110. A New Combined Purification/Phosphorylation Procedure for Oligodeoxynucleotides

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Dedicated to Dr. *O. Isler* on the occasion of his 80th birthday

(6.IV.90)

We describe a new principle for the purification of oligodeoxynucleotides using a purification handle which leads to a covalent linkage of the desired DNA sequence to a solid matrix. Truncated sequences are removed by washing steps. The release of the pure target sequence is performed in such a way that it is obtained directly in its 5′-phosphorylated form which represents a further advantage. In addition, the system can be used for 5′-thiol modifications of synthetic DNA.

1. Introduction. – For many applications in gene technology, synthetic DNA fragments have to be purified after their preparation in order to remove incomplete sequences from the desired oligodeoxynucleotide. This purification is commonly performed by HPLC or by polyacrylamide gel electrophoresis [1].

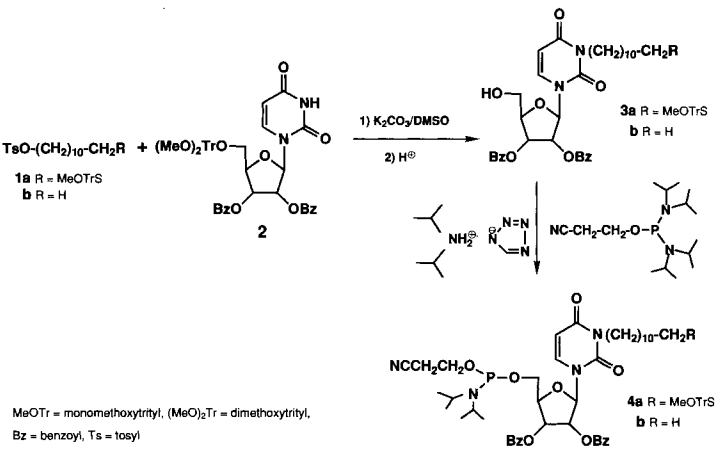
In order to obviate these relatively time-consuming methods, simple purification principles based on affinity chromatography on small cartridges have been developed [2]. The application of lipophilic purification handle molecules on small cartridges has attracted most attention [3–5]. The handle molecule is introduced at the end of the synthesis, and, therefore, only the desired oligodeoxynucleotide will be equipped with this unit because all the truncated sequences are capped in the course of the oligonucleotide synthesis. Since the lipophilicity of oligonucleotides differs depending on the base composition and since the isolation capacity of the lipophilic handle decreases with chain length, the conditions are difficult to adjust and the method is limited to short oligomers. Our intention was to develop a purification system for oligonucleotides synthesized by solid-phase methodology which is based on covalent bonds rather than physical interactions. Such an approach should not be influenced by the physical properties of the oligonucleotide nor by the chain length of the oligomer. From several possible functional groups, we have selected the thiol group, since 5′-thiol-modified DNA fragments can be linked by a fast reaction to solid supports bearing activated thiol functions by a disulfide bridge [6]. As linker molecule between the thiol group and the oligonucleotide, we have chosen a ribonucleotide unit so that the oligomer can be released after fixation onto the solid support and the removal of the truncated sequences by an oxidation/elimination process. This cleavage reaction leads then directly to the corresponding 5′-phosphorylated DNA fragment which represents a further advantage because for most application in molecular biology, the oligonucleotides need to be phosphorylated at their 5′-end after their synthesis either by polynucleotide kinase [7] or by chemical means in combination with the phosphoramidite procedure [8–11]. A similar approach for the triester strategy but based on lipophilic interactions rather than chemical-bond formation, in combination with an

acid-cleavable phosphoramidate linkage yielding also the 5′-phosphorylated DNA fragment, was developed recently [12].

As a suitable molecule for the introduction of the protected thiol function and the ribose moiety, we have synthesized the phosphoramidite **4a**. The compound was coupled to the DNA fragment still attached in the protected form to the solid support during a standard cycle for phosphoramidites. Capping of the truncated sequences guaranteed that only the desired DNA reacted with **4a**. After cleavage of the base-labile protecting groups and removal from the support (→ **5**), the 4-monomethoxytrityl group of the handle was cleaved (→ **6**) releasing the thiol group which could form a disulfide bridge with the activated support. Truncated sequences were removed by washing steps, and the desired 5′-phosphorylated fragment was released from the support.

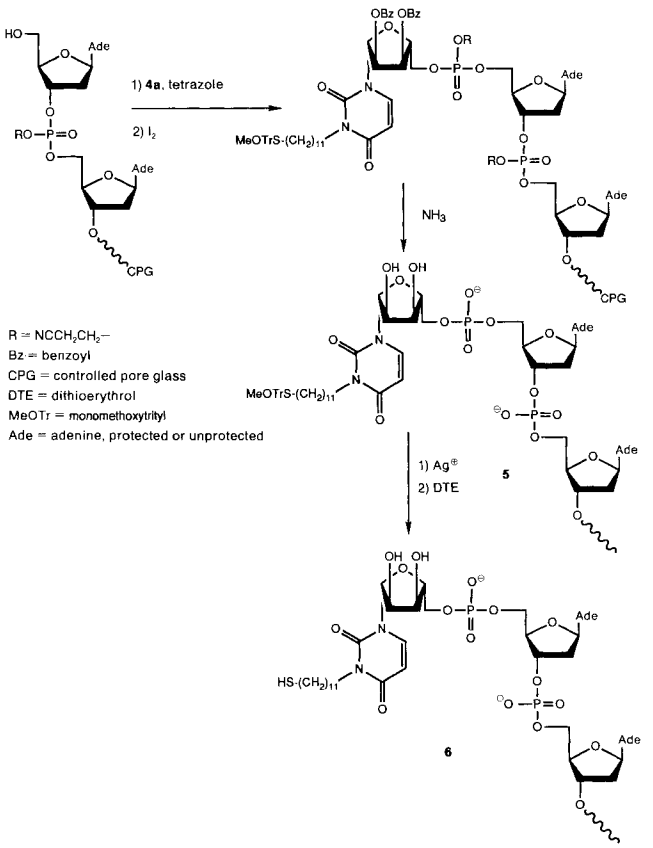
2. Results and Discussion. – 2.1. *Synthesis.* The starting thioether **1a** was prepared from 4-monomethoxytrityl thioalcohol which in turn was obtained in 52% yield according to [13]. Reaction of this thiol with 11-bromoundecan-1-ol [14] gave 11-[(4′-monomethoxytrityl)thio]undecan-1-ol (70%), and tosylation yielded **1a** in 60% yield *(Scheme 1)*. Next, uridine was protected at the 5′-position by reaction with 4,4′-dimethoxytrityl chloride and then benzoylated at the 2′- and 3′-position leading to **2** in 82% overall yield. Compounds **1a** and **2** were reacted together in DMSO by the addition of K_2CO_3, and the 4,4′-dimethoxytrityl group was removed from the product by treatment with 3% dichloroacetic acid in 1,2-dichloroethane yielding **3a** (75% overall). The desired phosphoramidite **4a** was obtained from **3a** with (2-cyanoethoxy)bis(diisopropylamino)phosphine in the presence of diisopropylammonium tetrazolide [15] [16].

Scheme 1



2.2. *Evaluation of the Purification Principle.* The applicability of the anchor molecule **4a** for a high-resolution purification was tested with a mixture of synthetic oligodeoxynucleotides. To this purpose, hexadecamer **6** equipped with the purification handle (see *Scheme 2*), the hexadecamer **7** without purification handle, as well as the truncated

Scheme 2



sequences **8**–**10** (see below, *Scheme 3*) were needed. These sequences were prepared by the solid-phase methodology on controlled pore glass (CPG) as solid support [17] and cyanoethyl phosphoramidites [15] as building units and our standard techniques [18]. At the 7mer, 10mer, 13mer, and the 16mer level, part of the material was removed and worked up separately. The synthesis of these fragments was checked by prep. gel electrophoresis (*Fig. 1*, lanes *1–4*). From part of the 16mer, the 4,4′-dimethoxytrityl group was removed, and one batch was reacted with bis(cyanoethoxy)(diisopropylamino)-phosphine according to our published phosphorylation procedure [10] to yield, after oxidation and deprotection, the 5′-phosphate of **7** for comparison (*Fig. 1*, lane *7*). In another batch, **4a** was coupled in a standard cycle *(Scheme 2)*. The reaction proceeded with high efficiency leading, after cleavage of the protecting groups and removal from the support with conc. ammonia, to **5** showing a high retardation in polyacrylamide-gel electrophoresis (*Fig. 1*, lane *5*). From **5**, the 4-monomethoxytrityl group was cleaved off with Ag^+, followed by DTE to give **6** (*Fig. 1*, lane *6*), showing that the huge retardation in gel electrophoresis was mainly caused by the 4-monomethoxytrityl group at the handle.

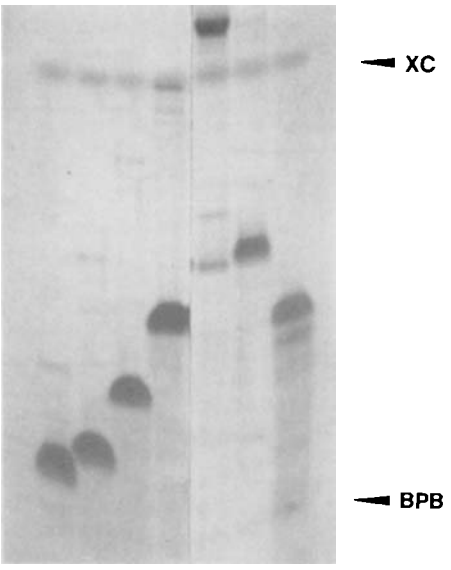


Fig. 1. *UV shadowing electrophoresis gel of the stepwise synthesis of the different oligomers used for the evaluation of the purification principle.* Lane *1*: crude **10**; lane *2*: crude **9**; lane *3*: crude **8**; lane *4*: crude **7**; lane *5*: **5** after coupling of **4a** to the hexadecamer before cleavage of the MeOTr group; lane *6*: **6** with the free thiol group; lane *7*: crude 5′-phosphate of **7** synthesized for comparison.

To compound **6** were added the hexadecamer **7** (without purification handle) and the truncated sequences **8**–**10** in relatively large amounts. All these were obtained by removing, at each of the corresponding sequence, part of the support material which was then deprotected separately. The relative amounts of these compounds can be judged from the preparative polyacrylamide gel (*Fig. 2*, lane *1*). The mixture **6**–**10** (corresponding roughly to 0.1–0.13 μmol each) in 200 ml of H_2O was applied to 200 mg of disulfide-activated CPG. Compound **6** bound itself *via* a disulfide bond to the solid support with release of 5-nitropyridine-2-(1*H*)-thione *(Scheme 3)*. After separation of the support, several washings completely removed the truncated sequences (*Fig. 2*, lane *2*). The desired pure 5′-phosphorylated DNA fragment corresponding to **7** was released from the support by oxidation with $NaIO_4$ followed by treatment with Et_3N. *Fig. 2* demonstrates that the truncated sequences can be removed completely by this purification procedure, even if the desired thiol-modified DNA sequence is highly underrepresented. In gel electrophoresis, the 5′-phosphorylated DNA fragment has a higher mobility than the parent 16mer oligonucleotide **7** due to the additional negative charges resulting from the terminal phosphate group. The mobility is identical with the 5′-phosphate of this DNA fragment prepared independently by our standard phosphorylation procedure (*Fig. 1*, lane *7*). These results were also confirmed by HPLC (data not shown). Thus, the procedure should be amenable for the purification of oligodeoxynucleotides on cartridges equipped with activated support and yielding the desired pure 5′-phosphorylated DNA fragments.

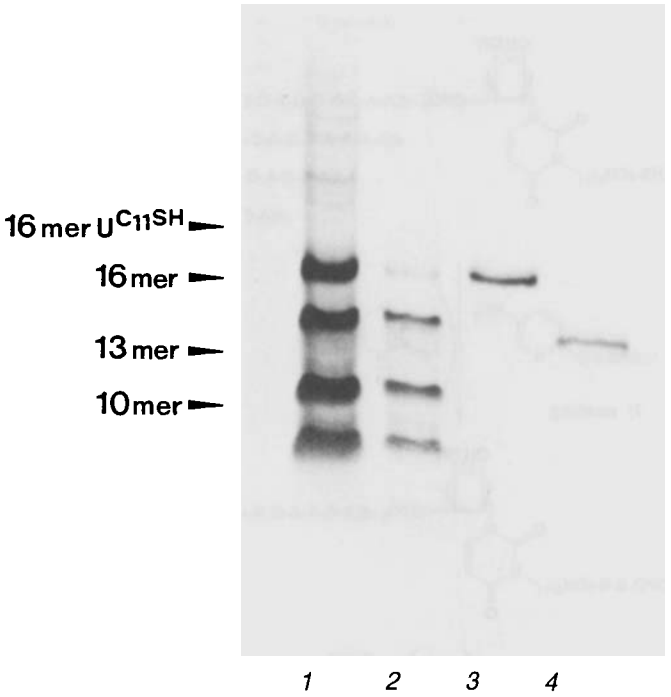
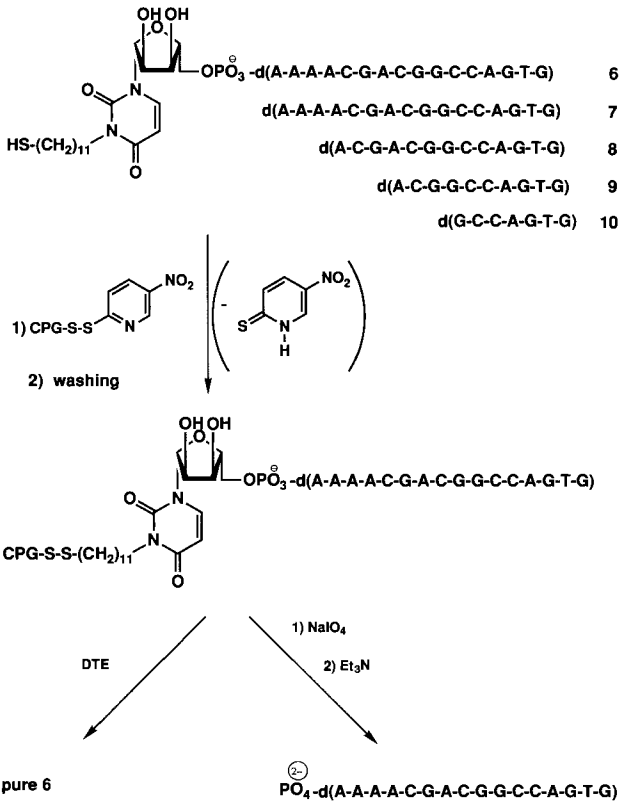


Fig. 2. *Polyacrylamide gel electrophoresis* (stains all) *of the evaluation of* **4a**. Lane *1*: crude mixture **6**–**10** applied to the activated matrix for purification (**10** not visible because of elution during staining); lane *2*: washings containing the truncated sequences; lane *3*: release of **6** from the matrix after DTE treatment; lane *4*: release of the pure 5′-phosphate of **7** after oxidation/elimination.

In a separate experiment, the release was performed just with DTE leading to the pure oligodeoxynucleotide **6** carrying the thiol group, thus, demonstrating that the desired fragment had been coupled indeed specifically. Modified oligonucleotides of type **6** carrying a thiol group can be also employed to perform cross-links between this DNA and other molecules of interest, *e.g.* for the attachment of dyes.

To test the purification procedure based on covalent-bond formation against one using lipophilic interactions, we have synthesized the building block **4b** starting from 2′,3′-dibenzoyluridine derivative **2** and undecanyl tosylate (**1b**) [4] *(Scheme 1)*. Compound **4b** allows the introduction of the undecanyl residue as lipophilic handle into synthetic DNA. Phosphoramidite **4b** was used in exactly the same way as the handle molecule **4a** and with the same sequences and sequence mixtures as mentioned above, but with small columns containing reversed-phase silica instead of CPG-support with an activated thiol function. The conditions for the purification and the oxidation/elimination were exactly as described in [4] where the same system but with an octyl instead of an undecanyl residue was described and tested for the purification of DNA fragments. Even after optimization of the conditions, the expected 5′-phosphate of the hexadecamer **7** could never be obtained in absolutely pure form (data not shown). These experiments

Scheme 3



demonstrated the superiority of the purification principle based on covalent-bond formation *vs.* the one based on lipophilic interactions.

3. Conclusion. – We have synthesized a phosphoramidite **4a** which allows the introduction of a purification handle into synthetic oligodeoxynucleotides during their synthesis on a solid support and proved its usefulness in a simple purification procedure for oligodeoxynucleotides. With this anchor molecule, it is possible to attach the desired DNA fragment after the deprotection procedure by a covalent bond to a solid support. All failure sequences can be removed by washing steps due to their lack of the anchor molecule. The removal of the pure DNA fragment by an oxidation/elimination process yields directly the 5′-phosphorylated DNA fragment. The method is amenable to the current methods employed for solid-phase DNA synthesis and, in principle, does not depend on chain length or base composition of the oligonucleotides. It should also find its application for the purification of modified DNA fragments, especially in the anti-sense area. Furthermore, the method could be useful for automated purification on small cartridges equipped with activated support material and for the purification of large amounts of DNA. Besides that, phosphoramidite **4a** can also be applied to the preparation of thiol-modified DNA fragments for the attachment of dyes and enzymes and the like.

We would like to thank *P. Iaiza* for excellent technical assistance. Furthermore, our thanks are due to our colleagues from the Central Research Units for C,H,N analysis (Dr. *S. Müller*) and ^{1}H- and ^{31}P-NMR spectra (Dr. *G. Englert*, Dr. *W. Arnold*) and to Mrs. *J. Lobsiger* for typing the manuscript.

Experimental Part

1. *General.* All solvents were of the highest purity available. Diisopropylammonium tetrazolide was prepared according to [21], (2-cyanoethoxy)bis(*N*,*N*-diisopropylamino)phosphine according to [10], 5′-*O*-(4,4′-dimethoxytrityl)-2′,3′-*O*-dibenzoyluridine (**2**) according to [1], and 4-monomethoxytrityl thioalcohol according to [13] in 52% yield. The latter was reacted with 11-bromoundecan-1-ol essentially as described in [14] (→ 70%), and undecanyl tosylate (**1b**) was obtained from undecanol and tosyl chloride. DNA synthesis was performed on controlled pore glass (CPG) as solid support as described earlier [18] [19]. CPG functionalized with a thiol group (*Pierce*). Activation of the thiol group with 5,5′-dinitro-2,2′-dithiobispyridine was performed as described [6]. Small *Sephadex* columns (*NAP*) were from *Pharmacia*. Small columns with reversed-phase silica gel were from *Baker*. Polyacrylamide gels were stained with a soln. of 3,3′-diethyl-9-methyl-4,5,4′,5′-dibenzo-thiacarbocyanine bromide (stains-all; *Fluka*). This was much more sensitive than visualization by UV shadowing and showed even smallest amounts of impurities. Short column chromatography (CC) [20]: silica gel *60* (0.063–0.040 mm, *Merck*). TLC: HPTLC silica plates (*Merck*). ^{1}H-NMR (250 MHz): chemical shifts in δ (ppm) relative to TMS. ^{31}P-NMR: chemical shifts rel. to H_3PO_4 (extern).

2. *11-[(4-Monomethoxytrityl)thio]undecyl 4-Toluenesulfonate* (**1a**). To a soln. of 25 mmol (1.2 g) of 11-[(4-methoxytrityl)thio]undecanol in 50 ml of anh. pyridine, 35 mmol (0.67 g) of tosyl chloride were added, and the soln. was stirred at r.t. for 3 h. The soln. was coevaporated 3 times with toluene. The resulting oil was taken up in Et_2O (300 ml) and washed 3 times with sat. $NaHCO_3$ soln. The org. phase was dried (Na_2SO_4) and evaporated: 2.1 g of crude **1a**. This oil was purified by CC (50 g of silica gel, pentane with increasing amount of AcOEt). The pure fractions (TLC (Et_2O/pentane 1:2): R_f 0.24) yielded 950 mg of **1a**. Colourless oil. ^{1}H-NMR ($CDCl_3$): 1.06–1.69 (*2m*, CH_2 (CH_2)$_9$$CH_2$); 2.13 (*t*, CH_2S); 2.44 (*s*, Me); 3.79 (*s*, MeO); 4.00 (*t*, CH_2O); 6.80 (*d*, 2 arom. H (C_6H_4)); 7.16–7.44 (*m*, 14 arom. H); 7.79 (*d*, 2 arom. H (C_6H_4)). Anal. calc. for $C_{38}H_{46}S_2O_4$ (630.90): C 72.34, H 7.35; found: C 71.89, H 7.51.

3. *2′,3′-*O*-Dibenzoyl-3-{11-[(4-monomethoxytrityl)thio]undecyl}uridine* (**3a**). Compound **2** (0.3 mmol, 226 mg) was twice taken up in anh. MeCN and evaporated. To the residue in 10 ml of DMSO, 0.36 mmol (227 mg) of **1a** and 1 mmol (140 mg) of K_2CO_3 were added. The suspension was stirred at r.t. for 24 h, then poured into sat. $NaHCO_3$ soln., and extracted with CH_2Cl_2 (3 × 100 ml), and the combined org. layer dried (Na_2SO_4) and evaporated. After coevaporation with toluene (3 times), 430 mg of an oil (R_f 0.82, CH_2Cl_2) were obtained. To this oil in 50 ml of CH_2Cl_2, 50 ml of dichloroacetic acid in 1,2-dichloroethane (3%) were added (immediately orange soln.). After 5 min at r.t., 3 ml of pyridine were added, and the soln. was poured into 50 ml of sat. $NaHCO_3$ soln., and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. layer was dried (Na_2SO_4) and evaporated: 450 mg of crude **3a** as an oil. CC (25 g of silica gel, Et_2O/pentane 9:1) and coevaporation of the pure fraction (TLC (CH_2Cl_2): R_f 0.25) with MeCN (several times) gave 205 mg (75%) of pure **3a** as an oil. ^{1}H-NMR ($CDCl_3$): 1.06–1.64 (*m*, CH_2(CH_2)$_9$$CH_2$); 2.13 (*t*, CH_2S); 4.40 (*t*, OH–C(5′)); 3.78 (*s*, MeO); 3.91 (*t*, CH_2N); 3.96–4.12 (*m*, 2H–C(5′)); 4.43–4.50 (*m*, H–C(4′)); 5.82–5.85 (*m*, H–C(2′), H–C(3′), H–C(5)); 6.29 (*dd*, H–C(1′)); 6.80 (*d*, 2 arom. H (C_6H_4)); 7.13–7.46 (16 arom. H (C_6H_5, C_6H_4)); 7.50–7.64 (*m*, 2 arom. H); 7.73 (*d*, H–C(6)); 7.91–8.02 (*m*, 4 arom. H). Anal. calc. for $C_{54}H_{58}N_2SO_9 \cdot 0.5$ MeCN (931.64): C 70.94, H 6.44, N 3.75; found: C 70.77, H 6.82, N 3.79.

4. *2′,3′-*O*-Dibenzoyl-3-{11-[(4-monomethoxytrityl)thio]undecyl}uridine 5′-[(2-Cyanoethyl) Diisopropylamino Phosphite]* (**4a**). A mixture of 0.9 mmol (820 mg) of **3a** and 0.45 mmol (77 mg) of diisopropylammonium tetrazolide was twice taken up in anh. MeCN and evaporated. To the residue in 50 ml of anh. CH_2Cl_2, 1.1 mmol (0.36 g) of (2-cyanoethoxy)bis(diisopropylamino)phosphine were added with stirring. Stirring was continued for 3 h at r.t. Then, the mixture was poured into 100 ml of sat. $NaHCO_3$ soln. and extracted with CH_2Cl_2 (3 × 100 ml), and the combined org. layer dried and evaporated: 1.6 g of crude **4a** as an oil. CC (35 g of silica gel, Et_2O/pentane/Et_3N 79:20:1) and coevaporation of the pure fractions (TLC (Et_2O): R_f 0.63 and 0.70; diastereoisomers) with MeCN (several times) gave 860 mg of pure **4a** as diastereoisomers. ^{31}P-NMR ($CDCl_3$): 154.7, 155.8 (*2s*, 1:1, diastereoisomers). Anal. calc. for $C_{63}H_{75}N_4O_{10}PS \cdot 0.5$ H_2O (1120.355): C 67.54, H 6.84, N 5.00; found: C 67.49, H 7.09, N 5.21.

5. *2′,3′-*O*-Dibenzoyl-3-undecyluridine 5′-[(2-Cyanoethyl) Diisopropylamino Phosphite]* (**4b**). Compound **3b** (1.3 mmol, 789 mg) was evaporated 3 times after the addition of anh. MeCN. The residue was taken up in 50 ml of

CH_2Cl_2, and 2.1 mmol (0.65 ml) of (2-cyanoethoxy)bis(diisopropylamino)phosphine and 1.05 mmol (180 mg) of diisopropylammonium tetrazolide were added. After 5 h at r.t. under Ar, the mixture was poured into 50 ml of sat. $NaHCO_3$ soln. and extracted with CH_2Cl_2. The combined org. layers were dried (Na_2SO_4) and evaporated: 1.5 g of crude **4b**. This oil was purified by CC (silica gel, Et_2O). From the pure fractions (TLC (Et_2O): R_f 0.64 and 0.71; diastereoisomers), 830 mg (81%) of **4b** were obtained. ^{31}P-NMR: 149.6, 150.8 (2*s*, 1.0:1.5, diastereoisomers). Anal. calc. for $C_{42}H_{59}N_4PO_9 \cdot 0.5$ MeCN (815.45): C 63.34, H 7.48, N 7.72; found: C 63.28, H 7.50, N 7.67.

6. *d(A-A-A-A-C-G-A-C-G-G-C-C-A-G-T-G)* (**7**) *and Fragments* **8**–**10** *thereof.* The synthesis was started with 600 mg of CPG-support derivatized with G (18 μmol). For each elongation, we applied 120 μmol (100 mg) of the corresponding (2-cyanoethyl) phosphoramidite and 470 μmol (33 mg) of tetrazole in 1 ml of anh. MeCN. At the heptamer, the decamer, the tridecamer, and the final hexadecamer stage, 100 μg of the support were removed after cleavage of the $(MeO)_2$Tr group, washed with MeCN and Et_2O, and dried. For the further deprotection, 30 mg of support of each sequence was treated with 700 μl of conc. NH_3 at 67° for 1.5 h 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 100 μl of H_2O and centrifuged (10 min, 0°). From the supernatant, the DNA was precipitated by addition of 200 μl of dioxane and 600 μl of THF. After centrifuging (15 min, 0°), the pellet was dissolved in H_2O and part of the material of each sequence (corresponding to 1 mg of support) was checked by gel electrophoresis on a 20% denaturing polyacrylamide gel (*Fig. 1*, lanes *1–4*).

7. *Coupling of the Purification Handle* **4a** *and Release of the Thiol Function.* To 100 mg of support to which the hexadecamer was attached in the completely protected form but lacking the 5′-$(MeO)_2$Tr group, the purification handle **4a** (15 μmol, 15 mg) was coupled in the presence of 235 μmol (17 mg) of tetrazole in 0.5 ml of anh. MeCN during 5 min *(Scheme 2)*. After several washings with anh. MeCN, the coupling was repeated with the same amounts. After oxidation with I_2 soln. (30 s), the support was washed with MeCN and Et_2O and dried. The deprotection of 30 mg of support was performed as described above for the sequences **7**–**10** and yielded **5**. Part of the material was checked by gel electrophoresis (*Fig. 1*, lane *5*). Crude material from sequence **5** corresponding to 20 mg of support was dissolved in 400 μl of 0.1M TEAA (= triethylammonium acetate; pH 7.0) and treated with 180 μl of 100 mM aq. $AgNO_3$ (2 h). Then, 210 μl of 100 mM DTE were added, and after 1 h at r.t., the suspension was centrifuged for 15 min at 0°. The supernatant was evaporated at the speed-vac concentrator, **6** taken up in H_2O, and part of the material checked by gel electrophoresis (*Fig. 1*, lane *6*).

8. *Purification.* A mixture **6**–**10** was prepared by mixing *ca.* 0.13 μmol of each crude oligonucleotide in a total volume of 200 μl of H_2O. The activated thiol support (200 mg) was washed with DMF, *Tris* · HCl (pH 7, 50 mM), H_2O, MeOH, Et_2O and dried. It was transferred into an *Eppendorf* tube with a screw cap, and the mixture of the oligonucleotide as well as 600 μl of *Tris* · HCl (pH 7.0, 50 mM) were added. After gentle shaking for 1 h, the supernatant was removed and the support washed with H_2O (4 × 200 μl). The washing solns. were evaporated and investigated by gel electrophoresis showing that they contained predominantly **7**–**10** (*Fig. 2*, lane *2*). The support was washed with H_2O, and MeOH; and Et_2O and dried.

The support (100 mg) was treated with 1 ml of 50 mM $NaIO_4$ (15 min) and washed with H_2O (4 × 250 μl). Then it was treated for 30 min with 1 ml of 5% aq. Et_3N soln. The Et_3N soln. was removed and the support washed with H_2O (4 × 250 μl). The combined Et_3N and H_2O solns. were evaporated on a speed-vac concentrator. The pellet was dissolved in H_2O and desalted by passing through a short *Sephadex* column (*NAP*). Part of this material was checked by gel electrophoresis showing that the pure 5′-phosphorylated d(A-A-A-A-C-G-A-C-G-G-C-C-A-G-T-G) was obtained by this procedure (*Fig. 2*, lane *4*).

In a separate experiment, the other 100 mg of support were taken up in 0.2M DTE in 300 μl *Tris* · HCl (pH 8.5). After treatment for 1 h, the suspension was centrifuged and the support washed with H_2O (5 × 200 μl). The combined solns. were evaporated at the speed-vac concentrator. Desalting was performed as described above and the material checked by gel electrophoresis showing that by this treatment pure **6** was obtained (*Fig. 2*, lane *3*).

In another set of experiments for the evaluation of the purification, based on lipophilic interactions, **4b** was coupled exactly in the same way as described for **4a** to the corresponding hexadecamer, and after deprotection and isolation, the same sequence mixtures were prepared as mentioned above. The purification was then tried on small cartridges filled with reversed-phase silica gel using the following procedure: The columns were first washed with MeOH/H_2O 1:1 and then with 1 ml of 0.1M TEAA (pH 7.0). The mixture of oligomers was taken up in 0.5 ml of 0.1M TEAA and then applied onto the prewashed reversed-phase column. This was followed by 3 × 0.3 ml of 15% MeCN in 0.1M TEAA, 2 × 0.5 ml of 0.1M TEAA, 3 × 0.3 ml of 50 mM $NaIO_4$, 3 × 0.3 ml of H_2O, 4 × 0.3 ml of 0.1M Et_3N, 3 × 0.3 ml of 15% MeCN in 0.1M TEAA, and 3 × 0.3 ml of 30% MeCN in 0.1M TEAA. The different eluents were collected, evaporated, and investigated by gel electrophoresis. In these experiments, no absolutely pure 5′-phosphate of **7** could be obtained.

REFERENCES

[1] M. J. Gait, in 'Oligonucleotide Synthesis', Ed. M. J. Gait, IRL Press, Oxford, 1984.
[2] H. Seliger, M. Holupirek, H.-H. Görtz, *Tetrahedron Lett.* **1978,** *19,* 2115.
[3] H. Seliger, H.-H. Görtz, *Angew. Chem.* **1981,** *93,* 709.
[4] J. Wippler, Dissertation, University of Munich, 1988.
[5] K.-M. Lo, S. S. Jones, N. R. Hackett, H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.A.* **1984,** *81,* 2285.
[6] R. Bischoff, J. M. Coull, F. E. Regnier, *Anal. Biochem.* **1987,** *164,* 336.
[7] J. Sambrook, E. F. Fritsch, T. Maniatis, in 'Molecular Cloning', 2nd edn., Ed. C. Nolan, Cold Spring Harbor Laboratory, New York, 1989.
[8] E. Uhlmann, J. Engels, *Tetrahedron Lett.* **1986,** *27,* 1023.
[9] T. Horn, M. S. Urdea, *DNA* **1986,** *5,* 421.
[10] W. Bannwarth, A. Trzeciak, *Helv. Chim. Acta* **1987,** *70,* 175.
[11] B. A. Connolly, *Tetrahedron Lett.* **1987,** *28,* 463.
[12] T. Tanaka, Y. Yamada, M. Ikehara, *Chem. Pharm. Bull.* **1987,** *35,* 2726.
[13] D. Vorländer, E. Mittag, *Ber. Dtsch. Chem. Ges.* **1913,** *46,* 3450.
[14] B. Connolly, P. Rider, *Nucleic Acids Res.* **1985,** *13,* 4485.
[15] N. D. Sinha, J. Biernat, J. McManus, H. Köster, *Nucleic Acids Res.* **1984,** *12,* 4539.
[16] L. J. McBride, M. H. Caruthers, *Tetrahedron Lett.* **1983,** *24,* 245.
[17] S. P. Adams, K. S. Kavka, E. J. Wykes, S. B. Holder, G. R. Galluppi, *J. Am. Chem Soc.* **1983,** *105,* 661.
[18] W. Bannwarth, *Chimia* **1987,** *41,* 302.
[19] H. R. Kiefer, W. Bannwarth, 'Immunol. Methods 3', Eds. I. Lefkovits and B. Pernis, Academic Press, New York, 1985, p. 69.
[20] B. J. Hunt, W. Rigby, *Chem. Ind. (London)* **1967,** 1868.
[21] A. D. Barone, J. Y. Tang, M. H. Caruthers, *Nucleic Acids Res.* **1984,** *12,* 4051.