Efficient Solid Phase Synthesis of Cleavable Oligodeoxynucleotides Based on a Novel Strategy for the Synthesis of 5′-*S*-(4,4′-Dimethoxytrityl)-2′-deoxy-5′-thionucleoside Phosphoramidites

by **Kerstin Jahn-Hofmann** and **Joachim W. Engels***

Institut für Organische Chemie und Chemische Biologie, Johann Wolfgang Goethe-Universität, Marie-Curie-Strasse 11, D-60439 Frankfurt am Main
(e-mail: joachim.engels@chemie.uni-frankfurt.de)

The incorporation of a specific cleavage site into an oligodeoxynucleotide can be achieved by utilizing the four 5′-*S*-(4,4′-dimethoxytrityl)-2′-deoxy-5′-thionucleoside 3′-(2-cyanoethyl diisopropylphosphoramidites) **5** and **15a–c** (*Fig. 1*). Based on the silver ion assisted cleavage of P–S and C–S bonds, we synthesized oligodeoxynucleotides with an achiral 5′-phosphorothioate linkage 3′–O–P–S–5′ by the solid-phase phosphoramidite procedure. The efficient cleavage of these modified oligodeoxynucleotides can be detected by HPLC, PAGE, and surface plasmon resonance (SPR) spectrometry. The liberated 5′-thiol moiety can be used directly for post-reaction labeling with appropriately functionalized reporter groups.

1. Introduction. – Modified oligodeoxynucleotides are versatile tools to solve a variety of problems in molecular biology. The cleavage of oligodeoxynucleotides with restriction enzymes is selective and quantitative. For an equivalent chemical cleavage, the oligodeoxynucleotides must be modified beforehand. We selected the thio-modification because of its electronic and steric similarity with the natural congener and the possibility for later derivatization. These backbone modified oligodeoxynucleotides can be cleaved selectively and quantitatively at the P–S or C–S bonds by Ag^+ and Hg^{2+} ions or by a concentrated I_2 solution under mild conditions [1]. The cleavage with I_2 however, does not occur on relatively low functionalized CPG material but fortunately has been shown to cause no problem during the oxidation step in DNA synthesis [1b]. Furthermore, oligodeoxynucleotides containing a free SH group can be derivatized with thiol-specific probes [1a][2a–d][3]. Thus, the thiol group is particularly useful for post-synthetic modifications and can also be used to reversibly form disulfide bonds.

Currently, we are developing a new concept for the detection and construction of DNA arrays using 5′-thio-modified phosphoramidites **5** and **15a–c** based on the incorporation of a selective cleavage site [4].

For the synthesis of a phosphorothioate linkage, most references [1][5] describe a 5′-*S*-trityl-2′-deoxy-5′-thionucleoside 3′-phosphoramidite building block. Since we intended to use the 4,4′-dimethoxytrityl group ($(MeO)_2$Tr-group) for the 5′-protection of all four 2′-deoxynucleosides, the aim of this work was to develop an easy procedure for incorporating a 3′–O–P–S–5′ internucleotide linkage that can be adapted to a fully automated synthesis *via* phosphoramidite chemistry. Originally, for the coupling of the 5′-*S*-trityl building block, the *S*-trityl group had to be removed by Ag^+ ions. This

heavy-metal-ion cleavage is necessary because the *S*-trityl function cannot easily be cleaved by mild acids (used in the standard solid-phase phosphoramidite procedure [6]). The advantage of the *S*-[$(MeO)_2$Tr] function as 5′-protecting group over the S-trityl function is its higher lability against acid. Thus, for the 5′-*S*-[$(MeO)_2$Tr]-protected amidites during oligodeoxynucleotide synthesis, we can now apply the standard detritylation solution also for the 5′-*S*-[$(MeO)_2$Tr] cleavage. With aqueous heavy-metal-ion solutions, we observed some problems during the oligodeoxynucleotide synthesis in the synthesizer. Oxidation by an I_2 solution and several CH_2Cl_2 washes used during the synthesis gave rise to small amounts of free halogen resulting in a detrimental precipitate in the tubings. In addition, working with aqueous $AgNO_3$ solutions on CPG material made further reaction under anhydrous conditions more difficult to proceed. For these reasons, most of the users do not carry out this cleavage in the synthesizer but remove the column from the synthesizer, place a syringe on each side of the column, and flush the aqueous heavy metal ion solutions between two syringes. After this *S*-trityl cleavage, the resin (CPG material) is treated with dithiothreitol solution (reduction) to ensure that no disulfide formation has occurred. Then the column is washed, dried by several washes with CH_2Cl_2 and MeCN, and placed back in the machine, and the synthesis can be continued.

The four modified amidites **5** and **15a**–**c** described here now allow the synthesis of modified oligodeoxynucleotides by the solid-phase phosphoramidite procedure completely in the synthesizer.

Results and Discussion. – 1. *Phosphoramidite Synthesis.* To incorporate the 3′–O–P–S–5′ linkage into 2′-deoxyoligonucleotides, the phosphoramidite solid-phase synthesis was used. Therefore, we synthesized the four 5′-*S*-(4,4′-dimethoxytrityl)-2′-deoxy-5′-thionucleoside 3′-(2-cyanoethyl diisopropylphosphoramidites) **5** and **15a**–**c** (*Fig. 1*). For the 5′-*S*-(4,4′-dimethoxytrityl)-5′-thiothymidine 3′-(2-cyanoethyl diisopropylphosphoramidite) (= T amidite; **5**), we found two different synthetic approaches (see below, *Schemes 1* and *2*). But these two strategies were possible only for the $\underline{T}_d$ amidite **5**, we were unable to synthesize the $\underline{A}_d$ amidite by these strategies, which yielded a large amount of by-products, the major one being the depurinated nucleoside.

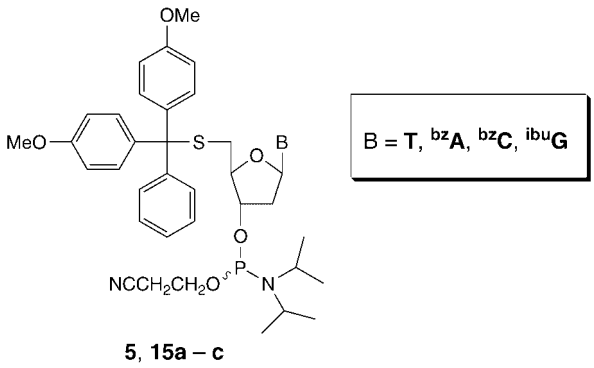

Fig. 1. *Modified monomer building blocks* **5** *and* **15a**–**c**

Thus, we needed a general method for the conversion of nucleosides into the correspondending thiols (see below, *Scheme 3*).

The starting material for the synthesis of **5** by the strategy of *Scheme 1* is thymidine (**1a**). Treatment of **1a** with TsCl gave the *p*-toluenesulfonate **2** (in 68% yield [7]), which was converted in acceptable yield (58%) with potassium thioacetate in acetone to compound **3** [8]. Using basic medium (NaOH, NaOMe, NH_3 in MeOH) for the 5′-deprotection of **3**, we isolated the symmetric disulfide that was formed in high yield. But when we used an acidic medium for deprotection (HCl in MeOH), the free 5′-thiol was liberated [7], which reacted with 4,4′-dimethoxytrityl chloride ($(MeO)_2$Tr-Cl) in H_2O and AcOH to give compound **4** in 68% yield, after purification by flash chromatography [9]. The transformation of the 5′-*S*-acetyl-5′-thiothymidine **3** to the 5′-*S*-(4,4′-dimethoxytrityl)-5′-thiothymidine (**4**) was achieved under conditions usually applied for standard deprotection of the 5′-*O*-(4,4′-dimethoxytrityl) group. The greater nucleophilicity of the SH in contrast to the OH group is the reason why we were successful under these conditions. Treatment of **4** with 2-cyanoethyl diisopropylphosphoramidochloridite in the presence of $^{i}Pr_2NH$ at room temperature gave the corresponding modified phosphoramidite **5** in 73% yield [1b].

The second method for the synthesis of $\underline{T}_d$ amidite **5** (*Scheme 2*) started with the same material, thymidine (**1a**). *Reese* and co-workers introduced 9-(4-methoxyphenyl)-9*H*-xanthene-9-thiol (AXT) for inserting the thio modification preferentially in the synthesis of 2′-thioadenosine [10]. We followed his method for the synthesis of our 2′-deoxy-5′-thionucleosides **4** and **14 a**–**c**. Treatment of the 5′-OH group with CCl_4 and triphenylphosphine gave **6** in 73% yield [8]. The 5′-chloro-thymidine (**6**) reacted with 9-(4-methoxyphenyl)-9*H*-xanthene-9-thiol (AXT) and 1,1,3,3-tetramethylguanidine in 70–95% yield to 5′-*S*-[9-(4-methoxyphenyl)-9*H*-xanthen-9-yl]-5′-thiothymidine (**7**) [10]. The easiest way would be to phosphitylate this compound and to use it in an automated synthesis *via* phosphoramidite chemistry. But we could not apply the standard solution for detritylation (3% $CHCl_2COOH$ in CH_2Cl_2); the reactivity towards this acid was too low. So we needed the 5′-*S*-[$(MeO)_2$Tr]-protected amidites for an automated synthesis. We converted the 5′-*S*-[9(4′-methoxyphenyl)-9*H*-xanthen-9-yl]-5-thiothymidine (**7**) into the 5′-*S*-(4,4′-dimethoxytrityl)-5′-thiothymidine (**4**) [11] by treatment with $AgNO_3$ in MeOH and pyridine, trituration of the obtained Ag salt with MeOH/AcOH prior to H_2S bubbling (→**8**; yield 68% after workup), and treatment of the intermediate thiol **8** with $(MeO)_2$TrCl in pyridine. The final phosphitylation of compound **4** with 2-cyanoethyl diisopropylphosphoramidochloridite in the presence of $^{i}Pr_2NEt$ at 4° gave the corresponding modified phosphoramidite **5** in 73% yield [1b].

The synthetic procedures according to *Schemes 1* and *2* could not be applied to the other amidites. Thus, we envisaged to prepare the four natural 5′-thionucleosides by introducing the $(MeO)_2$Tr group with the thiol reagent **12** according to *Scheme 3*. Since we did not find thiol **12** in the literature, we synthesized it from 4,4′-dimethoxytriphenylmethanol (**11**)[1]) which was obtained in turn by a *Grignard* reaction of *p*-bromoanisole (**9**) and methyl benzoate (**10**) in THF. We converted **11** to methanethiol **12** under the conditions used by *Reese* and co-workers for the synthesis of the AXT reagent [10].

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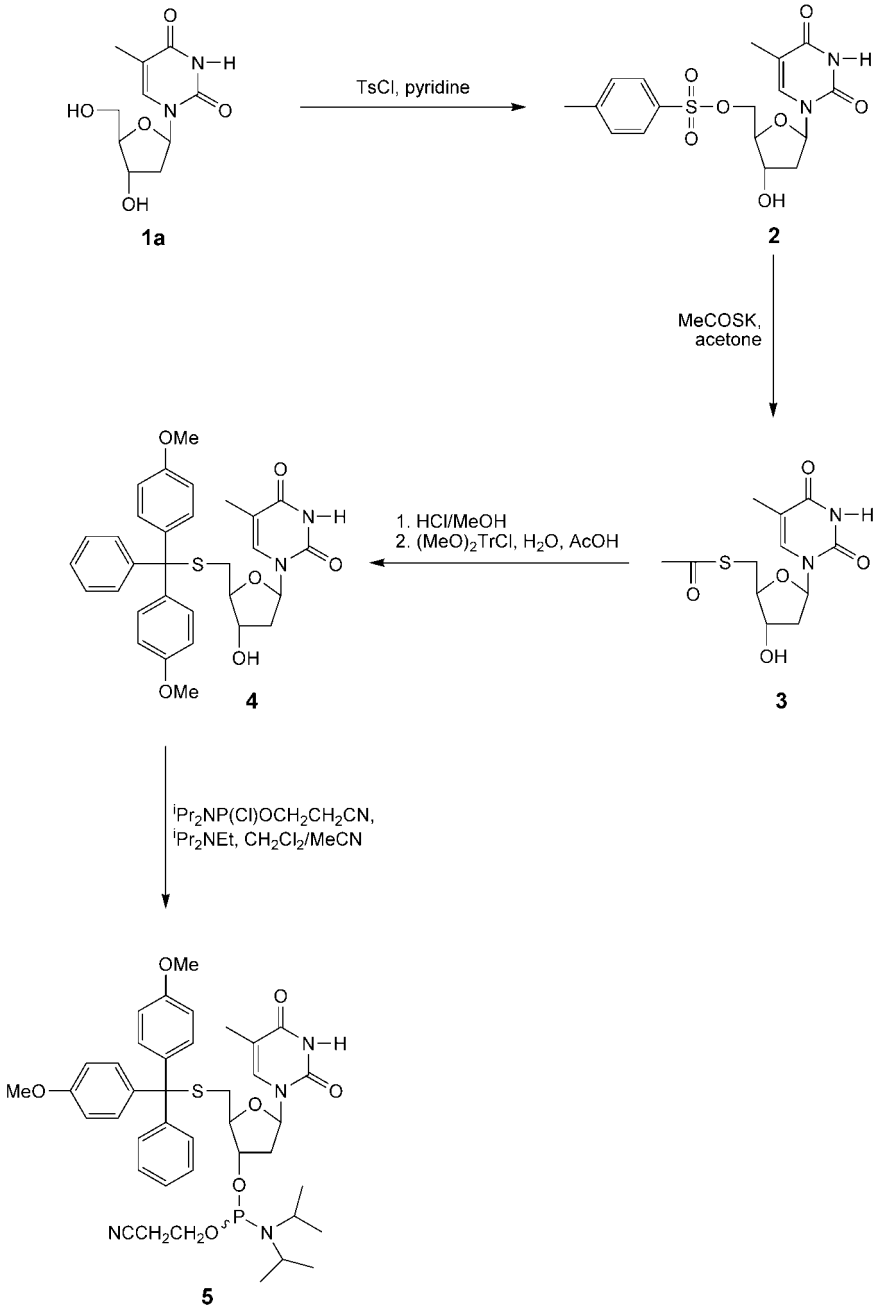
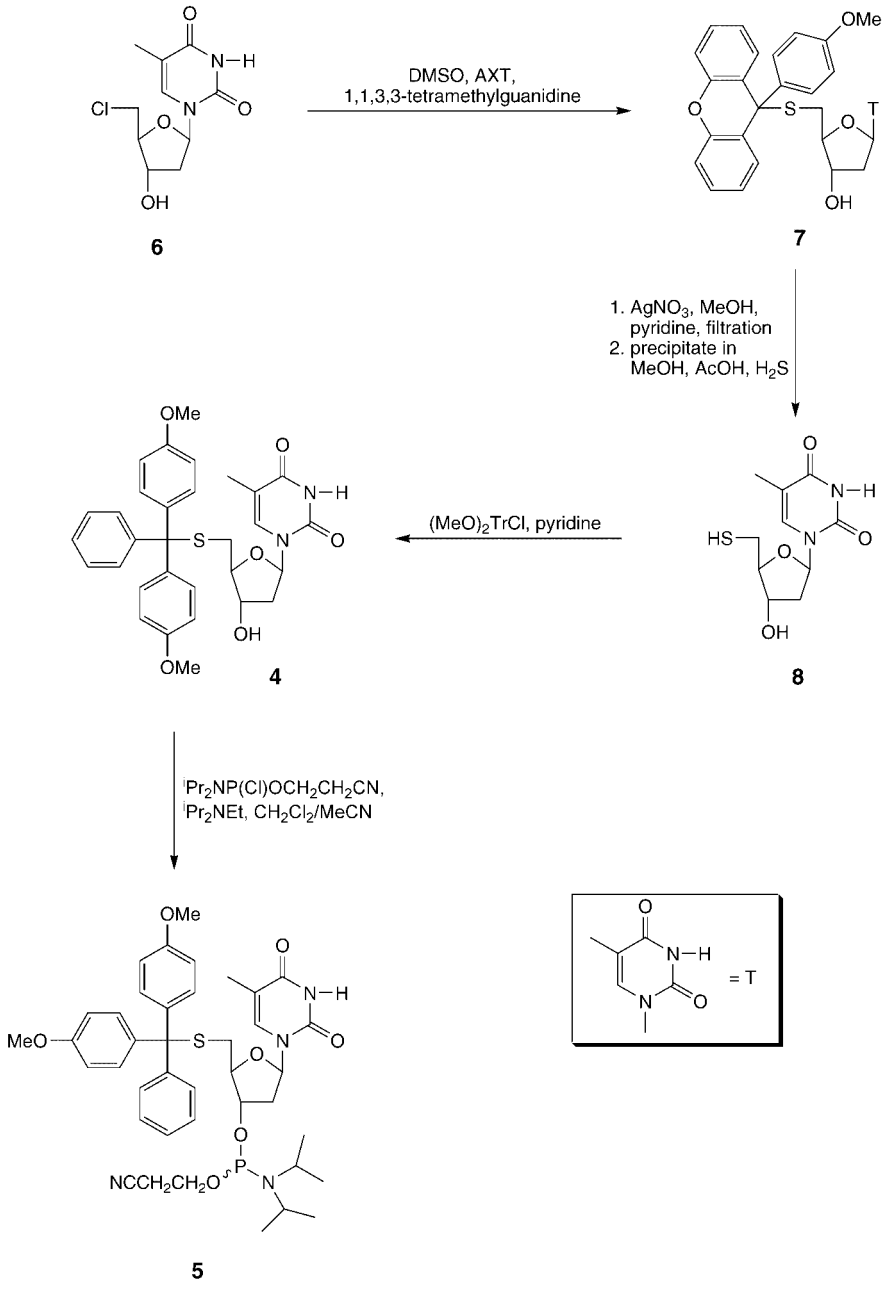
[1]) First step: reaction to $(MeO)_2$TrCl.

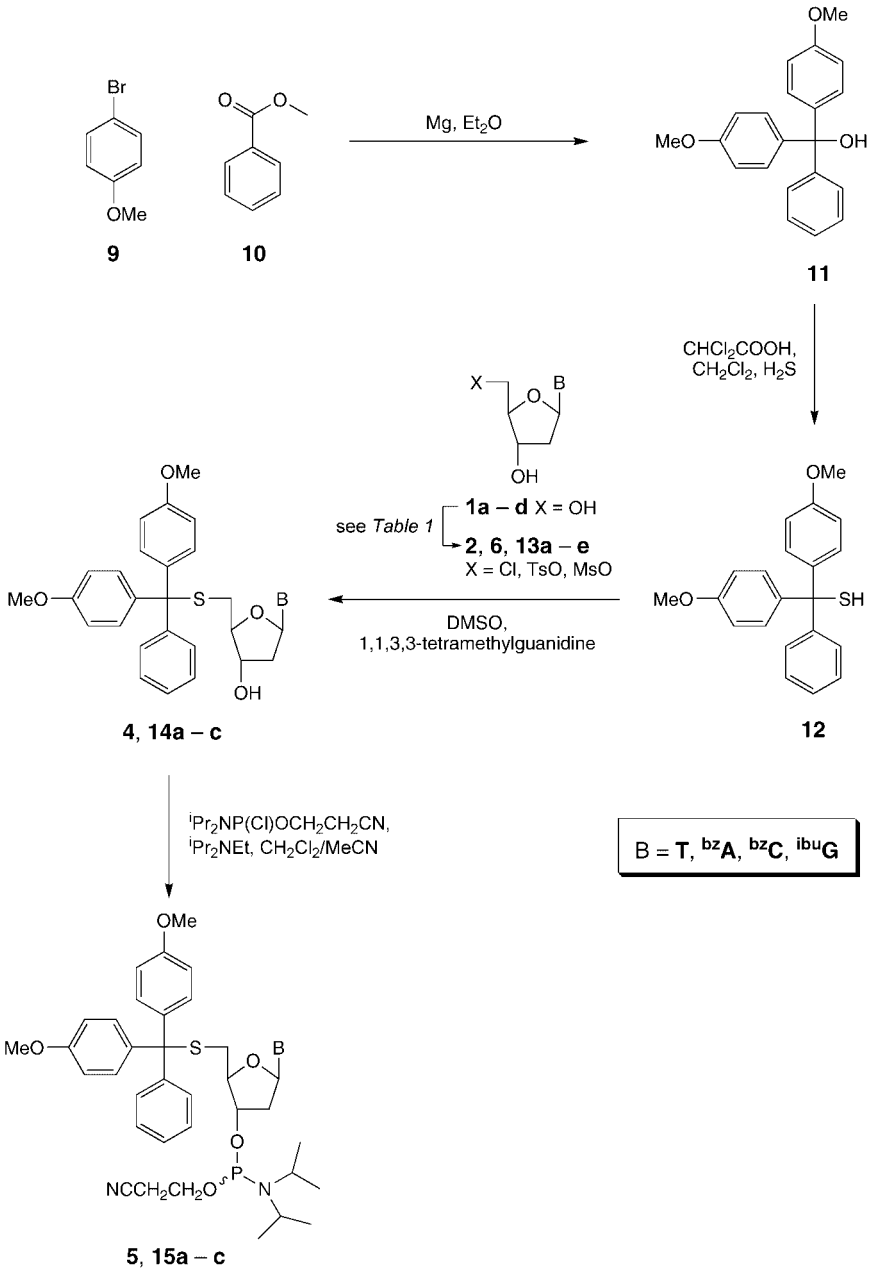
Scheme 1. *Synthesis of 5′-S-(4,4′-Dimethoxytrityl)-5′-thiothymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5**)



Scheme 2. *Synthesis of 5′-S-(4,4′-Dimethoxytrityl)-5′-thiothymidine 3′-(2-Cyanoethyl Diisopropylphosphoramidite)* (**5**)



Scheme 3. *Synthesis of 5′-S-(4,4′-Dimethoxytrityl)-2′-deoxy-5′-thionucleoside 3′-(2-Cyanoethyl Diisopropyl-phosphoramidites)* **5** *and* **15a – c**



Then, the methanethiol **12** reacted with the different activated 5′-modified nucleosides **2**, **6**, or **13a**–**e** and 1,1,3,3-tetramethylguanidine to the 5′-*S*-(4,4′-dimethoxytrityl)-5′-thionucleosides **4** and **14a**–**c** [10] (see *Table 1*). Subsequent phosphitylation (described for compound **5**) afforded the desired 3′-phosphoramidite building blocks **5** and **15a**–**c** [1b]. The kind of leaving group (activation) to be used depended on the nucleoside and the base-protecting group. The base protecting group has an effect on the solubility and, therefore, on the reactivity too. In conclusion, we demonstrate that we can insert a selective cleavage site at every position in an oligonucleotide.

Table 1. *Yields of Reactions in* Scheme 3

Nucleoside		5′-Activated nucleoside			5′-Thionucleoside		5′-Thionucleoside phosphoramidite	
No.	B	No.	5′-leaving group X	Yield [%]	No.	Yield [%]	No.	Yield [%]
1a	**T**	**6**	Cl	72	**4**	63	**5**	
1a	**T**	**2**	TsO	66	**4**	94	**5**	73
1a	**T**	**13a**	MsO	64	**4**	97	**5**	
1b	^{bz}**A**	**13b**	TsO	39	**14a**	94	**15a**	72
1b	^{bz}**A**	**13c**	MsO	79	**14a**	41	**15a**	
1c	^{bz}**C**	**13d**	MsO	48	**14b**	82	**15b**	72
1d	^{ibu}**G**	**13e**	MsO	93	**14c**	45	**15c**	76

2. *Oligonucleotides.* 2.1. *Synthesis.* The oligonucleotides were synthesized on an *Expedite* (*PerSeptive Biosystems*) or an *ABI-392* synthesizer (*Applied Biosystems*) by the phosphoramidite chemistry. Coupling times for modified amidites **5** and **15a**–**c**, 5′-biotin amidite (from *Glen Research*), and *5′-Amino-Modifier C6* (from *Glen Research*) were enhanced to 300 s. For **5** and **15a**–**c**, we used further modifications in the synthesis cycle, described in *Table 2*. We changed the time for detritylation, and we needed an additional reduction step (DTT (dithiothreitol) solution) after the detritylation. We used *tert*-butyl hydroperoxide [12] or a lower concentrated I_2 solution as oxidation reagent for improved yields. Thiols, especially on highly loaded supports are very reactive, so it is useful to minimize the time between the removal of the DTT solution and the addition of the next phosphoramidite. The [5′$(MeO)_2$Tr-on]-synthesized oligonucleotides were deprotected (base protecting groups) and cleaved from the controlled-pore-glass (CPG) support with ammonia at room temperature for 24 h. Crude DNA oligomers were purified by reversed-phase HPLC (*Poros®-R3* column, see *Exper. Part*) with an eluting gradient of 0–25% MeCN in 0.1M $(Et_3NH)OAc$ (pH 7.0) within 12 min (flow rate: 4 ml/min). Then the 5′-[$(MeO)_2$Tr group was removed with 80% aqueous AcOH within 60 min. After evaporation of the acid, the residue was diluted in 2M NH_4OAc and precipitated with EtOH under ice cooling (2 h). The precipitate was filtered off and dried. *Fig. 2* shows the purity-control HPLC of oligodeoxynucleotide **16** (modified model oligodeoxynucleotide). Pure oligonucleotides were characterized by mass spectroscopy, and the detected masses were in good agreement with the calculated values. *Table 3* shows the characteristic data of the oligodeoxynucleotides **16**–**23**, **26**, and **29**–**34**.

The model oligodeoxynucleotide **16** (24mer) was synthesized to examine the cleavage of the C–S bond, where 5′-*S*-(4,4′-dimethoxytrityl)-5′-thiothymidine 3′-(2-

Table 2. *Standard Cycle and Modified Cycle for the Oligonucleotide Synthesis* (*ABI-394* synthesizer)

Step	Reagent	Standard cycle		Modified cycle	
		t [s][a])	wait time [s]	*t* [s][a])	wait time [s]
Detritylation	3% CCl_3COOH in CH_2Cl_2	125	20	375	60
Reduction	50 mM DTT (dithiothreitol)	–	–	60	600
Washing	MeCN	23	–	60	–
	CH_2Cl_2	–	–	60	–
Coupling	amidite + 1*H*-tetrazole	12	25	12	300
Capping	Ac_2O/NMI (1-methyl-1*H*-imidazole)/THF	10	30	10	30
Oxidation	I_2/H_2O/pyridine	8	15	8	15

[a]) Time reagent to column.

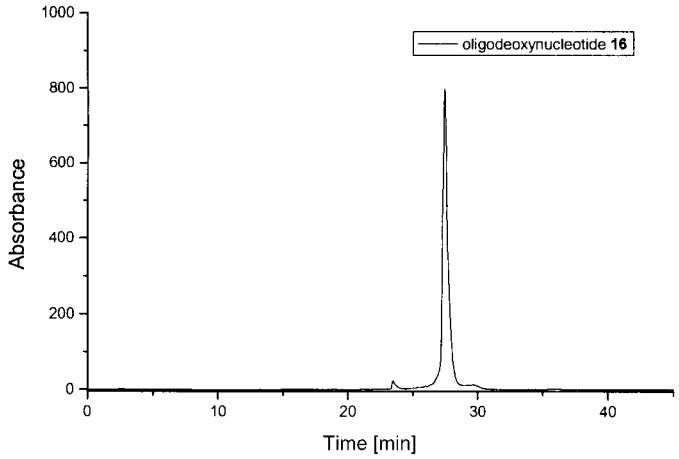


Fig. 2. *Reversed-phase HPLC profile of modified oligodeoxynuleotide* **16**. Conditions, see *Exper. Part.*

cyanoethyl-diisopropylphosphoramidite ($\underline{T}_d$) **5** was inserted as a modified building block. The sequence of the model oligodeoxynucleotide (24mer) **16** is:

5′-d(AGC CCT TAC T$\underline{T}$T GAC GGT ATA TCT)-3′ (**16**)

We carried out several tests for the cleavage, and the cleavage was detected by gel chromatography for the oligodeoxynucleotides **17** and **21**–**23**, by analytical HPLC for oligodeoxynucleotide **16**, on a chip for the oligodeoxynucleotides **21** and **22**, and by surface plasmon resonance spectroscopy (SPR) for the oligodeoxynucleotides **17**–**20**. The oligodeoxynucleotides **23**, **30**, and **32**–**34** were synthesized as controls for the following experiments. *Table 4* shows the synthesized oligodeoxynuleotides.

2.2. *Cleavages.* 2.2.1 *Cleavage of Modified Oligonucleotides in Solution.* First, we evaluated specific chemical conditions for the cleavage of the C–S internucleotide bond by using heavy-metal ions. Therefore, the modified oligodeoxynucleotide was diluted in H_2O and was treated with aqueous 50 mM $AgNO_3$. After 15 min at room

Table 3. *Characteristic Data of the Oligonucleotides* **16**–**23**, **26**, *and* **29**–**31**

	Synthesis cycle[a])	Reversed-phase HPLC		MALDI- or ESI-MS		Thermal stability	
		gradient	yield[g])	calc.	exper.	No.	T_m [°][c])
16	modified	0–30% *B* in 10 min[b])	66.1 *OD* (61.2%)	7309.8	7331.94	–	–
17	modified	0–30% *B* in 10 min[b])	40.1 *OD* (64.4%)	7715.3	7717.97	–	–
18	standard	0–25% *B* in 10 min[b])	60.7 *OD* (89.5%)	6110.0	6112.35	**17** + **18**	49.9
19	standard	0–25% *B* in 10 min[b])	63.4 *OD* (52.3%)	6125.1	6126.1	**17** + **19**	55.0
20	standard	0–25% *B* 10 min[b])	61.6 *OD* (48.5%)	6174.1	6175.71	**17** + **20**	58.0
21	modified	0–30 % *B* in 10 min[b])	71.5 *OD* (84.3%)	7489.0	7491.46	–	–
22	standard [d])	0–30% *B* in 10 min[b])	72.7 *OD* (66%)	7475.94	7475.64	–	–
23	standard [d])	0–30% *B* in 10 min[b])	72.4 *OD* (58.2%)	7699.24	7702.02	–	–
26	modified [e])	10–50% *B* in 25 min[f])	32.6 *OD* (43%)	4672.24	4672.8	–	–
29	modified [e])	10–50% *B* in 25 min[f])	46.9 *OD* (58%)	4600.18	4601.55	–	–
30	standard [d])	0–30% *B* in 10 min[b])	72.4 *OD* (58.2%)	7699.24	7702.02	–	–
31	standard [d])	0–30% *B* in 10 min[b])	72.7 *OD* (84.3%)	7489.00	7491.35	–	–
32	standard [d])	0–30% *B* in 10 min[b])	72.7 *OD* (66%)	7472.94	7475.64	–	–
33	standard	0–30% *B* in 10 min[b])	66.1 *OD* (75.5%)	4268.8	4267.03	–	–
34	standard	0–30% *B* in 10 min[b])	35 *OD* (55.6%)	2963.0	2961.42	–	–

[a]) See *Table 1*. [b]) Column *PorosR3*. B = MeCN, in 0.1M (Et_3NH)OAc. [c]) Buffer 0.2M NaCl, 10 mM NaH_2PO_4, pH 7.4. [d]) Wait time for 5′-modification was 300 s. [e]) Post-labeling on CPG material. [f]) Column *RP18*; *B* = MeCN, in 0.1M (Et_3NH)OAc. [g]) Yield of crude; *OD* = optical density.

Table 4. *Synthesized Oligodeoxynucleotides* **16**–**25**, *and* **27**–**34**. $\underline{T}_d$ = modified 5′-thiothymidine unit; *CPG* = controlled-pore class linkage; AF = 5-(acetamido)fluorescein

Oligodeoxynucleotide	
16	5′-d(AGC CCT TAC T$\underline{T}$T GAC GGT ATA TCT)-3′
17	5′-Biotin-d(AGC CCT $\overline{T}$AC T$\underline{T}$T GAC GGT ATA TCT)-3′
18	5′-d(GCA GCT AGA TAT A$\overline{C}$C GTC AA)-3′
19	5′-d(GCT AGA TAT ACC GTC AAA GT)-3′
20	5′-d(GAT ATA CCG TCA AAG TAA GG)-3′
21	5′-Amino-d(AGC CCT TAC T$\underline{T}$T GAC GGT ATA TCT)-3′
22	5′-Amino-d(AGC CCT TAC T$\overline{T}$T GAC GGT ATA TCT)-3′
23	5′-d(ACG CCT TAC TTT GAC GGT ATA TCT)-3′
24	5′-[(MeO)$_2$Tr]-S-d($\underline{T}$T GAC GGT ATA TCT-$\underline{CPG}$)$_d$-3′
25	5′-HS-d($\underline{T}$T GAC GGT ATA TCT-$\underline{CPG}$)-3′
27	5′-[(MeO)$_2$Tr]-S-d($\underline{T}$T TTT TTT TTT TTT-$\underline{CPG}$)-3′
28	5′-HS-d($\underline{T}$T TTT TTT TTT TTT-$\underline{CPG}$)-3′
29	5′-AF-d(TT TTT TTT TTT TTT)-3′
30	5′-Biotin-d(AGC CCT TAC TTT GAC GGT ATA TCT)-3′
31	5′-Cholesterol-d(AGC CCT TAC T$\underline{T}$T GAC GGT ATA TCT)-3′
32	5′-Cholesterol-d(AGC CCT TAC T$\overline{T}$T GAC GGT ATA TCT)-3′
33	5′-d(TT GAC GGT ATA TCT)-3′
34	5′-d(AGC CCT TAC T)-3′

temperature, 220 mM dithiothreitol in H_2O was added, and the precipitated silver salt was removed after 15 min by centrifugation. The supernatant was analyzed by polyacrylamide-gel electrophorese (PAGE; 1 mm, 15% PAA, 7M urea, 200 V) for the oligodeoxynucleotides **17**–**21**, **31**, and **32** (*Fig. 3*), and by reversed-phase HPLC (*Gen-*

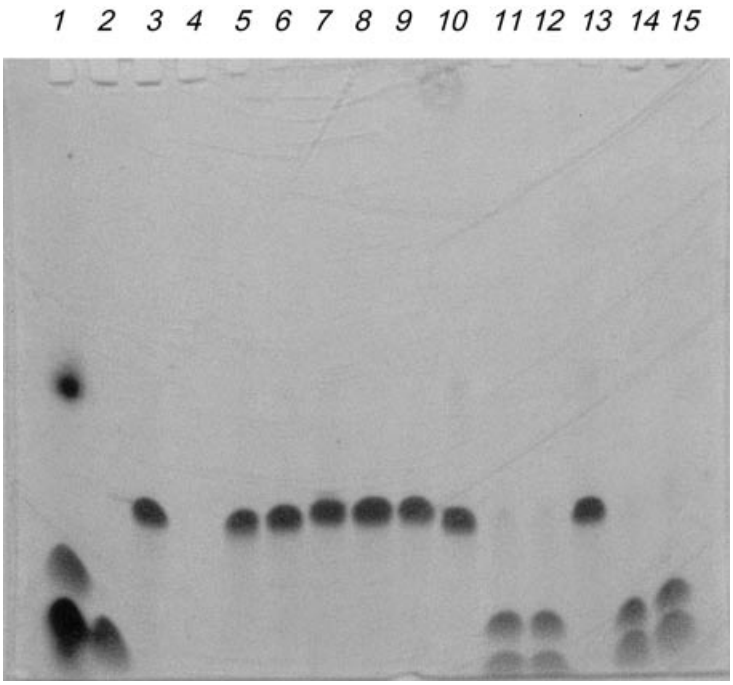


Fig. 3. *Cleavage of the modified model oligodeoxynucleotide* **17** and of **21**–**23** *detected by PAGE*. Conditions, see *Exper. Part. Lane 1:* **33** (14mer) + two markers; *Lane 2:* **34** (10mer); *Lane 3:* **32** (control); *Lane 4:* empty; *Lanes 5* and *6:* **32** after treatment with $AgNO_3$; *Lane 7:* **30** (control); *Lanes 8* and *9:* **30** after treatment with $AgNO_3$; *Lane 10:* **31** (control); *Lanes 11* and *12:* **31** after treatment with $AgNO_3$; *Lane 13:* **17** (control); *Lanes 14* and *15:* **17** after treatment with $AgNO_3$.

Pak Fax (column; eluent; see *Exper. Part*)) for the oligodeoxynucleotide **16** (*Fig. 4*). We can show that 50 mM aqueous $AgNO_3$ cleaves the model oligodeoxynucleotide within 5 min completely.

2.2.2 *Cleavage of Modified Oligonucleotides on a Chip.* We synthesized the two oligodeoxynucleotides **21** and **22** for immobilization on the surface of epoxy-modified glass slides (*Quantifoil Micro Tools GmbH*, Jena, Germany). For selective cleavage of the scissile bond, the oligodeoxynucleotides **21** and **22** were incubated for 30 min at room temperature in 50 mM aqueous $AgNO_3$. In experiments where a double-stranded DNA hybrid was formed prior to the cleavage reaction, the ionic strength of the cleaving solution was raised to keep the hybrid stable during the cleavage reaction. This was done by adding aqueous $NaNO_3$ solution (soluble Ag salt!) to a final concentration of 1M.

2.2.3 *Cleavage of Modified Oligonucleotides on a* Biacore *Chip. Biacore* (*Pharmacia Biosensor*, Sweden) takes advantage of surface plasmon resonance spectroscopy (SPR) to measure reactions as a function of mass changes [13]; we used the unmodified *CM5* sensor chip [14]. First we coupled streptavidin as ligand to the derivatized dextran matrix located on the sensor-chip surface. *Fig. 5 s*hows the construct for this experiment. Streptavidin immobilized on the sensor-chip surface can be used to

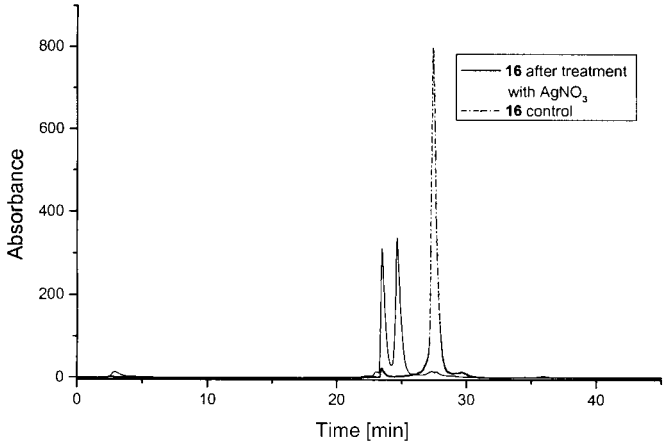


Fig. 4. *Reversed-phase HPLC profile of the cleavage* (modified model oligodeoxynucleotide **16**). Conditions, see *Exper. Part.*

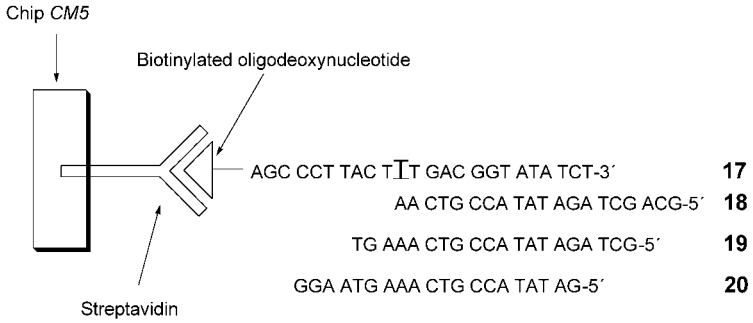


Fig. 5. *Principle of the experiment by* Biacore *measurement*. See text. The nucleoside units A, C, G, and T stand for A_d, C_d, G_d, and T_d. Conditions, see *Exper. Part.*

capture biotinylated ligands. For that, we synthesized the biotinylated oligodeoxynucleotide **17** (which contained the amidite **5**, and biotin at the 5′-end).

The streptavidin–biotin affinity is very high and serves to immobilize the modified oligodexynucleotide **17** for the following hybridization and the subsequent cleavage. The partly complementary oligodeoxynucleotides **18**–**20** were added in three different experiments, and the successful hybridization with the biotinylated probe **17** at room temperature was detected by mass change. *Scheme 4* exemplifies the hybridization of the oligodeoxynucleotides **17** and **18** and the following cleavage of the internucleotide S–C bond by $AgNO_3$, and *Table 5* shows the yield of hybridization of the oligodeoxynucleotide **17** with **18**–**20** followed by cleavage with $AgNO_3$. Here we can demonstrate a successful hybridization and cleavage detected by *Biacore* measurement at room temperature. Silver ions on the chip surface can be removed by an EDTA solution.

Scheme 4. *Cleavage of the Modified Oligonucleotide* **17** *Detected by a* Biacore *Measurement.* The nucleoside units A, C, G, and T stand for A_d, C_d, G_d, and T_d. Conditions, see *Exper. Part.*

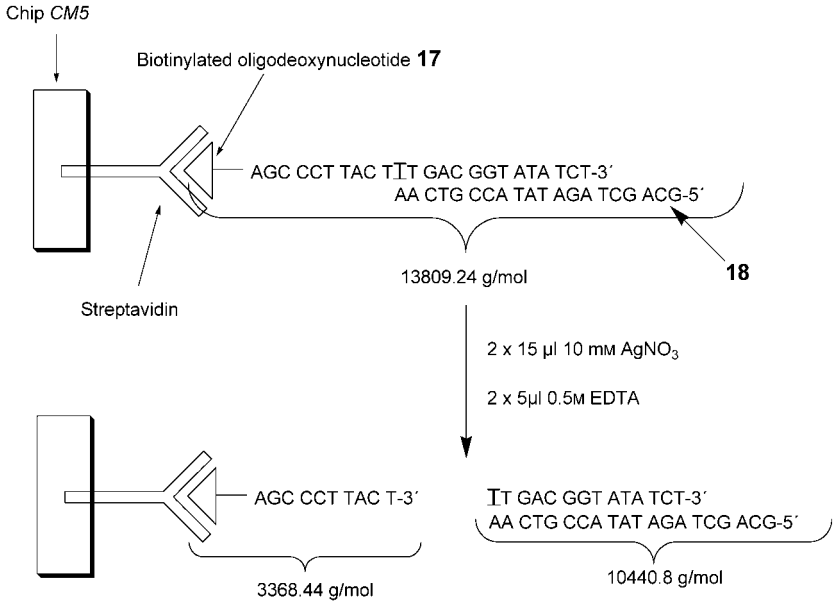


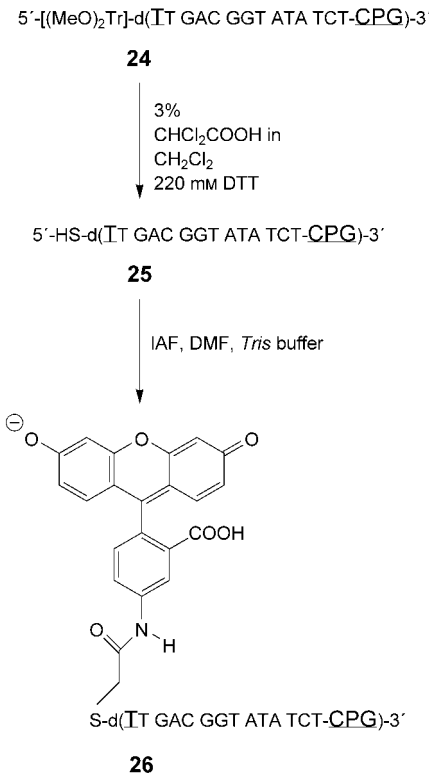
Table 5. *Data from the* Biacore *Experiment*[a]). Conditions, see *Exper. Part.*

Streptavidin labeling	Reaction with **17**		Hybridization			Cleavage	
RU abs.	RU abs.	RU rel.	No.	RU abs.	RU rel.	RU abs.	RU rel.
22237.4	23943.8	1706.4	**18**	25007.3	2769.9 (78.7%)	23233.8	996.4 (85.9%)
21684.5	23429.1	1674.6	**19**	24492.8	2738.3 (80.0%)	22766.0	1011.5 (83.4%)
19562.9	20578.0	1015.1	**20**	21327.0	1764.1 (92.2%)	20206.8	643.9 (83.7%)

[a]) RU abs. = response unit absorption.

2.3. *Post-Labeling.* For the post-labeling with 5-(iodoacetamido)-fluorescein (IAF) (*Scheme 5*), we synthesized the two modified oligodeoxynucleotides **24** and **27**. The [$(MeO)_2$Tr]-S group at the 5′-end of the oligodeoxynucleotides could be detritylated with 3% $CHCl_2COOH$ in CH_2Cl_2. The oligodeoxythionucleotides **25** and **28** dimerized quickly and had to be reduced prior to use. We used a 220 mM aq. DTT solution for this reduction step. The DTT solution had to be fully removed before the next coupling reaction (side reactions). This is the reason why we washed the CPG material with H_2O, MeOH, and MeCN. The free SH group was very reactive; thus, we minimized the time between the removal of the DTT and the addition of 5-(iodoacetamido)-fluorescein (IAF). The post-labeling on the CPG material saved time because the washing steps (filtration) were fast and easy to perform. Thus, the CPG-oligonucleotide was treated with the fluorescein derivate IAF at room temperature for 24 h. Then, we washed the CPG material with MeOH and MeCN and cleaved the oligodeoxynucleo-

Scheme 5. *Post-Reaction Labeling of Oligonucleotide* **25**. CPG = controlled-pore glass.



tides from the CPG support with ammonia at room temperature, for 1 h in the case of **29** and for 24 h in the case of **26** (cleavage and base deprotection). The crude modified oligodeoxynucleotides were purified by reversed-phase HPLC (*RP-18* column). *Fig. 6* shows the HPLC profile for the post labeling of oligodeoxynucleotide **25** with 5-(iodoacetamido)fluorescein (IAF). The free SH group of the oligodeoxynucleotides **25** and **28** reacted in acceptable yields to the oligodeoxynucleotides **26** (43%) and **29** (58%).

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Experimental Part

General. All starting materials were obtained from *Fluka or Aldrich*, and were used without further purification. Moisture-sensitive reactions were conducted in dried glassware under a positive pressure of dry Ar. Dry MeCN (H_2O < 30 ppm) for the phosphitylation reaction was purchased from *PerSeptive Biosystems* or *Biosolve*. Oligodeoxynucleotide synthesis: *Expedite-8909* synthesizer from *PerSeptive Biosystems* or *ABI-392* synthesizer from *Applied Biosystems*. Oligodeoxynucleotide reagents: *PerSeptive Biosystems* and *Glen Research*. Flash column chromatography (FC): silica gel *60* (40–63 μm) from *Merck*. TLC: silica gel *60* F_{254}

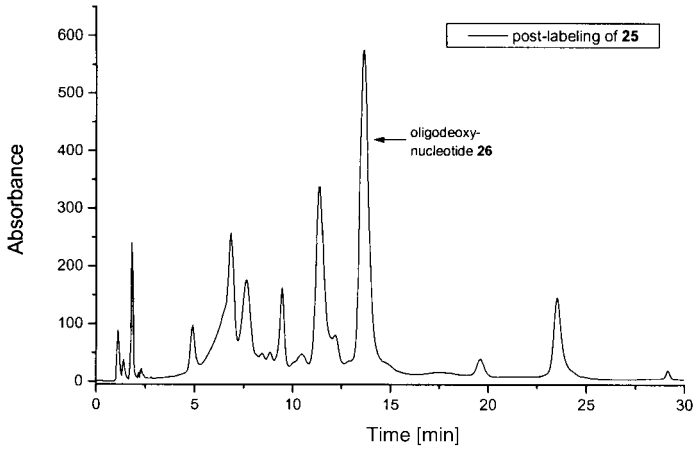

Fig. 6. *HPLC Profile of the product of post labeling of oligodeoxynucleotide* **25**. Conditions, see *Exper. Part.*

plates from *Merck*. Prep. HPLC: *Poros® Oligo™-R3* column (4.6 mm × 100 mm) from *Applied Biosystems* (order-No. material 1-1339-03) *or LiChroCart® 250-10, RP-18e* (5 μm) from *Merck* (order-no. 50257); eluent: buffer $A = 0.1$M $(Et_3NH)OAc$, buffer $B =$ MeCN. Anal. HPLC: *EcoCART® 125-3-RP-18* column (5 μm) from *Merck* (order-no. 51232); eluent: buffer $A = 0.1$M $(Et_3NH)OAc$, buffer $B =$ MeCN; and *Gen-Pak Fax* column (4.6 mm × 100 mm) from *Waters* (order-no. 15490); eluent: buffer $A = 10$ mM NaH_2PO_4 in $MeCN/H_2O$ 1:4 (*v*/*v*), buffer $B = 1.5$M NaCl, 10 mM NaH_2PO_4 in $MeCN/H_2O$ 1:4 (*v*/*v*). Gel chromatography (PAGE): 1 mm, 15 cm, 15% PAA, 7M urea, 220 V. UV (for *OD* measurements): *Varian Cary-1*-UV/VIS spectrophotometer or *Hitachi U-1100* spectrophotometer: 10-mm cuvette. NMR: *Bruker AMX-250* (^{1}H) and *Bruker AMX-400* (^{1}H,^{31}P) spectrometers; δ in ppm, *J* in Hz; primed locants are given to the monosaccharide moiety and unprimed ones to the nucleobase moiety. MS: *PerSeptive Biosystems* MALDI-TOF spectrometer *Voyager DE*; ESI = electrospray ionization.

5′-S-Acetyl-5′-thiothymidine (**3**). To MeCOSK (18 g, 124.8 mmol) and **2** (12 g, 30.3 mmol) was added acetone (250 ml). The suspension was stirred under Ar at 50° for 6 h and then r.t. overnight. The suspension was cooled (ice-water bath) before filtration, and the filtrate was evaporated. The residual oil was subjected to FC (silica gel, CH_2Cl_2/MeOH (9:1, *v*/*v*)): **3** (8.6 g, 94.5%). Colorless foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.37. ^{1}H-NMR ((D_6)DMSO): 11.03 (*s*, NH); 7.45 (*d*, H–C(6)); 6.15 (*t*, H–C(1′)); 5.41 (*d*, OH–C(3′)); 4.1 (*m*, H–C(3′)); 3.75 (*m*, H–C(4′)); 3.23 (*m*, 1 H–C(5′)); 3.1 (*m*, 1 H–C(5′)); 2.37 (*s*, Ac); 2.23 (*m*, 1 H–C(2′)); 2.07 (*m*, 1 H–C(2′)); 1.81 (*s*, Me–C(5)). ESI-MS (pos.) 301.2 ($[M+H]^+$, $C_{12}H_{17}N_2O_5S^+$; calc. 301.33).

5′-S-(4,4′-Dimethoxytrityl)-5′-thiothymidine (**4**). *a*) A soln. of **3** (6 g, 20 mmol) in 1M HCl/MeOH (145 ml) under Ar was stirred for 2 h at 45°. The soln. was concentrated to 1/2 the volume and added to a soln. of AcOH (40 ml), H_2O (20 ml), and $(MeO)_2$TrCl (3 g, 35.4 mmol). The soln. was stirred at r.t. for 3 h and then concentrated to nearly 100 ml. After addition of H_2O (200 ml), the pH was adjusted to 10 with 2M NaOH. The mixture was extracted with CH_2Cl_2 (3 × 150 ml), the combined org. phase washed with 5% aq. $NaHCO_3$ soln. (150 ml) and brine (200 ml), dried (Na_2SO_4), and evaporated, and the residual oil subjected to FC (silica gel, CH_2Cl_2/MeOH 95:5 + 0.5% Et_3N). **4** (7.61 g, 68%). Foam.

b) To a soln. of **8** (1.29 g, 5 mmol) in pyridine (20 ml) was added $(MeO)_2$TrCl (2.03 g, 6 mmol) in pyridine (5 ml) at r.t. The soln. was stirred overnight, and the reaction was quenched with MeOH. The mixture was diluted with CH_2Cl_2 (200 ml) and washed with 5% aq. $NaHCO_3$ soln. (100 ml), the aq. phase extracted with CH_2Cl_2 (100 ml), the combined org. phase washed with brine (100 ml), dried (Na_2SO_4), and evaporated, and the residual oil subjected to FC (silica gel, CH_2Cl_2/MeOH 95:5 + 0.5% Et_3N): **4** (2.21 g, 79%). Foam.

c) Dry 1,1,3,3-tetramethylguanidine (0.14 ml, 1.11 mmol) was added dropwise to a stirred soln. of a 5′-X-thymidine **2**, **6**, or **13a** (X = leaving group; 1 mmol; **2**: 0.396 g; **6**: 0.261 g; **13a**: 0.320 g) and 4,4′-dimethoxytriphenylmethanethiol (0.504 g, 1.5 mmol) in dry DMSO (10 ml) under Ar at r.t. After 3 h, cooled CH_2Cl_2 (300 ml) was added, and the mixture was washed with sat. aq. $NaHCO_3$ soln. (150 ml). The aq. layer was extracted with CH_2Cl_2 (100 ml), the combined org. layer washed with H_2O (4 × 100 ml), dried ($MgSO_4$), and

evaporated, and the residue subjected to FC (silica gel, CH_2Cl_2/MeOH 97:3): **4** (from **2**, 0.52 g (94%); from **6**, 0.356 g (63.6%); from **13a**; 0.546 g (97.5%)). Foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.51. ^{1}H-NMR ($CDCl_3$): 7.22–7.43 (*m*, 9 arom. H); 6.83 (*m*, H–C(6), 4 arom. H); 6.16 (*t*, H–C(1′)); 4.1 (*m*, H–C(3′)); 3.78 (*m*, H–C(4′), 2 MeO); 2.54 (*m*, 1 H–C(5′)); 2.5 (*m*, 1 H–C(5′)); 2.29 (*m*, 1 H–C(2′)); 2.06 (*m*, 1 H–C(2′)); 1.86 (*s*, Me–C(5)). ESI-MS (neg.): 560.2 ($[M+H]^-$ $C_{31}H_{33}N_2O_6S^-$; calc. 560.66).

5′-S-(4,4′-Dimethoxytrityl)-5′-thiothymidine 3′-(2-Cyanoethyl-Diisopropylphosphoramidite) (**5**). A soln. of **4** (0.56 g, 1 mmol) in MeCN (4 ml) and CH_2Cl_2 (4 ml) in the presence of ^{i}Pr$_2$NEt (0.424 ml, 3 mmol) was cooled with ice, and 2-cyanoethyl diisopropylphosphoramidochloridite (0.219 g, 1.2 mmol) was added dropwise under Ar. The reaction was quenched after 1 h at r.t. by adding BuOH (0.5 ml). The soln. was diluted with CH_2Cl_2 (100 ml), washed with 5% aq. $NaHCO_3$ soln. (100 ml) and brine (100 ml), dried (Na_2SO_4), and evaporated and the residual oil subjected to FC (short column of silica gel, CH_2Cl_2/MeOH 97:3): **5** (0.56 g, 73%). Foam. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.65. ^{31}P-NMR ($CDCl_3$ + 0.1% ^{i}Pr$_2$NEt) 149.55; 149.35. ESI-MS (pos.): 761.2 ($[M+H]^+$, $C_{40}H_{50}N_4O_7PS^+$; calc. 761.86).

5′-Chloro-thymidine (**6**). A soln. of thymidine (**1a**; 9.6 g, 40 mmol), PPh_3 (14 g, 54 mmol), and CCl_4 (20 ml, 200 mmol) in DMF (200 ml) was kept at r.t. for 24 h and then quenched with MeOH. The soln. was evaporated, and the residue was crystallized from MeOH: pure **6** (7.56 g, 72.6%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.38. ^{1}H-NMR ((D$_6$)DMSO): 11.38 (*s*, NH); 7.55 (*d*, H–C(6)); 6.22 (*t*, H–C(1′)); 4.25 (*m*, H–C(3′)); 4.24 (*m*, H–C(4′), 2 H–C(5′)); 3.34 (*d*, OH–C(3′)); 2.32–2.21 (*m*, 1 H–C(2′)); 2.14–2.06 (*m*, 1 H–C(2′)); 1.8 (*s*, Me–C(5)). ESI-MS (pos.): 261.9 ($[M+H]^+$; $C_{17}H_{21}N_2O_7S^+$; calc. 261.68).

*5′-S-[9-(4-Methoxyphenyl)-9*H*-xanthen-9-yl]-5′-thiothymidine* (**7**). Anh. 1,1,3,3-tetramethylguanidine (2.42 ml, 18.92 mmol) was added dropwise to a stirred soln. of **6** (4.4 g, 17 mmol) and 9-(4-methoxyphenyl)-9*H*-xanthene-9-thiol (AXT; 8.17 g, 25.5 mmol) in dry DMSO (150 ml) under Ar at r.t. After 3 h, cooled CH_2Cl_2 (1 l) was added, and the mixture was washed with sat. aq. $NaHCO_3$ soln. (800 ml) (*Caution*!), the aq. layer extracted with CH_2Cl_2 (200 ml), and the combined org. layer washed with H_2O (4 × 400 ml), dried ($MgSO_4$), and evaporated, and the residue subjected to FC (silica gel, CH_2Cl_2/MeOH 97:3): **7** (7.1 g, 95%). Foam. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.183. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.46. ^{1}H-NMR ($CDCl_3$): 8.82 (*s*, NH); 7.38–7.35 (*m*, 2 arom. H); 7.2–7.02 (*m*, 7 arom. H); 6.93–6.9 (*m*, 2 arom. H); 6.81–6.78 (*m*, 2 arom. H); 6.06–6.01 (*dd*, 1 H–C(1′)); 3.86–3.82 (*m*, 1 H–C(4′)); 3.73 (*s*, MeO); 3.6–3.52 (*m*, H–C3′)); 2.21–2.11 (*m*, 2 H–C(5′)); 1.9–1.79 (*m*, 2 H–C(2′)); 1.84 (*d*, Me–C(5)). ESI-MS (neg.): 543.3 ($[M-H]^-$, $C_{30}H_{27}N_2O_6S^-$; calc. 543.55).

4,4′-Dimethoxytriphenylmethanethiol (**12**). H_2S was bubbled through a cooled (ice-water bath), stirred, anh. soln. of $CHCl_2COOH$ (19.75 ml, 240 mmol) in CH_2Cl_2 (400 ml), while a soln. of **11** (38.44 g, 120 mmol) in anh. CH_2Cl_2 (400 ml) was added dropwise during 1 h. After H_2S had been bubbled through the mixture for a further 15 min, the H_2S gas was replaced by N_2 gas for 15 min. Then the soln. was washed with sat. aq. $NaHCO_3$ soln. (2 × 1 l) and H_2O (2 × 1 l), dried ($MgSO_4$), and evaporated and the residue subjected to FC (silica gel, hexane/AcOEt 4:1): **12** (36.33 g, 90%). Foam. TLC (hexane/AcOEt 3:1): R_f 0.66. ^{1}H-NMR ($CDCl_3$): 7.24–7.06 (*m*, 9 arom. H); 6.75–6.69 (*m*, 4 arom. H); 3.72 (*s*, 2 MeO). MALDI-MS: 336.45 (M^+, $C_{21}H_{20}O_2S^+$; calc. 336.45).

5′-S-(4,4′-Dimethoxytrityl)-2′-deoxy-5′-thionucleosides **4** and **14a–c**). Dry 1,1,3,3-tetramethylguanidine (0.142 ml, 1.11 mmol) was added dropwise to a stirred soln. of an activated 5′-X-nucleoside **13a–d** (X = leaving group, see *Table 1*; 1 mmol; 0.320 g, 0.509 g, 0.433 g, 0.409 g, and 0.415 g, resp.) and 4,4′-dimethoxytriphenylmethanethiol (0.505 g, 1.5 mmol) in anh. DMSO (10 ml) under Ar at r.t. After 3 h, cooled CH_2Cl_2 (300 ml) was added, the mixture washed with sat. aq. $NaHCO_3$ soln. (150 ml), the aq. layer extracted with CH_2Cl_2 (100 ml), the combined org. layer washed with H_2O (4 × 100 ml), dried ($MgSO_4$), and evaporated, and the residue subjected to FC (silica gel, CH_2Cl_2/MeOH 97:3): Foam.

Data of **14a**: 0.63 g (93%) from **13b**; 0.26 g (41%) from **13c**. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.45. ^{1}H-NMR ($CDCl_3$): 9.08 (*s*, NH); 8.65, 8.22 (*s*, H–C(2), H–C(8)); 7.93–7.92 (*m*, 2 H$_o$ (bz)); 7.51–7.11 (*m*, 12 arom. H); 6.74–6.68 (*m*, 4 arom. H); 6.27–6.22 (*m*, H–C(1′)); 4.33–4.29 (*m*, H–C(3′)); 3.77–3.74 (*m*, H–C(4′)); 3.69 (*s*, 2 MeO); 2.74–2.36 (*m*, 2 H–C(5′), 2 H–C(2′)). ESI-MS (neg.): 672.6 ($[M-H]^-$, $C_{38}H_{34}N_5O_5S^-$; calc. 672.77).

Data of **14b**: 0.54 g (83%) from **13d**. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.44. ^{1}H-NMR ($CDCl_3$): 8.17–8.15 (*m*, H–C(5), H–C(6)); 7.86–7.04 (*m*, 2 H$_o$ (bz), 14 arom. H ((MeO)$_2$Tr); 7.81–6.77 (*m*, 2 arom. H); 6.06–6.01 (*d*, 1 H–C(1′)); 3.90–3.83 (*m*, H–C(3′), H–C(4′)); 3.73 (*s*, 2 MeO); 2.57–2.29 (*m*, 2 H–C(5′), 2 H–C(2′)). ESI-MS (neg.): 643.4 ($[M-Me]^-$, $C_{34}H_{34}N_5O_6S^-$; calc. 640.73).

Data of **14c**: 0.3 g, (45%) from **13e**. TLC (CH_2Cl_2/MeOH 9:1): R_f 0.43. ^{1}H-NMR ($CDCl_3$): 10.24 (*s*, NH); 7.66 (*s*, H–C(8)); 7.29–7.02 (*m*, 9 arom. H); 6.75–6.62 (*m*, 4 arom. H); 5.98–5.93 (*t*, H–C(1′)); 4.98 (*s*, OH–C(3′)); 4.63 (*m*, H–C(3′)); 4.09–4.01 (*m*, H–C(4′)); 3.64 (*s*, 2 MeO); 2.89–2.84 (*m*, Me$_2$*CH*CO);

2.53–2.45 (*m*, 2 H–C(5′)); 2.31 (*m*, 2 H–C(2′)); 1.21–1.16 (*dd*, *Me*$_2$CHCO). ESI-MS (pos.): 656.1 ([*M* + H]$^+$, $C_{35}H_{38}N_5O_6S^+$; calc. 656.76).

5′-S-(4,4′-Dimethoxytrityl)-2′-deoxy-5′-thionucleoside 3′-(2-Cyanoethyl Diisopropyl)phosphoramidite) **15a–c**. A soln. of **14a–c** (see *Table 1*; 1 mmol; 0.674 g, 0.650 g, and 0.655 g, resp.) in MeCN (4 ml) and CH_2Cl_2 (4 ml) in the presence of ^{i}Pr$_2$NEt (0.424 ml, 3 mmol) was cooled with ice, and 2-cyanoethyl diisopropylphosphoramidochloridite (0.219 g, 1.2 mmol) was added dropwise unter Ar. After stirring for 1 h at r.t., the reaction was quenched by adding BuOH (0.5 ml). The soln. was diluted with CH_2Cl_2 (100 ml), washed with 5% aq. $NaHCO_3$ soln. (100 ml) and brine (100 ml), dried (Na_2SO_4), and evaporated and the residual oil subjected to FC (short column of silica gel, CH_2Cl_2/MeOH 97:3): Foam.

Data of **15a**: 0.59 g (68%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.90. TLC (CH_2Cl_2/MeOH 95:5): R_f 0.81, 0.77. ^{31}P-NMR ($CDCl_3$ + 0.1% ^{i}Pr$_2$NEt): 149.99; 149.74. ESI-MS (pos.): 858.5 ([*M* – Me]$^+$, $C_{46}H_{49}N_7O_6PS^+$; calc. 858.94).

Data of **15b**: 0.65 g (76.4%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.76, 0.67. ^{31}P-NMR ($CDCl_3$ + 0.1% ^{i}Pr$_2$NEt): 150.43; 149.99. ESI-MS (pos.): 832.5 ([*M* – Me]$^+$, $C_{45}H_{49}N_5O_7PS^+$; calc. 834.96).

Data of **15c**: 0.62 g (72%). TLC (CH_2Cl_2/MeOH 9:1): R_f 0.71. ^{31}P-NMR ($CDCl_3$ + 0.1% ^{i}Pr$_2$NEt): 150.13; 148.93. ESI-MS (pos.): 856.6 ([*M* + H]$^+$, $C_{44}H_{55}N_7O_7PS^+$; calc. 857.6).

Cleavage of Oligodeoxynucleotide **16**. A soln. of 1 A_{260} unit of oligodeoxynucleotide **16** in H_2O (100 µl) was treated with 50 mM aq. $AgNO_3$ (20 µl) at r.t. for 15 min. Then 220 mM DTT in H_2O (5 µl) was added, and the precipitated silver salt was removed after 15 min by centrifugation. Aliquots (60 µl) were used for HPLC analysis (*Gen-Pak Fax* 4.6 mm × 100 mm); *cf. General:* 5% buffer *B* to 60% *B* in 30 min (*Fig. 4*).

Cleavage of Oligodeoxynucleotides **17** and **21–23**. As described above for **16**, with 1 A_{260} unit of oligodeoxynucleotide, H_2O (40 µl), 50 mM aq. $AgNO_3$ (10 µl) and 220 mM DTT in H_2O (2.5 µl). After centrifugation, the soln. (10 µl) was analyzed by PAGE (1 mm, 15% PAA, 7M urea, 220 V) after adding 10 µl of formamide (see *Fig. 3*).

Biacore *Measurement* (*Biacore 1000*). 1) Conditions for streptavidin immobilization (*Chip CM 5*): *a*) Continuous-flow buffer HBS, flow rate 5 µl/min; *b*) 50 mM EDC/200 mM NHS, inject 35 µl (7 min); *c*) streptavidin (50 µg/ml), inject 35 µl (7 min); *d*) 0.05% SDS, inject 35 µl (7 min).

2) Conditions for binding (*a*)), hybridization (*b*)), and cleavage (*c*) and *d*)): *a*) 25 µl of oligodeoxynucleotide **17** (0.1 µg/ml); *b*) 25 µl of oligodeoxynucleotide **18–20** (500 nM); *c*) 2 × 15 µl of 10 mM $AgNO_3$; *d*) 2 × 5 µl of 0.5M EDTA. The results are shown in *Table 5*.

Immobilization and Cleavage of Oligonucleotides **21** *and* **22** *on the Chip* (*Clondiag Chip Technology GmbH*; Jena, Germany). The oligonucleotides **21** (phosphorothioate oligonucleotide) and **22** (all-phosphate-backbone oligonucleotide) were immobilized on the surface of epoxy modified glass slides (*Quantifoil Micro Tools GmbH*; Jena, Germany). Both oligonucleotides carried a primary NH_2 group at the 5′-terminus that was introduced during synthesis by using a *5′-Amino-Modifier C6*. The oligonucleotides were dissolved at a final concentration of 10 µM in 0.5 mM sodium phosphate buffer (pH 8.0). The slides were diced in squares (3 × 3 mm in size). Three 0.2-µl droplets were applied by using a hand-held pipet (*Eppendorf*) to each square, representing either the all-phosphate or the phosphorothioate oligonucleotide. The squares were incubated at r.t. until the droplets were dried. Then the squares were incubated at 60° for 20 min, washed twice in 1.5-ml reaction vessels (*Eppendorf*) with 50 mM aq. KCl and once with dist. H_2O, and dried in a *Speed Vac* concentrator for storage.

For selective cleavage of the scissile linkage, the squares were incubated at r.t. for 30 min in 50 mM $AgNO_3$. In experiments where a double-stranded DNA hybrid was formed prior to the cleavage reaction, the ion strength of the cleaving soln. was raised by adding aq. $NaNO_3$ soln. to a final concentration of 1M to keep the hybrid stable during the cleavage. After cleavage of the scissile bond, the squares were washed 3 times in dist. H_2O (immobilized single-stranded DNA) or in 1M aq. $NaNO_3$ (DNA hybrids).

Post-labeling of Oligodeoxnucleotides **25** *and* **28**. The (MeO)$_2$Tr-on (1 µmol) synthesis by phosphoramidite chemistry, and the final deblocking were carried out on the synthesizer (*Table 2*). Then the CPG material was suspended in 220 mM DTT for 10 min and then washed with H_2O and MeCN (→ **25** or **28**). A soln. of 1 mg of 5-(iodoacetamido)fluorescein (IAF) in DMF, 100 µl of H_2O and 10 µl of 1M *Tris* · HCl (pH 8) were added to **25** (or **28**), and the mixture was kept at r.t. for 24 h. The CPG **26** (or **29**) was washed with H_2O, CH_2Cl_2, and MeCN. Conc. aq. ammonia was added for cleavage and deprotection (for **26**: 24 h at r.t.; for **29**: 1 h at r.t.). The labeled oligodeoxynucleotides **26** and **29** were purified by HPLC (*LiChroCart®-250-10* column, *RP-18e* (5 µm); *cf. General*): 15% buffer *B* to 60% in 25 min (see *Fig. 6*). The labeled oligodeoxynucleotides were isolated in 43% (**26**) and 58% (**29**) yield and characterized by mass spectra, see *Table 3*.

REFERENCES

[1] a) B. A. Connolly, P. Rider, *Nucleic Acids Res.* **1985**, *13*, 4485; b) B. S. Sproat, B. Beijer, P. Rider, P. Neuner, *Nucleic Acids Res.* **1987**, *15*, 4837; c) R. Cosstick, J. S. Vyle, *Nucleic Acids Res.* **1990**, *18*, 829; d) M. Mag, S. Lücking, J. W. Engels, *Nucleic Acids Res.* **1991**, *19*, 1437; e) R. G. Kuimelis, L. W. McLaughlin, *Nucl. Acids Res.* **1995**, *23*, 4753.

[2] a) R. Zuckerman, D. Corey, P. Shultz, *Nucleic Acids Res.* **1987**, *15*, 5305; b) B. S. Sproat, B. Beijer, P. Rider, P. Neuner, *Nucleosides Nucleotides* **1988**, *7*, 651; c) P. Li, P. P. Medon, D. C. Skingle, J. A. Lanser, R. H. Symons, *Nucleic Acids Res.* **1987**, *15*, 5275; d) R. Bischoff, J. M. Coull, F. E. Regnier, *Anal. Biochem.* **1987**, *164*, 336.

[3] A. B. Burgin Jr., B. N. Huizenga, H. A. Nash, *Nucleic Acids Res.* **1995**, *23*, 2973; V. N. Rybakov, M. I. Rivkin, V. P. Kumarev, *Nucleic Acids Res.* **1981**, *9*, 189; A. B. Burgin Jr., *Methods Mol. Biol.* **2000**, *95*, 119.

[4] K. Jahn-Hofmann, J. W. Engels, Collect. *Symposium Series* **2002**, *5*, 335; K. Jahn-Hofmann, J. W. Engels, *Nucleosides, Nucleotides, Nucleic Acids* **2003**, *5–8*, 1479.

[5] K. A. Henningfeld, T. Arslan, S. M. Hecht, *J. Am. Chem. Soc.* **1996**, *118*, 11701.

[6] M. H. Caruthers, G. Geaton, J. V. Wu, W. Wiesler, *Methods Enzymol.* **1992**, *211*, 3.

[7] E. J. Reist, A. Benitez, L. Goodman, *J. Org. Chem.* **1964**, *29*, 554.

[8] J. P. H. Verheyden, J. G. Moffatt, *J. Org. Chem.* **1972**, *37*, 2289.

[9] B. Greiner, G. Breipohl, E. Uhlmann, *Helv. Chim. Acta* **1999**, *82*, 2151.

[10] J. H. Marriott, M. Mottahedeh, C. B. Reese, *Tetrahedron Lett.* **1990**, *31*, 7485; J. H. Marriott, M. Mottahedeh, C. B. Reese, *Carbohydr. Res.* **1991**, *216*, 257; P. R. J. Gaffeney, L. Changsheng, M. V. Rao, C. B. Reese, J. G. Ward, *J. Chem. Soc., Perkin Trans. 1* **1991**, *6*, 1355.

[11] K. J. Divakar, A. Mottoh, C. B. Reese, Y. S. Sanghvi, *J. Chem. Soc., Perkin Trans. 1* **1990**, 969.

[12] J. W. Engels, A. Jäger, *Angew. Chem., Int. Ed.* **1982**, *21*, 912.

[13] S. J. Wood, *Microchem. J.* **1993**, *47*, 330; R. Gambari, N. Bianchi, C. Rutigliano, E. Borsetti, M. Tomassetti, G. Feriotto, F. Zorzato, *Int. J. of Oncology* **1997**, *11*, 145.

[14] http://www.biacore.com; K. Nagata, H. Handa, 'Real-Time Analysis of Biomolecular Interactions', Springer, Berlin, 2000.

[15] J. P. Horwitz, J. Chua, M. Noel, *J. Org. Chem.* **1964**, *29*, 2076.

[16] B. Sprunkel, Ph.D. Thesis Johann-Wolfgang-Goethe Universität, Frankfurt am Main, **1994**.

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