J* = 5.7, 9.3), 5.66 (*dd*, *J* = 5.5, 9.5), 5.88 (*dd*, *J* = 5.6, 9.4), 5.94 (*dd*, *J* = 3.0, 5.7), 5.97 (*dd*, *J* = 2.6, 5.6), 6.07 (*t*, *J* = 6.6), 6.11 (*t*, *J* = 6.6, 5 H–C(1′) of A^{1-4}, A^6); 7.46, 7.47, 7.52, 7.53, 7.54, 7.78, 7.87, 7.88, 7.89, 7.90, 8.03, 8.06, 8.07, 8.08 (14 *s*, H–C(2), H–C(8) of A^{1-4}, A^6). ^{31}P-NMR (D_2O, 20 mM sodium cacodylate (pH 6.0)): –0.603 (2P), 0.061 (3P).

Cleavage Reactions (see *Fig. 4*). The crude apurinic oligomers **12** (0.4 μmol), **13** (0.15 μmol), and **14** (0.4 μmol), prepared as described above, were each dissolved in 5 μl of 1M NaOH and left at r.t. for 14 h. The probes were loaded onto the gel after dilution with 10 μl of formamide.

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