166. Solid-Phase Synthesis of Oligodeoxynucleotides Containing Phosphoramidate Internucleotide Linkages and their Specific Chemical Cleavage

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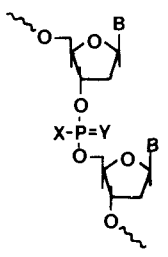
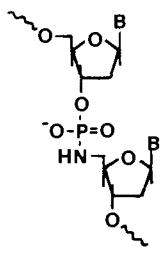
Oligonucleotides bearing phosphoramidate internucleotide linkages can be prepared chemically by standard solid-phase DNA synthesis. Thus, phosphoramidate internucleotide bonds can be placed at will into specific positions within a given DNA fragment. The backbone-modified DNA fragments prepared in this way are susceptible to a specific chemical cleavage.

1. Introduction. – Currently, there is a great interest in the chemical synthesis of oligonucleotides bearing modifications at the phosphodiester internucleotide linkages. These changes in the backbone of DNA fragments often lead to an increased stability towards enzymatic hydrolysis by nucleases and a greater overall lipophilicity without influencing too much the hybridisation conditions to a complementary single-stranded DNA or RNA sequence. Therefore, such backbone-modified DNA fragments seem to be ideal tools for being applied in the control of gene expression on the mRNA level known as anti-sense approach [1]. This could finally lead to agents capable of stopping the growth of viruses or malignant cells [2].

The most prominent candidates for this purpose are oligonucleotides of type **1b–e**. Compared to the standard phosphodiester internucleotide linkage (see **1a**), they bear phosphorothioate (**1b**), phosphoramidate (**1c**), methylphosphonate (**1d**), or phosphotriester (**1e**) internucleotide linkages. We were interested in the chemical solid-phase synthesis of oligonucleotides of type **1f** which bear one or more phosphor amidate links at distinct positions within a given DNA fragment. In this case, the backbone modification is situated directly between the P-atom and the 2′-deoxyribose moiety. Such modifications lead to DNA fragments with a number of interesting properties. They do not form



1a-e



1f

	X	Y
1a	O	O
b	O	S
c	RNH	O
d	CH_3	O
e	RO	O

diastereoisomers like the backbone-modified DNA fragments **1b–e** [3], and they still hybridize very specifically to a target strand. Furthermore, they can function as templates and primers in enzymatic reactions [4–6]. Restriction endonucleases recognition sites containing phosphoramidate linkages at the cleavage point show also interesting properties [7].

On the one hand, the phosphoramidate linkage should be sufficiently stable to allow the handling of such modified DNA fragments without precaution. On the other hand, it is known that phosphoramidate linkages are susceptible to a chemical cleavage under mild acidic conditions [8–10] resulting in a phosphate and an amino moiety. In backbone-modified fragments of type **1f**, this could then be used to create one or more nicks at specific positions within a given DNA fragment by chemical means.

Generally, the synthesis of DNA containing phosphoramidate internucleotide linkages can be performed enzymatically. This was demonstrated for the incorporation of 5′-amino-5′-deoxythymidine 5′-phosphate by DNA polymerase I [5]. But with this procedure, a random incorporation takes place according to the template used for the enzymatic reaction. An incorporation into specific positions of a single-stranded DNA is thereby not possible. Here, a chemical procedure would be favourable, especially if it could be applied to standard solid-phase oligonucleotide synthesis approaches.

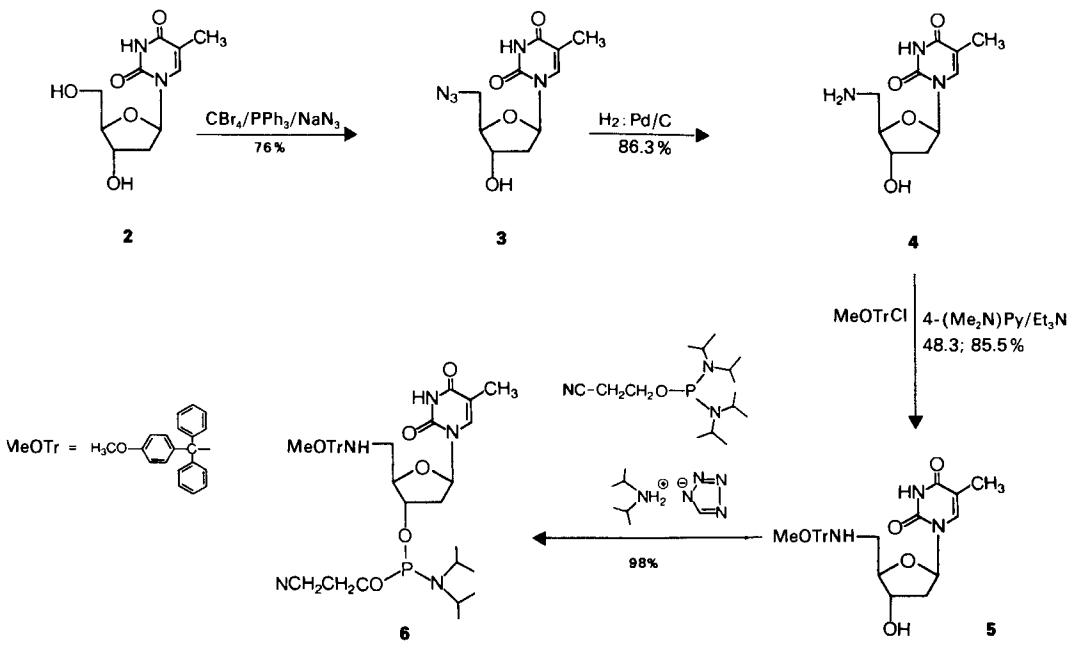
The chemical synthesis of dinucleotide monophosphates in solution containing a phosphoramidate linkage was reported by *Letsinger* and *Hata* by coupling 5′-azido-5′-deoxythymidine with thymidine 3′-phosphite intermediates [11] [12]. Lately, *Shabarova* reported also the synthesis of such modified dinucleotide monophosphates in solution by the *Atherton-Todd* coupling procedure [13–14].

Here, we would like to report the synthesis of DNA fragments containing phosphoramidate internucleotide linkages at specific positions by the phosphoramidite procedure on a solid support as well as the behaviour of such compounds towards a specific chemical cleavage of phosphoramidate internucleotide linkages under acidic conditions.

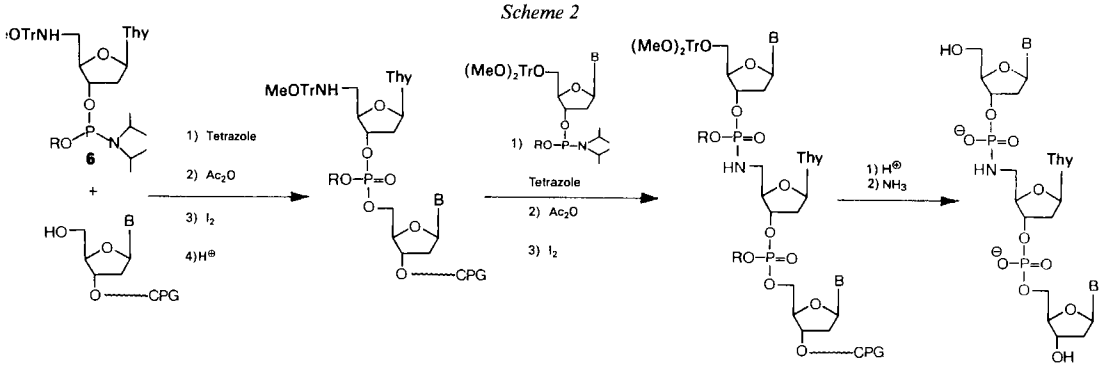
2. Results and Discussion. – 2.1. *Synthesis.* Our key building block for the synthesis of DNA fragments with inserted phosphoramidate internucleotide bonds is the phosphoramidite **6**. This compound was synthesized according to *Scheme 1*. Thymidine (**2**) was transformed to 5′-azido-5′-deoxythymidine (**3**) as published by *Hata* [15] with a slightly modified procedure. The reduction to 5′-amino-5′-deoxythymidine (**4**) was performed by hydrogenation over Pd/C. The regioselective introduction of the 4-methoxytrityl (MeOTr) group yields the corresponding 5′-protected nucleoside **5**. This compound was then transformed to the desired phosphoramidite **6** by phosphinylation with (2-cyanoethoxy)bis(diisopropylamino)phosphine in the presence of diisopropylammonium tetrazolide [16] [17]. The phosphoramidite **6** could be purified by short-column chromatography and is stable enough to be handled without greater precautions.

The MeOTr group was used for the protection of the 5′-NH_2 group in **4** since protection with the 4,4′-dimethoxytrityl group ($(MeO)_2Tr$) normally applied in solid-phase DNA synthesis led to very unstable products due to an increased acid sensitivity. The MeOTr group can be cleaved from the 5′-NH_2 group under similar conditions as the $(MeO)_2Tr$ group in standard DNA synthesis with 3% dichloroacetic acid in 1,2-dichloroethane. The MeOTr cation produced by the cleavage allows monitoring by UV giving an idea about the coupling performance.

Scheme 1



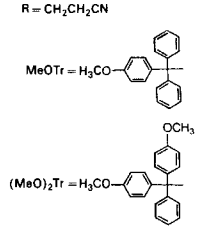
Scheme 2

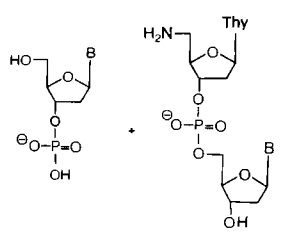


CPG = Controlled pore glass

B = Base, *i.e.* N^6-benzoyladenine, N^4-anisoylcytosine, N^2-isobutyrylguanine, thymine, or the corresponding unprotected parents

R = CH_2CH_2CN





The modified building unit **6** can be introduced in the course of a standard cycle into a growing DNA fragment being synthesized on a solid support, wherever one wants to place a phosphoramidate internucleotide linkage or produce a DNA fragment with a 5′-terminal NH_2 group. After the cleavage of the MeOTr group from the 5′-NH_2 group, the synthesis is continued with standard phosphoramidites *(Scheme 2)*. With this procedure, we have synthesized the following sequences: d(T-T-C-A-T-G-C-A-A-nh-T-C-C-G-A-T-G) (**11**) and d(G-nh-T-nh-T-G-nh-T-A-A-A-A-C-G-A-C-G-G-C-C-A-G-T-G) (**12**). The sequence **11** contains just one phosphoramidate internucleotide linkage (marked as -nh-), whereas in **12** we have inserted three such modifications.

The DNA fragment **11** was synthesized in a stepwise mode according to the *Table*, starting from the hexamer sequence d(C-C-G-A-T-G) (**7**) which was synthesized on controlled pore glass as solid support using our standard procedures [18] [19]. The attachment of the modified building block **6** was performed during a standard cycle, with the exception that slightly longer times for the coupling of **6** and the cleavage of the MeOTr protecting group were applied. After the cleavage of the MeOTr group (→**8**), part of the support was removed, and with the residual support material, the synthesis was continued with standard phosphoramidites as building units up to the desired 16-mer sequence **11**. At the level of the decamer **9** and the 13-mer **10**, again part of the support was removed. Then all the fragments **7**–**11** were worked up individually. Since the MeOTr and the $(MeO)_2Tr$ protecting groups had been removed already during the synthesis cycle, only the treatment with conc. NH_3 was necessary to remove all the other protecting groups and to cleave the fragments off the support. After evaporation of the NH_3 solution, the purification was either performed by polyacrylamide gel electrophoresis *(Fig. 1)* or reversed-phase HPLC *(Fig. 2)*. Thus, the deprotection/purification procedure was the same as for unmodified DNA fragments.

The UV-shadowing gel shown in *Fig. 1* gives an idea about the performance of the synthesis. The crude products correspond to the lanes *1* (**7**), *2* (**8**), *3* (**9**), *6* (**10**), and *9* (**11**). The introduction of the terminal NH_2 group in **8** leads to a relatively strong retardation compared to **7** possibly due to zwitterion formation and thereby compensating one negative charge. The absence of an intensive band for **8** in the lanes *3*, *6*, and *9* clearly indicates that no cleavage of the phosphoramidate internucleotide linkage has occurred under the acidic conditions applied during the synthesis of these fragments. Furthermore, the absence of a band for **8** indicates that the coupling reaction between a phosphoramidite and the 5′-NH_2 group of the DNA fragment proceeds with high efficiency. As calculated from HPLC, the average coupling yield was at least 96%.

Table[a])

d(C-C-G-A-T-G)	**7**
d(h_2nT-C-C-G-A-T-G)	**8**
d(C-A-A-nh-T-C-C-G-A-T-G)	**9**
d(A-T-G-C-A-A-nh-T-C-C-G-A-T-G)	**10**
d(T-T-C-A-T-G-C-A-A-nh-T-C-C-G-A-T-G)	**11**

[a]) h_2n means replacement of the 5′-terminal OH by NH_2;
-nh-means replacement of the phosphate link by the phosphoramidate link.

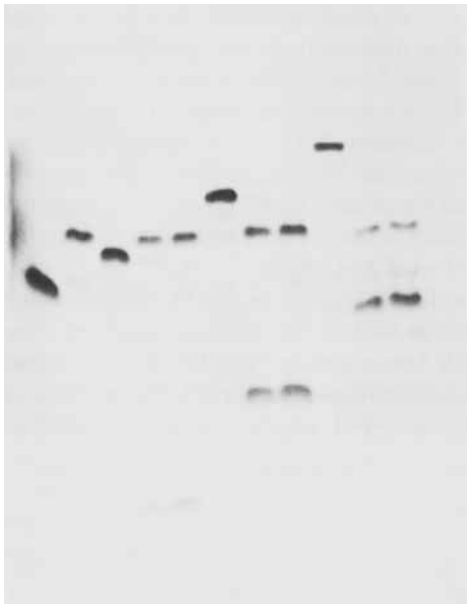


Fig. 1. *UV-Shadowing gel electrophoresis of the products in the stepwise synthesis of* **11** *and cleavage products thereof.* Lane *1*: crude **7**; lane *2*: crude **8**; lane *3*: crude **9**; lane *6*: crude **10**; lane *9*: crude **11**; lanes *4, 5*: cleavage of crude **9** with 80% AcOH after 2 and 6 h; lanes *7, 8*: cleavage of crude **10** with 80% AcOH after 2 and 6 h; lanes *10, 11*: cleavage of crude **11** with 80% AcOH after 2 and 6 h.

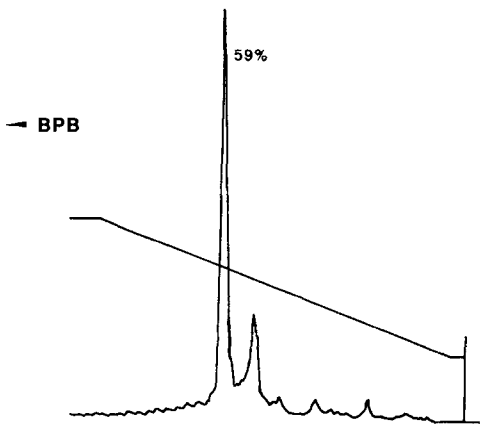


Fig. 2. *Reversed-phase HPLC of crude* **10**. *Bondapak C18*, 5–15% MeCN in 0.1M TEAA in 30 min, 40°.

With the same strategy, we have synthesized the backbone-modified fragment **12** which contained altogether three phosphoramidate linkages. The synthesis was started with 1.8 μmol of functionalized support, and a 15fold excess of the modified phosphoramidite **6** was applied for the introduction of the phosphoramidate internucleotide linkages. The deprotection procedure was the same as for **11**, and the pure fragment was obtained after purification by polyacrylamide gel electrophoresis.

2.2. *Specific Cleavage of the Backbone-Modified DNA Fragments.* In general, phosphoramidate linkages were reported to be very susceptible to cleavage reactions, even under very mild conditions [12]. This fact seems to limit the introduction of phosphoramidate linkages into synthetically prepared DNA fragments as well as the application of these backbone-modified DNA fragments. In section *2.1* we have demonstrated the possibility of the synthesis of phosphoramidate-linkage-containing DNA fragments by standard solid-phase technology. After isolation, we experienced that the fragments could be handled without special precautions. In order to evaluate whether a specific cleavage at the phosphoramidate linkage is still possible, we have treated them under acidic conditions. On the one hand, the conditions should perform the cleavage in a reasonable time, and on the other hand, these conditions should not harm unmodified DNA. AcOH (80%) at r.t. used routinely in the deprotection of synthetically prepared DNA fulfills these criteria.

2.2.1. *Cleavage of* **11** *and Fragments Thereof.* a) *Crude Material.* Part of the crude modified DNA fragments **9**–**11**, obtained after deprotection and precipitation, was treated with 300 µl of 80% AcOH, each at r.t. After 2 and 6 h, the reaction was stopped, and the cleavage products were investigated by polyacrylamide gel electrophoresis together with the untreated crude modified DNA fragments **9**–**11** (*Fig. 1*, lanes *3–11*). After 6 h, the cleavage was complete in all cases, whilst after 2 h, the fragments were cleaved almost completely. The unique cleavage product for all three compounds was as expected the 5′-NH_2 oligomer **8**. The other cleavage product is the 3′-phosphate of d(C-A-A) (for **9**), of d(A-T-G-C-A-A) (for **10**), and of d(T-T-C-A-T-G-C-A-A) (for **11**).

b) *Purified Material.* The cleavage of the purified fragments **10** and **11** was performed as described above. Then, the pure fragments as well as the cleavage products were labelled at the 5′-end by ^{32}P with polynucleotide kinase and (γ-^{32}P)ATP or at the 3′-end with (α-^{32}P)ddATP and terminal-deoxynuclotidyl transferase (see *Fig. 3*). The labelling of the 3′-end of the cleavage products reveals that the 5′-NH_2 oligomer **8** can be labelled in

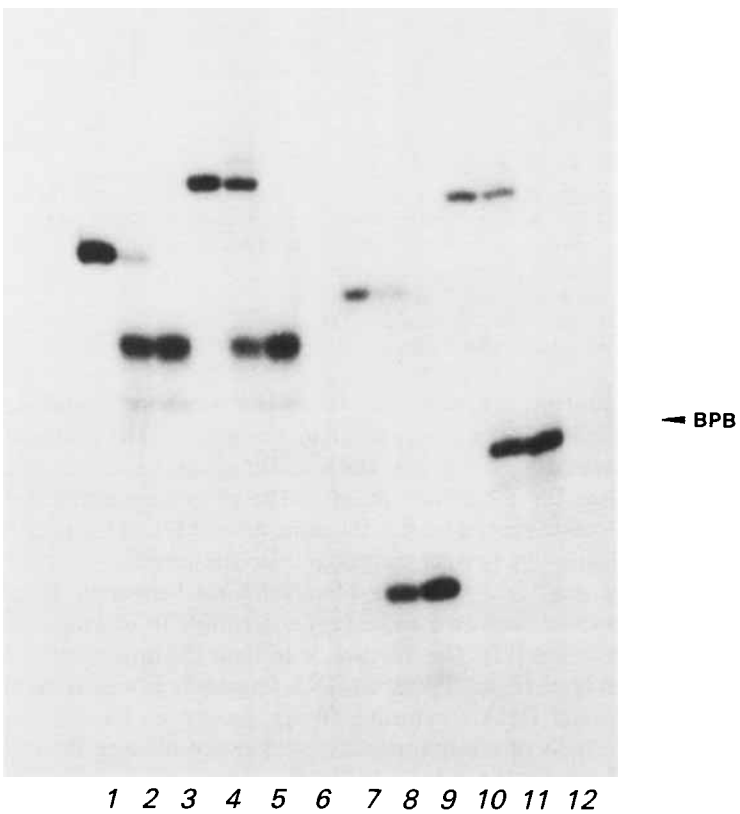


Fig. 3. *Autoradiograph of pure* **10** *and* **11** *and of their cleavage products after labelling with* ^{32}P *by terminal transferase* (lane *1–6*) *and* T_4 *polynucleotide kinase* (lanes *7–12*). Lane *1*: pure **10**; lane *2*: after cleavage of pure **10** with 80% AcOH; lane *3*: pure **8**; lane *4*: pure **11**; lane *5*: after cleavage of pure **11** with 80% AcOH; lane *6*: pure **8**; lane *7*: pure **10**; lane *8*: after cleavage of pure **10** with 80% AcOH; lane *9*: pure d(A-T-G-C-A-Ap); lane *10*: pure **11**; lane *11*: after cleavage of pure **11** with 80% AcOH; lane *12*: pure d(T-T-C-A-T-G-C-A-Ap).

contrast to the other cleavage product which carries a 3′-phosphate and is thereby not accessible to this labelling procedure. In contrast, the 5′-labelling is only possible for the cleavage product bearing at 3′-phosphate and a free 5′-OH group but is not possible with fragment **8** which bears a 5′-NH_2 group. The fact that only the expected cleavage products are formed without other side-products indicates that unmodified DNA is inert to the cleavage conditions which was also confirmed in other examples.

2.2.2. *Cleavage of* **12**. a) *After Labelling with* ^{32}P *at the 5′-End.* The oligonucleotide **12** was phosphorylated at the 5′-end by ^{32}P with polynucleotide kinase. Then, it was cleaved in the presence of the synthetically prepared template **13** (see *Fig. 4*, below) for 5 h with 80% AcOH. After separation by polyacrylamide gel electrophoresis, no radioactively labelled **12** could be seen anymore (*Fig. 4*, lane *4*) which indicates that a complete cleavage had occurred.

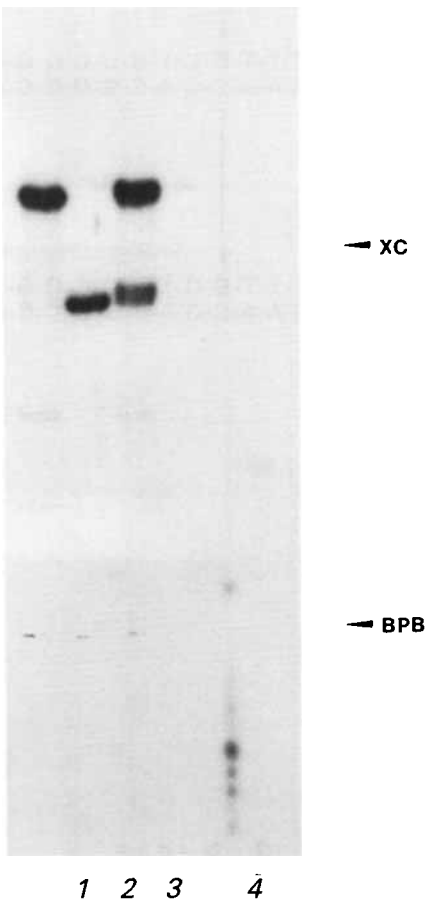
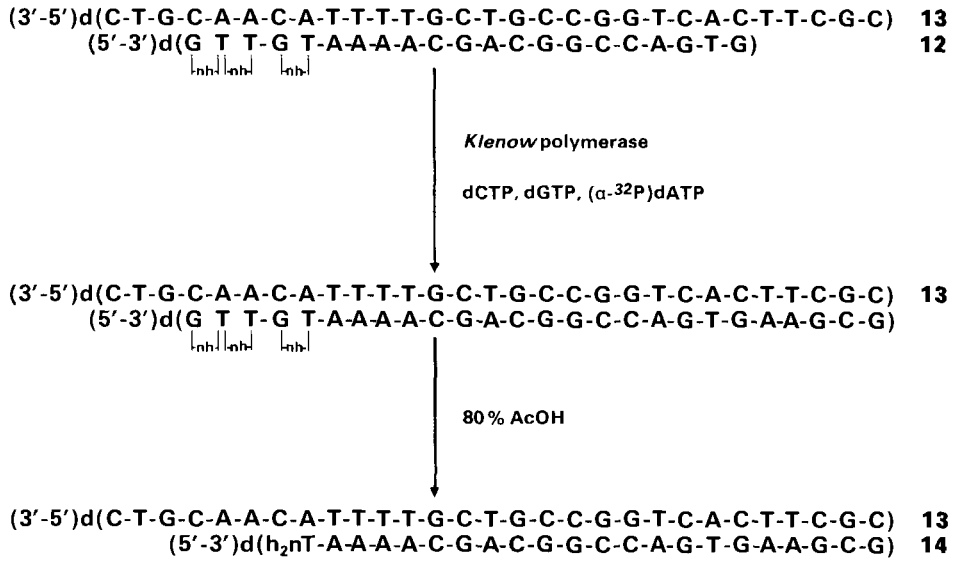


Fig. 4. *Autoradiograph of the cleavage products of* **12** *after labelling with* T_4 *polynucleotide kinase.* Lane *1*: pure labelled **13**; lane *2*: pure labelled **12**; lane *3*: pure labelled **13** and **12**; lane *4*: after treatment of pure labelled **12** and unlabelled **13** with 80% AcOH.

b) *After Labelling at the 3'-End.* In the presence of the synthetic template **13**, the oligonucleotide **12** was extended according to *Scheme 3* which adds an additional 5 bases to **12** and incorporates the radioactive label at the same time. This confirms also that oligonucleotides carrying phosphoramidate linkages can be used as primers for polymer-

Scheme 3



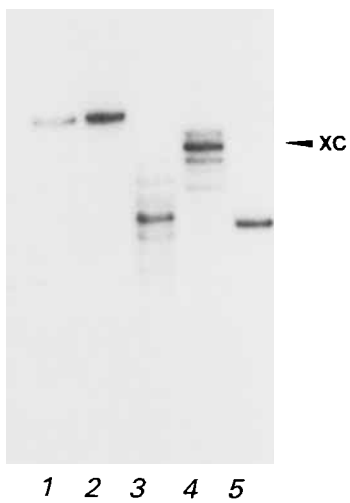


Fig. 5. *Autoradiograph of the cleavage products of* **12** *after labelling with* Klenow *DNA polymerase.* Lane *1*: pure **13** labelled at the 5'-end by T_4 polynucleotide kinase; lane *2*: after treatment of pure **13** labelled at the 5'-end by T_4 polynucleotide kinase, with 80% AcOH; lane *3*: cleavage with 80% AcOH of **12** after labelling with *Klenow* DNA polymerase according to *Scheme 3*; lane *4*: labelling of **12** with *Klenow* DNA polymerase according to *Scheme 3*; lane *5*: polynucleotide kinase 5'-labelled 21-mer as comparison.

ases. After the treatment with 80% AcOH, no elongated fragment could be seen anymore which indicates also a complete cleavage reaction *(Fig. 5)*.

3. Conclusion. – We have described a simple incorporation of phosphoramidate internucleotide linkages at specific positions of a given DNA fragment by standard solid-phase DNA synthesis on controlled pore glass using phosphoramidites as building units. The workup and purification procedures are identical with those of unmodified synthetic DNA fragments. Furthermore, these backbone-modified DNA fragments are stable enough to be handled without special precautions, but they can be cleaved specifically and quantitatively at the phosphoramidate linkages by chemical means without harming unmodified DNA. The nucleosides 2′-deoxyguanosine, 2′-deoxyadenosine, and 2′-deoxycytidine can be converted in similar ways to the 5′-amino-2′,5′-dideoxy-nucleosides as described for 5′-amino-5′-deoxythymidine (**4**). They can then be further reacted to give finally their corresponding phosphoramidites [20]. In this manner, phosphoramidate internucleotide linkages can be introduced at any desired position within a synthetic DNA fragment. This could have implications in anti-sense approaches, since these modified DNA fragments show a greater stability towards nucleolytic enzymes and do not create problems due to optical activity of the modified internucleotide linkages.

The specific cleavage reaction could be applied in DNA probe technology. This cleavage produces a primary amino group which can be detected with great sensitivity. Since the number of NH_2 groups produced in this way is proportional to the number of phosphoramidate linkages in a DNA probe, this could be used to amplify the signal if the cleavage is performed after the hybridisation of the probe to the complementary sequence.

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Experimental Part

1. *General.* Pyridine: refluxed and distilled from TsCl, refluxed over CaH_2, distilled, and kept over molecular sieves (4 Å) in tightly closed bottles. MeCN: refluxed over CaH_2 and distilled just prior to use. CH_2Cl_2: dried over Na_2SO_4. DMF: filtered through Al_2O_3 (grade I) and distilled. All other solvents or reagents were of *p.a.* grade. Diisopropylammonium tetrazolide and (2-cyanoethoxy)bis(diisopropylamino)phosphine for the preparation of **6** were synthesized according to [23] and [21]. The DNA fragment **13** used as a template was synthesized by standard procedures [18]. T_4-Polynucleotide kinase, terminal transferase, and DNA polymerase *(Klenow)* were from *Pharmacia,* (α-^{32}P)ddATP, (α-^{32}P)dATP, and (γ-^{32}P)dATP from *Amersham.* Short-column chromatography [22]: silica gel *60* (0.063–0.040 mm, *Merck*). TLC: HP-TLC silica gel plates *(Merck)*.

2. *5′-Amino-5′-deoxythymidine* (**4**). Thymidine (7.11 g, 29.4 mmol) was taken up in anh. pyridine and evaporated. This was repeated with anh. toluene. The residue was taken up in DMF (100 ml), and Ph_3P (7.80 g, 29.4 mmol), NaN_3 (9.63 g, 141 mmol), and CBr_4 (1.00 g, 29.4 mmol) were added (the latter portionwise). The mixture was stirred at r.t. for 16 h and then treated with 10 ml of MeOH. Stirring was continued for another h. After evaporation, the residue in EtOH was absorbed onto 20 g of silica gel and purified by short-column chromatography over 100 g of silica gel using a gradient of EtOH in CH_2Cl_2 (2–10%): 6 g (76%) of **3** as colourless powder.

To a soln. of **3** in 500 ml of EtOH, 0.5 g of 10% Pd/C were added and hydrogenated at r.t. Fresh H_2 was added several times to replace the N_2 formed during the hydrogenation (TLC monitoring). After 6 h, more EtOH was

added and heated to get the partly crystallized product completely into soln. Then, the catalyst was filtered off. Crystallization in the refrigerator afforded 4.66 g (86.3%) of **4** as colourless crystals. M.p. 178–180° ([12]: 175–177°). Anal. calc. for $C_{10}H_{15}N_3O_4$ (241.25): C 49.79, H 6.27, N 17.42; found: C 49.54, H 6.44, N 17.03.

3. *5'-Amino-5'-deoxy-5'-*N*-(4-methoxytrityl)thymidine* (**5**). Compd. **4** (6.03 g, 25 mmol) was twice taken up in anh. pyridine and evaporated. Then, it was again dissolved in anh. pyridine (150 ml), and 4-(dimethylamino)pyridine (2.04 g, 16.7 mmol). Et_3N (1.70 g, 16.7 mmol, 2.3 ml), and 4-methoxytrityl chloride (18.53 g, 60 mmol) were added at r.t. and with stirring. After 2 h, MeOH (20 ml) was added, and after additional 15 min, the mixture was poured into sat. $NaHCO_3$ soln. (250 ml), extracted with $CHCl_3$ (4 × 250 ml), and the combined org. layer dried (Na_2SO_4) and evaporated. The residue was separated over 200 g of silica gel by short-column chromatography. The residue of the pure product fractions was dissolved in 20 ml of $CHCl_3$ and 3 ml of Et_3N and precipitated into 2 l of pentane. The precipitate was dried: 6.2 g (48.3%) of **5**. A repetition of this reaction starting from 1.5 mmol (362 mg) of **4** resulted in a 85.5% yield of **5**. M.p. 126–129°. ^{1}H-NMR ($CDCl_3$) 1.25 (br. *s*, NH); 1.64 (*s*, CH_3); 2.05–2.60 (*m*, 2 H–C(2'), 2 H–C(5')); 3.78 (*s*, CH_3O); 3.96–4.04 (*m*, H–C(4')); 4.27–4.37 (*m*, H–C(3')); 6.25 (*dd*, H–C(1')); 6.80–7.50 (*m*, 2 C_6H_5, C_6H_4, H–C(5)). Anal. calc. for $C_{30}H_{31}N_3O_5 \cdot 0.8\ H_2O$ (528.01): C 68.24, H 6.22, N 7.96; found: C 68.12, H 6.28, N 7.98.

4. *5'-Amino-5'-deoxy-5'-*N*-(4-methoxytrityl)thymidine 3'-[(2-Cyanoethyl)* N,N*-Diisopropylphosphoramidite]* (**6**). Separately, **5** (2.01 g, 4 mmol) and bis(diisopropylammonium) tetrazolide (0.55 g, 3 mmol) were dried overnight under high vacuum. Then, **5** was taken up in 60 ml of anh. CH_2Cl_2, and the bis(diisopropylammonium) tetrazolide as well as 7.9 mmol (2.60 g) of (2-cyanoethoxy)bis(diisopropylamino)phosphine were added with stirring. Stirring was continued for 1 h, then the mixture poured into 70 ml of sat. $NaHCO_3$ soln. and extracted several times with CH_2Cl_2, and the combined org. layer extracted once with 50 ml of sat. NaCl soln. and evaporated. The residue was dissolved in 20 ml of CH_2Cl_2 and the product precipitated with 700 ml of pentane. The precipitate was dried: 2.8 g (98%) of **6** as colourless powder (diastereoisomers). ^{1}H-NMR ($CDCl_3$): 1.10–1.19 (2*d*, 2 $(CH_3)_2CH$); 1.86 (2*s*, CH_3); 2.22–2.60 (*m*, 2 H–C(2')); 2.45, 2.61 (2*t*, CH_2CH_2CN); 3.50–3.92 (*m*, 2 H–C(5'), 2 $(CH_3)_2CH$); 3.78 (*s*, CH_3O); 4.08 (*dd*, H–C(4')); 4.38–4.55 (*m*, H–C(3')); 6.30 (*dd*, H–C(1')); 6.78–7.55 (*m*, 2 C_6H_5, C_6H_4, H–C(6)); 8.25 (br. *s*, NH). Anal. calc. for $C_{39}H_{48}N_5O_6P \cdot 0.5\ H_2O$ (722.82): C 64.99, H 6.93, N 9.73; found: C 64.81, H 6.83, N 9.69.

5. *d(T-T-C-A-T-G-C-A-A-nh-T-C-C-G-A-T-G)* (**11**). The synthesis was performed on controlled pore glass (CPG) as solid support [24] starting with 60 mg (1.5 μmol). The synthesis up to the hexamer sequence d(C-C-G-A-T-G) (**7**) was performed using standard (2-cyanoethyl) phosphoramidites as building units [16] as described earlier [21]. At this stage and after cleavage of the $(MeO)_2Tr$ group, 10 mg of the support were removed, washed with MeCN and Et_2O and dried. The synthesis was continued by coupling the modified building unit **6** (42 μmol) in the presence of 200 μmol of tetrazole in 0.5 ml of anh. MeCN in a standard cycle, with the exception that the condensation time was 10 min, and the cleavage time for the MeOTr using 3% $CHCl_2$-COOH in 1,2-dichloroethane was also 10 min instead of 2–3 min which normally is sufficient. Again 10 mg of the support were removed, and the synthesis was continued using again standard (2-cyanoethyl) phosphoramidites up to the desired sequence **11**. At the level of the 10-mer **9** and the 13-mer **10**, again 10 mg of support had been removed after cleavage of the $(MeO)_2Tr$ group.

Then, all the fragments were worked up individually. Since the 5'-protecting group ($(MeO)_2Tr$, MeOTr) had been removed already, the support material was treated with 700 μl of conc. NH_3 at 56° overnight in tightly closed *Eppendorf* tubes. After removal of the support, the NH_3 soln. was evaporated on a speed-vac. concentrator. The residue was taken up in 300 μl of H_2O/dioxane 1:2 and precipitated by addition of 600 μl of THF. After centrifuging (15 min, 0°), the pellet was dissolved in H_2O and part of this soln. (corresponding to 1 mg of support material) was applied, after addition of dye soln., to gel electrophoresis on a 20% denaturing polyacrylamide gel. The bands of the products were visualized by UV shadowing *(Fig. 1)* and isolated from the UV-absorbing bands by electroelution followed by precipitation. Alternatively, we purified and isolated **11** also by HPLC *(Fig. 2)*.

6. *d(G-nh-T-nh-T-G-nh-T-A-A-A-A-C-G-A-C-G-G-C-C-A-G-T-G)* (**12**). The synthesis was performed in the same way as described in *Exper. 5*, but starting from 65 mg (1.6 μmol) of controlled pore glass as solid support. Whenever an elongation with **6** was performed, this was carried out with 28 μmol of **6** in the presence of 200 μmol of tetrazole in 500 μl of anh. MeCN with a condensation time of 7 min. The deprotection workup and isolation procedure was the same as described for **11** *(Exper. 5)*.

7. *Cleavage Reactions.* 7.1. *Cleavage of* **11**. a) *Crude Material.* Part of the crude modified DNA fragments **9**–**11** (0.063 μmol each), obtained after deprotection and precipitation as a pellet, was treated with 300 μl of 80% AcOH at r.t. After 2 and 6 h, the reaction was stopped by addition of 700 μl of Et_2O, whereupon the DNA precipitated. After centrifuging for 15 min at 0°, the pellets were taken up in loading buffer and separated on a denaturing polyacrylamide gel (40 × 20 × 0.2 cm, *Fig. 1*).

b) *Purified Material.* At r.t., 0.02 *OD* units of purified **10** or **11** were treated with 100 µl of 80% AcOH. After 6 h, the mixture was evaporated on a speed-vac. concentrator. This was repeated twice after addition of 20 µl of H_2O. The residue was taken up in 20 µl of H_2O, and 2 µl of this soln. (0.002 *OD*) were either labelled at the 5′-end with ^{32}P by polynucleotide kinase and (γ-^{32}P)ATP or at the 3′-end with terminal-deoxynucleotidyl transferase and (α-^{32}P)ddATP as described in [25]. After a precipitation step, it was separated on an anal. 20% polyacrylamide gel under denaturing conditions *(Fig. 3)*.

7.2. *Cleavage of* **12**. On the one hand, 0.002 *OD* of **12** which was labelled at the 5′-end by ^{32}P was treated in the presence of the complementary sequence **13** with 50 µl of 80% AcOH. After 5 h, the mixture was analysed by polyacrylamide gel electrophoresis as described in *Exper. 7.16 (Fig. 4)*. No radioactivity was detected in lane *4* which indicates complete cleavage.

On the other hand, **12** (0.003 *OD*) was elongated after hybridisation to 0.003 *OD* of the template sequence **13** with *Klenow* polymerase according to [25] *(Scheme 3)*. Half of the enzymatic reaction mixture was treated with 100 µl of 80% AcOH for 6 h at r.t. After evaporation on a speed-vac. concentrator, the pellet was taken up in 100 µl of H_2O and precipitated, after addition of 10 µl of 5M LiOAc and 250 µl of i-PrOH, at $-78°$. After centrifuging, the pellet was taken up in loading buffer and investigated by denaturing polyacrylamide gel electrophoresis *(Fig. 5)*. As a reference, the template DNA sequence **13** was also labelled with ^{32}P at the 5′-end and treated with 80% AcOH. No effect on this DNA could be seen (*Fig. 5*, lane *2*).

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