Noncovalent Attachment of Nucleotides by Fluorous–Fluorous Interactions: Application to a Simple Purification Principle for Synthetic DNA Fragments

by **Christian Beller** and **Willi Bannwarth***

Institut für Organische Chemie und Biochemie, Universität Freiburg, Albert-Strasse 21, D-79104 Freiburg (phone: +49 761 203-6073; fax: +49 761 203-8705; e-mail: willi.bannwarth@organik.chemie.uni-freiburg.de)

We report a highly efficient noncovalent immobilization of DNA fragments on surfaces by fluorous–fluorous interactions. The principle was applied to a simple and straightforward purification protocol for synthetic DNA. The method is completely compatible with standard solid-phase synthesis of DNA.

1. Introduction. – Fluorous biphasic systems (FBS) employing perfluoro-tagged compounds as reagents, catalysts, or products, which can be extracted out of organic phases with perfluorinated solvents, have gained widespread acceptance as efficient tools for the straightforward separation, recovery, and reuse of catalysts and reagents as well as for simplified product isolation [1–3]. Nevertheless, the principle is fraught with the problem of high costs of perfluorinated solvents as well as with their high environmental persistance. As a consequence, solid-phase extractions (SPEs) on fluorous reversed-phase silica gel (FRPSG) were developed as an alternative approach [4][5]. Furthermore, FRPSG was used to support catalysts in organic solvents [6][7] and in a 'catch-and-release' approach for a multistep synthesis [8]. All the above-mentioned examples were bound to perfluoro-alkyl tags in organic solvents. These tags reveal a higher hydrophobicity compared to linear alkyl chains. In addition, fluorous–fluorous interactions are unique, stronger, and clearly distinguishable from lipophilic interactions of alkyl units so that they can be dubbed as fluorophilic interactions [9][10].

Lipophilic interactions are the result of an increase of entropy of the solvent during self-organization of lipophilic entities. This effect is especially pronounced in H_2O as solvent and plays an important role in the stabilization of tertiary structures of proteins by lipophilic interactions. Due to the higher hydrophobicity and the rigid structure of perfluoroalkyl chains, the hydrophobic effect in H_2O should be even more intense than that of linear alkyl groups. Hence, perfluoroalkyl tags should represent ideal tools for effective noncovalent immobilization of H_2O-soluble biomolecules like DNA and RNA to perfluorinated surfaces. Until now, this potential has not been demonstrated or exploited for practical applications. Here, we report a first example illustrating a simple and effective purification principle for synthetic DNA fragments.

In DNA synthesis on solid support, truncated sequences are commonly removed from the desired oligomer after deprotection by HPLC or polyacrylamide-gel electrophoresis (PAGE). An alternative, which can be performed in parallel, is the

so-called trityl-on purification (TOP) on small cartridges containing lipophilic support material. The desired sequence still carries the lipophilic dimethoxytrityl ((MeO)Tr) group and is retained on the resin while the truncated sequences lacking the $(MeO)_2Tr$ group can be removed under stringent conditions. The desired sequence is eluted following acidic cleavage of the $(MeO)_2Tr$ group. The system has the disadvantage that the hydrophobicity of the $(MeO)_2Tr$ group is not very distinct so that the isolation efficiency decreases rapidly with increasing chain length. Furthermore, 5′-$(MeO)_2Tr$-protected but otherwise unprotected DNA fragments are very labile with respect to 5′-deprotection, resulting often in losses in yield of the desired fragment.

To circumvent these problems and to demonstrate the potential of fluorous–fluorous interactions in aqueous systems, we replaced the $(MeO)_2Tr$ group with a perfluoro-tagged monomethoxytrityl (F-(MeO)Tr) group and, to create even more stability towards acidic conditions, with a perfluoro-tagged trityl (F-Tr) group. We applied them in combination with FRPSG. For the chain length of the perfluoro tag, we selected C_8F_{17}, since longer tags create solubility problems and shorter tags show limited interaction properties. Fluorous–fluorous interactions can be drastically enhanced by cooperative effects, so we introduced two perfluoroalkyl chains in the anchor unit [9][10].

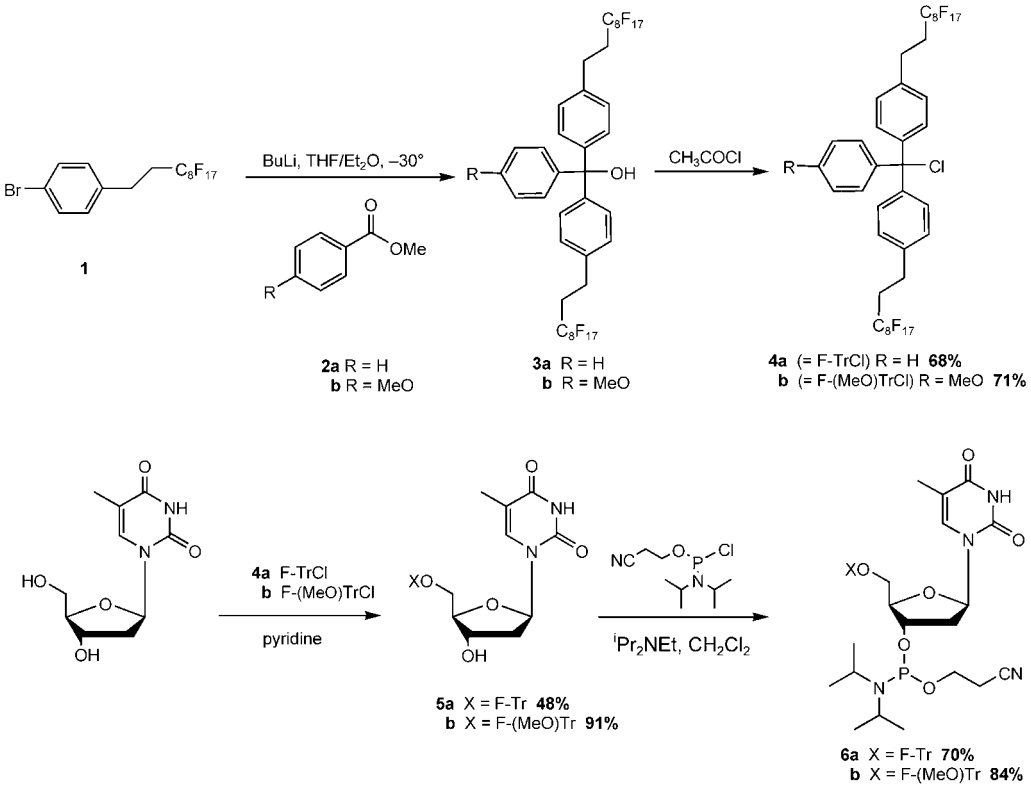
2. Results and Discussion. – Since the new purification system should be completely compatible with standard DNA synthesis on solid support, we synthesized building blocks **6a** and **6b** according to the *Scheme*. These can be introduced as the last building block in the course of a solid-phase synthesis by the phosphoramidite procedure. The corresponding perfluoro-tagged trityl chlorides (F-(MeO)TrCl and F-TrCl) were synthesized starting from 1-bromo-4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)benzene (**1**) [11] which was submitted to a Br/Li exchange and brought to reaction with either methyl benzoate (**2a**) or with methyl 4-methoxybenzoate (**2b**), yielding the desired trityl alcohols **3a** and **3b**, respectively, which were directly transformed to the corresponding chlorides F-TrCl **4a** and F-(MeO)TrCl **4b** with acetyl chloride in an overall yield of 68 and 71%, respectively.

The perfluoro-tagged trityl chlorides **4a** and **4b** were then regioselectively introduced at the 5′-position of thymidine. Phosphitylation with 2-cyanoethyl diisopropylphosphoramidochloridite in the presence of *N*,*N*-diisopropylethylamine yielded the desired phosphoramidites **6a** and **6b**, which were obtained after short column chromatography as a mixture of diastereoisomers. Although perfluorinated compounds show at times a high propensity for oxidation due to their ability to dissolve oxygen in perfluoroalkyl chains [12], no oxidation from P^{III} to P^{V} was observed as indicated by the ^{31}P-NMR spectra (see *Exper. Part*).

The perfluoro-tagged silica gels used in this study, *i.e.*, the FRPSGs **7a** and **7b** (*Fig. 1*), were prepared by our standard procedures [8].

Although we had synthesized both phosphoramidites **6a** and **6b**, the evaluation was first carried out with **6b**. During the synthesis of a $(dT)_{20}$ oligomer, we experienced that phosphoramidite **6b**, despite its high hydrophobicity, could be introduced as the last building block with high efficiency under standard conditions by activation with 5-(benzylthio)-1*H*-tetrazole (BTT) in MeCN [13]. This high coupling efficiency was also revealed by PAGE analysis of the ammonia-deprotected fragment (*Fig. 2*). This PAGE

Scheme. *Synthesis of Building Blocks* **6a** *and* **6b**



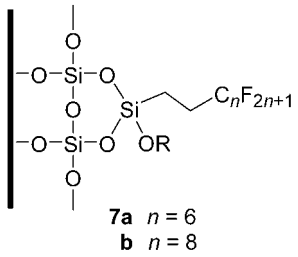


Fig. 1. *Fluorous reversed-phase silica gels applied in this study*

also indicated, that F-(MeO)Tr-$(dT)_{20}$ (**10**) showed no mobility at all and remained in the well, whereas the mobility of $(MeO)_2$Tr-$(dT)_{20}$ (**9**) was only slightly decreased as compared to that of $(dT)_{20}$ (**8**).

For an evaluation of the aspired purification method on the basis of fluorous–fluorous interactions as outlined in *Fig. 3*, *i.e.*, of fluorous trityl-on purification (F-TOP), we synthesized the DNA sequences **11**–**15**.

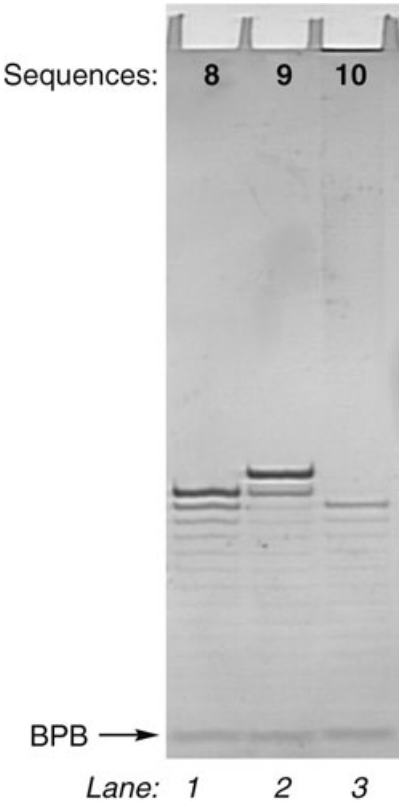


Fig. 2. *Polyacrylamide gel* (20%), *stained with 'Stains-all' solution: Synthesis of DNAs* **8**–**10**. *Lane 1:* Crude (dT_{20}) (**8**); *Lane 2:* $(MeO)_2$Tr-(dT_{20}) (**9**); *Lane 3:* F-(MeO)Tr-(dT_{20}) (**10**).

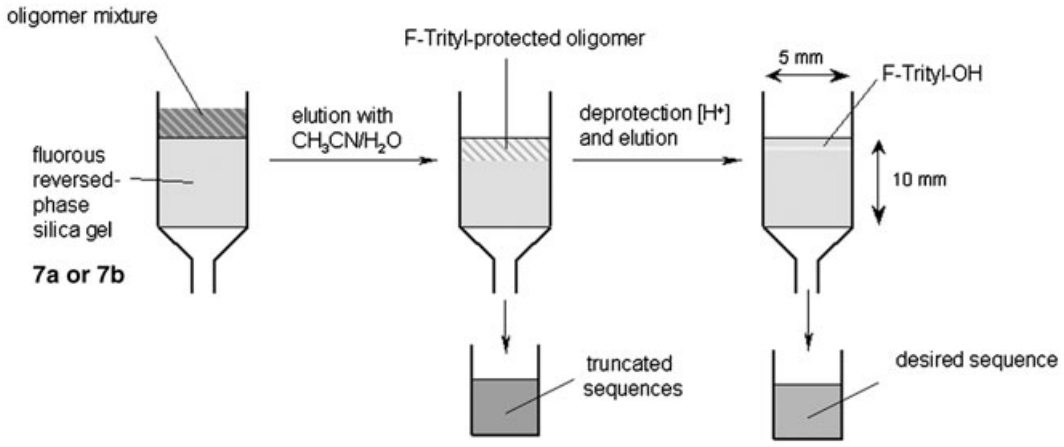


Fig. 3. *F-TOP Method*

5′-d(ACA GGA CCT)-3′ (**11**)
5′-d(AAC ACA GGA CCT)-3′ (**12**)
5′-d(TCG AAC ACA GGA CCT)-3′ (**13**)
5′-F-(MeO)Tr-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**14**)
5′-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**15**)

Again we were able to demonstrate that building block **6b** could be coupled under standard conditions with high efficiency. For the evaluation of the new purification principle, the perfluoro-tagged sequence **14** was spiked with the sequences **11**–**13** so that the actual concentration of **14** in the mixture was *ca.* 30%. The purification on FRPSG **7a** was then carried out according to the following protocol: *1.* Addition of an equal volume of binding buffer to the sample (2 ml maximum, total combined volume); *2.* tube conditioning (1 ml of MeCN, 1 ml of $(Et_3NH)OAc$ buffer solution); *3.* sample addition (1-ml aliquots); *4.* purification (1 ml of MeCN/$(Et_3NH)OAc$ solution 15:85); *5.* detritylation (1 ml of 5% CF_3COOH solution); *6.* rinsing (1 ml of $(Et_3NH)OAc$ solution, 1 ml of H_2O); *7.* elution (1 ml of MeCN/H_2O 50:50). The results were evaluated by PAGE (*Fig. 4*). The interaction between the perfluoro-tagged 30mer **14** and the FRPSG was so intense that all truncated sequences could be removed by a neutral aqueous-phase washing step (*Lane 7*). Cleavage of the perfluoro tag under

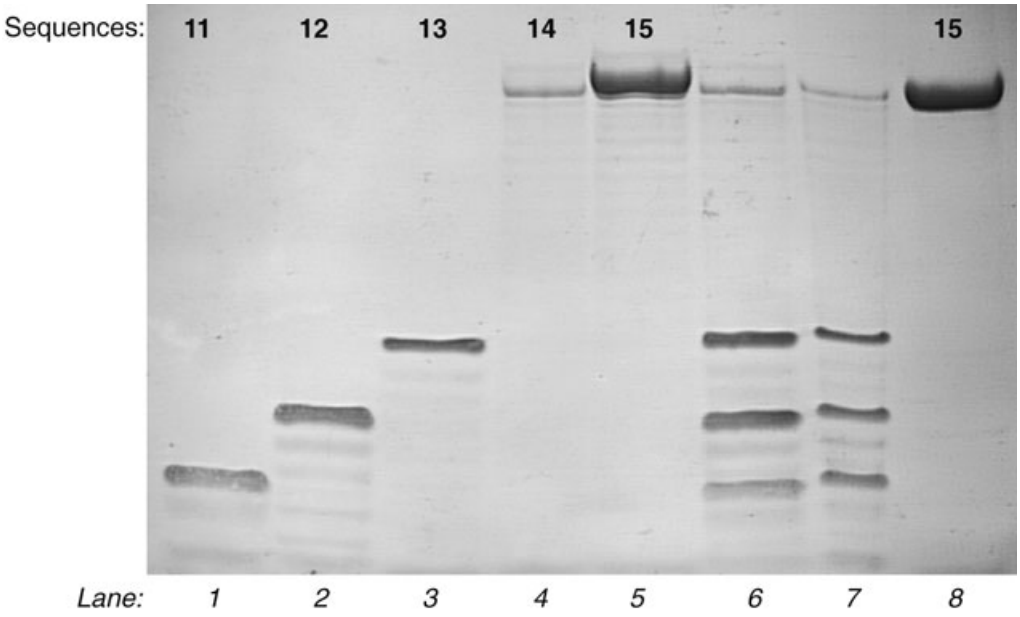


Fig. 4. *Polyacrylamide gel* (20%), *stained with 'Stains-all' solution: F-TOP results. Lane 1:* crude 5′-d(ACA GGA CCT)-3 (**11**); *Lane 2:* crude 5′-d(AAC ACA GGA CCT)-3 (**12**); *Lane 3:* crude 5′-d(TCG AAC ACA GGA CCT)-3 (**13**); *Lane 4:* crude 5′-F-(MeO)Tr-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**14**); *Lane 5:* crude 5′-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**15**); *Lane 6:* mixture **11**–**14**; *Lane 7:* eluted failure sequences; *Lane 8:* pure 5′-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**15**).

acidic conditions followed by a washing step yielded fragment **15** in pure form (*Lane 8*). Up to 40% of pure fragment with respect to the crude material after deprotection were obtained. These results were also confirmed by HPLC (*Fig. 5*).

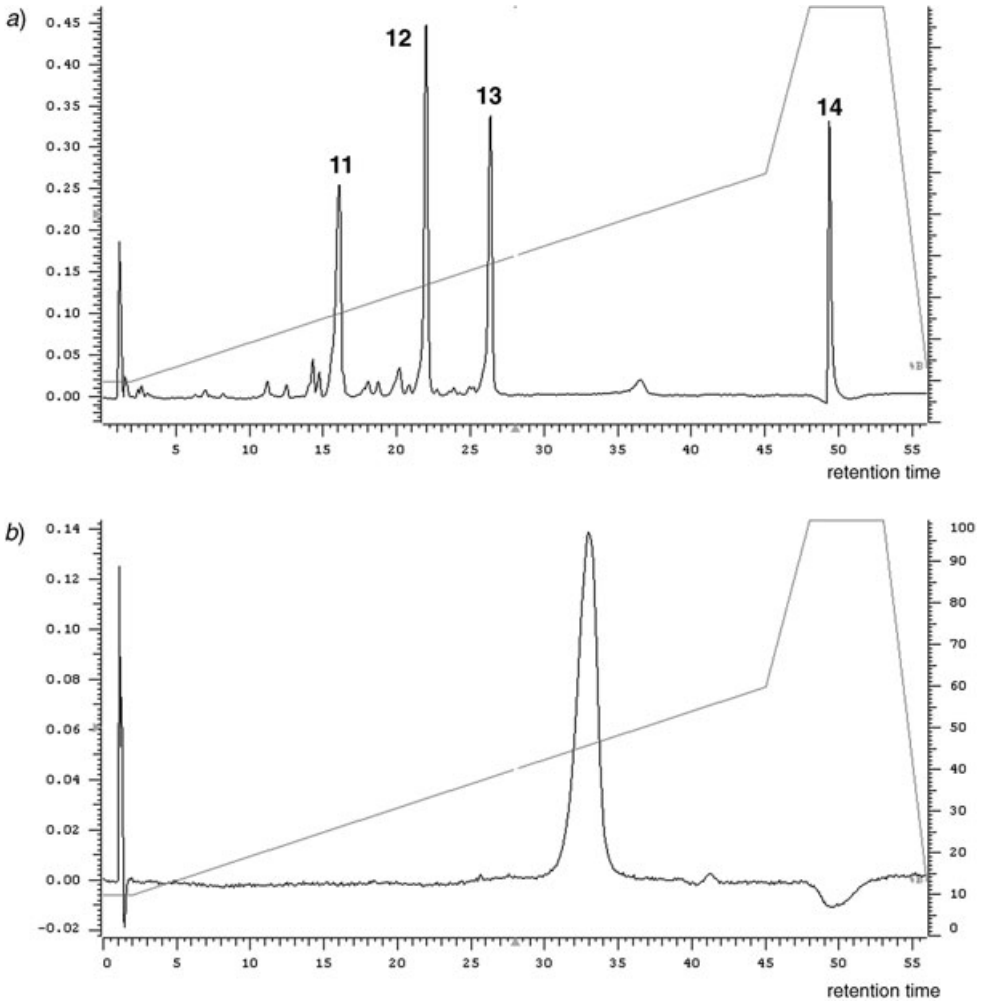


Fig. 5. *Anion-exchange HPLC* a) *of oligomer mixture* **11**–**14** *and* b) *of fluorous-trityl-on-purified* (F-TOP) *5′-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′* (**15**)

The exceptional efficiency of the purification method was further demonstrated when a mixture of the sequences **14**, $(dT)_9$ (**16**), $(dT)_{12}$ (**17**), and $(dT)_{15}$ (**18**) was additionally spiked with a sequence $(dT)_{40}$ (**19**): *Fig. 6* illustrates that the perfluoro-tagged 30mer **14** was attached so efficiently on the perfluorinated silica gel that even $(dT)_{40}$ (**19**) could be removed (*Lane 3*).

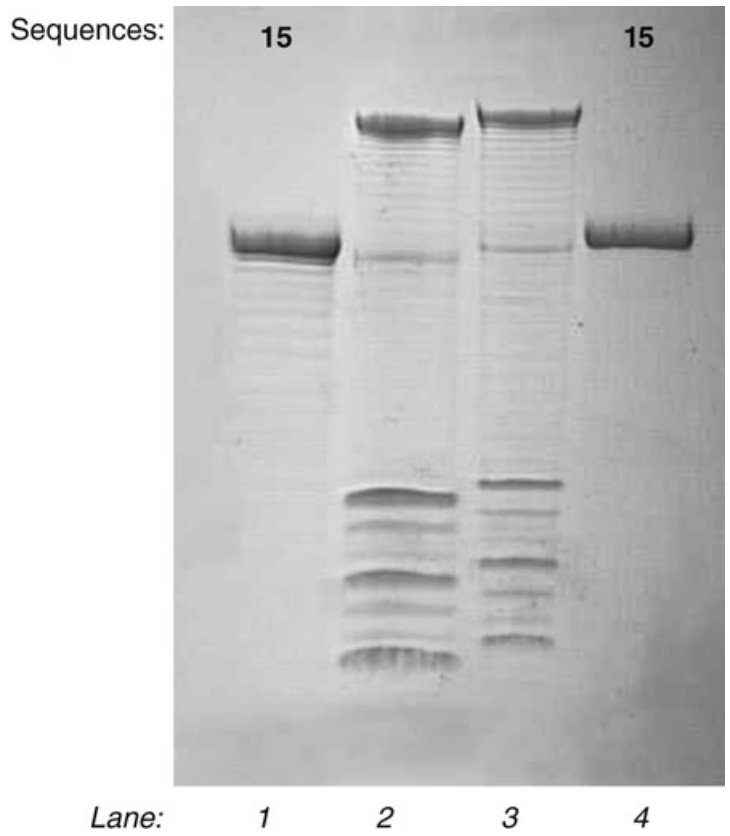


Fig. 6. *Polyacrylamide gel* (20%), *stained with 'Stains-all' solution: F-TOP results. Lane 1:* crude 5′-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**15**); *Lane 2:* mixture of crude $(dT)_9$ (**16**), crude $(dT)_{12}$ (**17**), crude $(dT)_{15}$ (**18**), crude 5′-F-(MeO)Tr-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**14**), and crude $(dT)_{40}$ (**19**); *Lane 3:* eluted failure sequences (**16**–**19**); *Lane 4:* pure 5′-d(TCG AAC ACA GGA CCT TCG AAC ACA GGA CCT)-3′ (**15**).

In our hands, no difference in efficiency was observed between the perfluorinated silica gels **7a** and **7b**. The same holds true for the phosphoramidites **6a** and **6b**.

Conclusions. – In aqueous phase, interactions of perfluoroalkyl tags can become so intense as to allow an efficient fixation of polyanionic DNA fragments on perfluorinated matrices. We have used this interaction for the development of a simple purification method for synthetic DNA fragments that is completely compatible with standard solid-phase synthesis. We are confident that noncovalent attachments in aqueous phase mediated by fluorous–fluorous interactions are not limited to DNA fragments and can be extended to the immobilization of RNA, oligosaccharides, peptides, and proteins.

We would like to thank Dr. *M. Keller*, Mrs. *M. Schonhard*, and Mr. *F. Reinbold* for recording NMR spectra, Mr. *C. Warth* and Dr. *J. Wörth* for recording mass spectra, *Mr. Hickl* for performing elemental analyses, and Mr. *A. Drechsle* and Mr. *R. Kramer* for excellent technical assistance.

Experimental Part

General. All reagents were of the highest purity available. THF was dried over Na/benzophenone and was freshly distilled before use. FRPSGs **7a** and **7b** were prepared from *sds* silica gel *Silice 60 A* (35–70 μm, 550 m^2/g) according to [8], 1-bromo-4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)benzene (**1**) according to [11], and 2-cyanoethyl diisopropylphosphoramidochloridite according to [14]. DNA Synthesis was performed on controlled-pore glass (CPG) as solid support as described in [15]. The syntheses of the DNA fragments were performed on an *Expedite™-8909* nucleic acid synthesizer. Ultrapure water was obtained by a *Direct-Q™-Millipore* system. Anion-exchange HPLC: *Merck-Hitachi* system: anal. *DNAPac®-PA-100* ion-exchange column (4 × 250 mm) from *Dionex*. F-TOP: FRPSG resins were packed into polypropylene tubes between 20 μm polyethylene frits from *Varian*; binding buffer and (Et_3NH)OAc soln. from *Varian*. PAGE: polyacrylamide gels were stained with a soln. of 3,3′-diethyl-9-methyl-4,5:4′,5′-dibenzothiacarbocyanine bromide ('Stains-all'; *Fluka*). UV Spectra: *Perkin-Elmer Lambda-35* UV/VIS spectrometer. ^{1}H- (300 MHz), ^{13}C- (125 MHz), and ^{31}P-NMR (121 MHz): chemical shifts δ in ppm rel. to $SiMe_4$ and H_3PO_4, resp., J in Hz. MS: *Finnigan-MAT8200* (EI), *-MAT 312* (CI), and *-TSQ-7000* (ESI) mass spectrometer; in m/z (rel. %).

1,1′-(Phenylmethylene)bis[4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)benzene] (**4a**). A soln. of **1** (4.64 g, 7.7 mmol) in Et_2O/THF 1:1 (60 ml) was cooled to −30°. Then 1.6M BuLi in hexane (4.8 ml, 7.7 mmol) and a soln. of methyl benzoate (481 μl, 2.5 mmol) in Et_2O (10 ml) were carefully added one after the other. The yellow mixture was allowed to warm up to r.t. and was stirred for 30 min. The mixture was quenched with an equal volume of sat. NH_4Cl soln. and extracted with Et_2O (3 × 50 ml). The org. phase was dried (Na_2SO_4) and evaporated. The crude product **3a** was converted into **4a** without further purification. For this, a soln. of **3a** in acetyl chloride (30 ml) was refluxed for 2 h. Recrystallization in MeCN/acetyl chloride 1:1 (20 ml) at −20°, gave 3.08 g (68%) of **4a**. ^{1}H-NMR ($CDCl_3$): 2.20–2.42 (*m*, 2 $C_8F_{17}CH_2CH_2$); 2.80–2.92 (*m*, 2 $C_8H_{17}CH_2CH_2$); 7.00–7.30 (*m*, 13 arom. H). ESI-MS (MeOH/MeCl 21:1, 400 μl/min, 3.5 kV): 1135 (100, $[M-Cl]^+$). Anal. calc. for $C_{39}H_{21}ClF_{34}$ (1170.98): C 40.00, H 1.81; found C 41.01, H 2.43.

1,1′-[Chloro(phenyl)methylene]bis[4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)benzene] (**4b**). As described for **4a**, with **1** (8.10 g, 13.45 mmol) 1.6M BuLi in hexane (8.40 ml, 13.45 mmol), and methyl 4-methoxybenzoate (1.12 g, 6.73 mmol) in Et_2O/THF 1:1 (100 ml). Reaction with acetyl chloride (50 ml) yielded 5.72 g (71%) of **4b**. ^{1}H-NMR ($CDCl_3$): 2.20–2.40 (*m*, 2 $C_8F_{17}CH_2CH_2$); 2.80–2.92 (*m*, 2 $C_8H_{17}CH_2CH_2$); 3.78 (*s*, MeO); 6.82 (*dd*, J = 9.0, 2 arom. H); 7.10–7.30 (*m*, 10 arom. H). CI-MS (NH_3, 200°, 160 eV): 1165 (42, $[M-Cl]^+$), 1075 (12), 659 (59), 331 (54), 299 (22), 266 (44), 249 (75), 259 (34), 206 (100). Anal. calc. for $C_{40}H_{23}ClF_{34}O$ (1201.01): C 40.00, H 1.93; found: C 40.15, H 2.14.

5′-O-{Bis[4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)phenyl]phenylmethyl}thymidine (**5a**). Thymidine (726 mg, 3.00 mmol) was dried by azeotropic co-evaporation with pyridine and dissolved in anh. pyridine (20 ml). A soln. of **4a** (4.14 g, 3.54 mmol) in benzotrifluoride (=(trifluoromethyl)benzene = BTF; 10 ml) was added to the mixture, which was stirred overnight at r.t. The reaction was quenched with MeOH (5 ml) and stirred for 30 min. The solvent was evaporated and the crude material purified by short column chromatography (silica gel, deactivation with 1% Et_3N, cyclohexane/AcOEt 98:2): 1.98 g (48%) of pure **5a**. ^{1}H-NMR ($CDCl_3$): 1.20 (*s*, Me); 1.95 (br. *s*, OH); 2.20–2.40 (*m*, 2 $C_8F_{17}CH_2CH_2$, 2 H–C(2′)); 2.78–2.88 (*m*, 2 $C_8F_{17}CH_2CH_2$); 3.30 (*dd*, J = 10.5, 3.4, 1 H–C(5′)); 3.40 (*dd*, J = 10.6, 3.4, 1 H–C(5′)); 3.96 (*dd*, J = 6.1, 3.4, H–C(4′)); 4.50 (*m*, H–C(3′)); 6.31 (*t*, J = 6.7, H–C(1′)); 6.78 (*d*, J = 8.9, 2 arom. H); 7.10 (*d*, J = 8.4, 4 arom. H); 7.18–7.39 (*m*, 9 arom. H); 7.43 (*s*, H–C(6)); 8.18 (*s*, NH). Anal. calc. for $C_{49}H_{34}F_{34}N_2O_5$ (1376.75): C 42.75, H 2.49, N 2.03; found: C 42.94, H 2.76, N 2.00.

5′-O-{Bis[4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)phenyl](4-methoxyphenyl)methyl}-thymidine (**5b**). As described for **5a**, with thymidine (960 mg, 3.97 mmol) and **4b** (5.70 g, 4.70 mmol): 5.09 g (91%) of **5b.** ^{1}H-NMR ($CDCl_3$): 1.20 (*s*, Me); 2.18–2.40 (*m*, 2 $C_8F_{17}CH_2CH_2$, 2 H–C(2′)); 2.78–2.90 (*m*, 2 $C_8F_{17}CH_2CH_2$); 3.29 (*dd*, J = 10.6, 3.4, 1 H–C(5′)); 3.39 (*dd*, J = 10.6, 3.4, 1 H–C(5′)); 3.73 (*s*, MeO); 3.97 (*dd*, J = 6.2, 3.4, H–C(4′)); 4.50 (*dd*, J = 6.2, 3.4, H–C(3′)); 6.32 (*t*, J = 6.8, H–C(1′)); 6.78 (*d*, J = 8.9, 2 arom. H); 7.09 (*d*, J = 8.3, 4 arom. H); 7.19 (*d*, J = 8.9, 2 arom. H); 7.28 (*d*, J = 8.3, 4 arom. H); 7.46 (*s*, H–C(6)); 8.38 (*s*, NH). Anal. calc. for $C_{50}H_{36}F_{34}N_2O_6$ (1406.78): C 42.69, H 2.58, N 1.99; found: C 43.15, H 2.88, N 1.91.

5′-O-{Bis[4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)phenyl]phenylmethyl}thymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**6a**). Compound **5a** (840 mg, 0.61 mmol) was dried by co-evaporation with MeCN (3 × 10 ml) and then dissolved in anh. CH_2Cl_2 (20 ml). Diisopropylethylamine (256 μl, 1.50 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (162 μl, 0.73 mmol) were added by syringe. The mixture was stirred at r.t. overnight. The reaction was quenched with sat. $NaHCO_3$ soln. and extracted with CH_2Cl_2 (3 × 30 ml). The org. layer was dried (Na_2SO_4) and evaporated. Purification of the crude product by short column

chromatography (silica gel, deactivation with 1% Et_3N, cyclohexane/AcOEt 2:1) yielded 680 mg (70%) of **6a**. ^{1}H-NMR ($CDCl_3$): 0.95–1.13 (*m*, 2 Me_2CH); 1.19 (*s*, 1 Me); 2.20–2.40 (*m*, 2 $C_8H_{17}CH_2CH_2$, 2 H–C(2′)); 2.57 (*t*, *J* = 6.4, CH_2CH_2CN); 2.80–2.85 (*m*, 2 $C_8H_{17}CH_2CH_2$); 3.22 (*m*, 1 H–C(5′)); 3.30(*m*, 1 H–C(5′)); 3.38–3.82 (*m*, CH_2CH_2CN, 2 Me_2CH_2); 4.08 (*m*, H–C(4′)); 4.61 (*m*, H–C(3′)); 6.32 (*m*, H–C(1′)); 7.10 (*m*, 4 arom. H); 7.18–7.35 (*m*, 9 arom. H); 7.44 (*s*, H–C(6)); 8.23 (*s*, NH). ^{31}P-NMR ($CDCl_3$): 149.9, 150.0 (mixture of diastereoisomers). ESI-MS (MeOH, 300 µl/min, 2.5 kV): 1599 (93, $[M+Na]^+$), 1460 (100), 1359 (20), 1135 (52).

5′-O-{Bis[4-(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecylfluorodecyl)phenyl](4-methoxyphenyl)methyl}-thymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite) (**6b**). To **5b** (1 g, 0.73 mmol), *N*,*N*-diisopropylethylamine (311 µl, 1.82 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (207 mg, 0.88 mmol) were added. After 16 h, workup as described for **6a** yielded 966 mg (84%) of **6b**. ^{1}H-NMR ($CDCl_3$): 0.93–1.15 (*m*, 2 Me_2CH); 1.20 (*s*, 1 Me); 2.20–2.38 (*m*, 2 $C_8F_{17}CH_2CH_2$, 2 H–C(2′)); 2.55 (*t*, *J* = 6.5, CH_2CH_2CN); 2.79–2.84 (*m*, 2 $C_8F_{17}CH_2CH_2$); 3.22 (*m*, 1 H–C(5′)); 3.38 (*m*, 1 H–C(5′)); 3.42–3.82 (*m*, CH_2CH_2CN, 2 Me_2CH, MeO); 4.04 (*m*, H–C(4′)); 4.58 (*m*, H–C(3′)); 6.32 (*m*, H–C(1′)); 6.77 (*d*, *J* = 8.9, 2 arom. H); 7.08 (*d*, *J* = 8.3, 4 arom. H); 7.18 (*d*, *J* = 8.9, 2 arom. H); 7.28 (*d*, *J* = 8.3, 4 arom. H); 7.47 (*s*, H–C(6)); 7.98 (*s*, NH). ^{31}P-NMR ($CDCl_3$): 149.9, 150.0 (mixture of diastereoisomers). ESI-MS (MeOH, 300 µl/min, 2.5 kV): 1629 (100, $[M+Na]^+$), 1165 (29).

Oligomer Synthesis. All oligonucleotide syntheses were performed starting with 1 µmol of immobilized nucleoside on the support. Commercially available phosphoramidites of dA, dC, dG, and dT were coupled automatedly on the synthesizer, while perfluorinated building blocks **6a** and **6b** were coupled manually on a synthesis frit [16] by using 65 mg of **6a** or **6b** in 0.3M BTT in MeCN (1 ml). Cleavage from the CPG support and removal of the base-labile protecting groups was done with 25% ammonia solution (1 ml; 2 h, 60°). The yield was determined by UV spectroscopy at 260 nm.

Fluorous Trityl-On Purification (F-TOP). F-TOPs were done by using the above mentioned protocol. Test samples were F-Tr- or F-(MeO)Tr-protected oligomers (5 *OD* units) spiked with nonfluorinated crude failure sequences (3 *OD* units of each).

Anion-Exchange HPLC Analysis. Aq. oligonucleotide solns. (20 µl, 0.2 *OD* units) were separated on an anal. ion-exchange column equilibrated with a mixture of solvent *A* (20 mM KH_2PO_4 in H_2O/MeCN 8:2, pH 6.0) and solvent *B* (1 mM KCl, 20 mM KH_2PO_4 in H_2O/MeCN 8:2, pH 6.0) at 30°. Gradient: 2 min 10% *B*, up to 60% *B* in 43 min, up to 100% *B* in 3 min, 5 min 100% *B*. Detection at 260 nm.

Analytical Gel Electrophoresis. Polyacrylamide gels (20%) of 0.4-mm thickness were used. Pre-electrophoresis was performed for 2 h at 500 V with a *Tris* borate running buffer. Oligonucleotides (1 µl, 0.1 *OD* units) and a bromophenol blue/xylenecyanol soln. (2 µl) were heated to 100° for 2 min and rapidly cooled to 0°. The samples were loaded with gel loader tips into the rinsed 10 × 5 mm wells of the gels. Electrophoretic separation was performed for 2 h at 500 V and 4 mA. DNA bands were visualized with 'Stains-all' soln.

REFERENCES

[1] I. T. Horváth, J. Rábai, *Science (Washington, D.C.)* **1994**, *266*, 72.
[2] A. Studer, S. Hodida, R. Ferritto, S.-Y. Kim, P. Jeger, P. Wipf, D. P. Curran, *Science (Washington, D.C.)* **1997**, *275*, 823.
[3] C. C. Tzschucke, C. Markert, W. Bannwarth, S. Roller, A. Hebel, R. Haag, *Angew. Chem.* **2002**, *114*, 4136; *Angew. Chem., Int. Ed.* **2002**, *41*, 3964.
[4] D. P. Curran, Z. Luo, *J. Am. Chem. Soc.* **1999**, *121*, 9069.
[5] D. P. Curran, *Synlett* **2001**, *9*, 1488.
[6] C. C. Tzschucke, C. Markert, H. Glatz, W. Bannwarth, *Angew. Chem.* **2002**, *114*, 4678; *Angew. Chem., Int. Ed.* **2002**, *41*, 4500.
[7] A. Biffis, M. Zecca, M. Basato, *Green Chem.* **2003**, *5*, 170.
[8] D. Schwinn, H. Glatz, W. Bannwarth, *Helv. Chim. Acta* **2003**, *86*, 188.
[9] I. de Miguel, S. Exbrayat, D. Samain, *Chromatographia* **1987**, *24*, 849.
[10] H. Glatz, C. Blay, H. Engelhardt, W. Bannwarth, *Chromatographia* **2004**, *59*, 567.
[11] S. Darsens, M. Pucheault, J.-P. Genet, *Eur. J. Org. Chem.* **2001**, *6*, 1121.
[12] S. Schneider, W. Bannwarth, *Angew. Chem.* **2000**, *112*, 4293; *Angew. Chem., Int. Ed.* **2000**, *39*, 4142.
[13] S. Pitsch, P. A. Weiss, L. Jenny, A. Stutz. X. Wu, *Helv. Chim. Acta* **2001**, *84*, 3773.
[14] M. V. Rao, C. B. Reese, V. Schehlmann, P. S. Yu, *J. Chem. Soc., Perkin Trans. 1* **1993**, 43.
[15] S. P. Adams, K. S. Kavka, E. J. Wykes, S. P. Holder, G. R. Galluppi, *J. Am Chem. Soc.* **1983**, *105*, 661.
[16] W. Bannwarth, *Chimia* **1987**, *41*, 302.

Received September 20, 2004