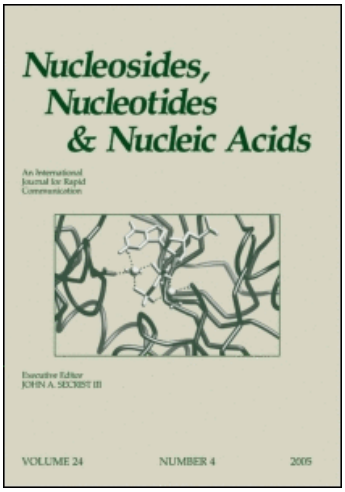

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A New Concept for DNA-Arrays

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ABSTRACT

We will insert a cleavage site in an oligodeoxynucleotide, which can be used for a selective and quantitative cleavage. For that reason we synthesized the four 5′-*S*-(4,4′-dimethoxytrityl)-mercapto-2′-deoxynucleotide-3′-*O*-(2-cyanoethoxydiisopropylamino)-phosphites (**5a–d**). The cleavage of P-S and C-S bonds is described (Mag, M.; Lücking, S.; Engels, J.W. Synthesis and selective cleavage of an oligodeoxy-nucleotide containing a bridged internucleotide 5′-phosphorthioate linkage. Nucleic Acids Res. 1991, *19* (7), 1437–1441; Marriott, J.H.; Mottahedeh, M.; Reese, C.B. 9-(4-methoxyphenyl)xanthen-9-thiol: A useful reagent for the preperation of thiols. Tetrahedron Lett. 1990, *31* (51), 7485–7488; Divakar, K.J.; Mottoh, A.; Reese, C.B.; Shanghvi, Y.S. Approaches to the synthesis of 2′ thio analogues of pyrimidine ribosides. J. Chem. Sc., Perkin Trans. 1 1990, 969–974). The oligodeoxynucleotides with an achiral bridged 5′-phosphorothioate linkage 5′-O-P-S-3′ are synthesized by the phosphoramidite procedure.

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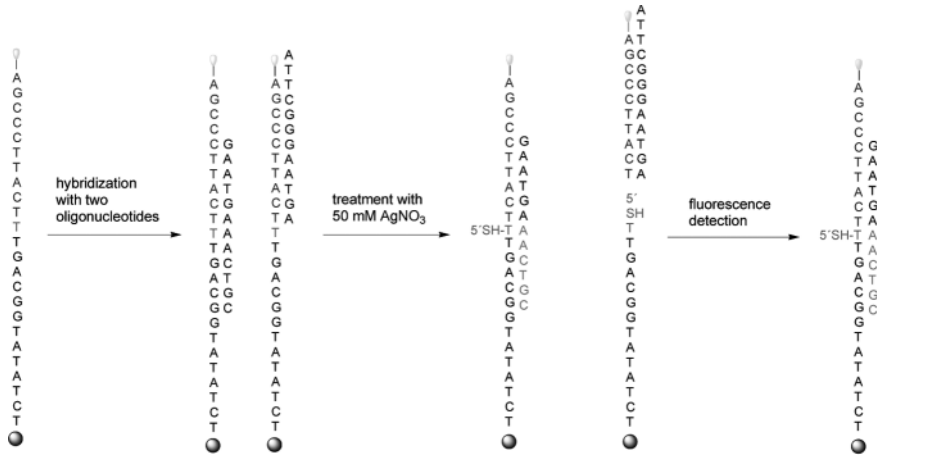

Figure 1. Concept of our new DNA-array.

With the completion of the human genome project, we are just at the beginning of a new time in genetic analysis. Wide scale DNA testing requires the development of fast, accurate, small, cheap and easy-to-use devices. We will create a new array system, which works with speed, is simple, has a high performance and low cost. The actual commercial standard tests are based on three different steps: the target-labeling, the hybridization with an immobilized probe, and the detection of the hybridization (target-probe).[1]

We are developing an array, which works without any target-labeling. Thus we modified the immobilized probe with a marker (label) for detection and moreover we have a control about the quality of the chip. Furthermore we inserted a cleavage site, which can be used for a selective and quantitative cleavage. During this cleavage the target should protect the section around the cleavage site, Fig. 1. shows this concept. The cleavage of P-S and C-S bonds is described and we prefer the thio-modification because of it's electronic and steric similarity with the natural congener and the

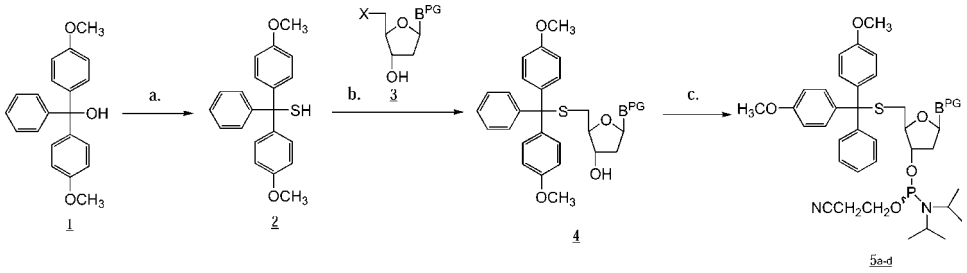

Figure 2. Shows the synthesis of 5′-*S*-(4,4′-dimethoxytrityl)-mercapto-2′-deoxynucleotides-3′-*O*-(2-cyanoethoxy-diisopropylamino)-phosphites (**5a-d**) (x = leaving group). (a) Dichloro-acetic acid, DCM, H_2S, 3 h, 4°C, 95%. (b) 5′-X-2′-deoxynucleotide **(3a-g)**, DMSO, 1,1,3,3-tetramethyl-guanidine, argon, rt, (yield shows table 1.). (c) 2-cyanoethyl-N,N′-diisopropyl-chloro-phosphoramidite, DIPEA, DCM:ACN, 1 h, rt, (yield shows Table 1.)

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Table 1. Yields of some reactions from Fig. 1.

B = Nucleoside	X = Leaving Group Yield 3 a–g	Yield 4 a–g	Yield 5 a–d
T	(3a) Chloro 72.6%	(4a) 63.6%	(5a) 73%
T	(3b) Tosyl 66%	(4b) 94%	
T	(3c) Mesyl 64%	(4c) 97%	
A^{bz}	(3d) Tosyl 39%	(4d) 94%	(5b) 72%
A^{bz}	(3e) Mesyl 79%	(4e) 41%	
C^{bz}	(3f) Mesyl 48%	(4f) 82%	(5c) 72%
G^{ibu}	(3g) Mesyl 93%	(4g) 45%	(5d) 76.4%

opportunity of a later derivatisation. We synthesized the four 5′-*S*-(4,4′-dimethoxytrityl)-mercapto-2′-deoxynucleotides-3′-*O*-(2-cyanoethoxy-diisopropylamino)-phosphites (5a-d), see Fig. 2. and Table 1.[2] To synthesize an oligodeoxynucleotide with an achiral bridged 5′-phosphorothioate linkage 5′-O-P-S-3′ we used the solid phase phosphoramidite procedure.

We started our work with the following model oligodeoxynucleotide: 5′-AGC CCT TAC T***T***T GAC GGT ATA TCT-3′(***T*** = 5′-*S*-(4,4′-DMTr)-mercapto-2′-deoxythymidine-3′-*O*-(2-cyanoethoxy-diisopropylamino)-phosphite).

The model oligodeoxynucleotide was synthesized by the phosphoramidite method on CPG material or directly on the surface of a chip. The coupling time for the modified ***T***-Amidite amounts 2×300 sec. (CPG) or 900 sec. (chip). After deblocking (***T***-Amidite) a reduction with 50 mM DTT solution is carried out to avoid the disulfide bond formation. The first tests of the cleavage were made in solution. For these tests we incubated the CPG material with ammonia (24 h, rt) and a preparative HPLC followed. Then we carried out several tests for the cleavage detected by HPLC and gel electrophoresis. 50 mM $AgNO_3$ solution cleaves the oligodeoxynucleotide in solution and on a surface of a biacore chip within 5 min completely. Then we performed the synthesis and the cleavage on the chip. During the treatment with silver nitrate all positions on the chip with a modified building block will be cleaved. The result is a free 5′-thiol and we postulate, that the target

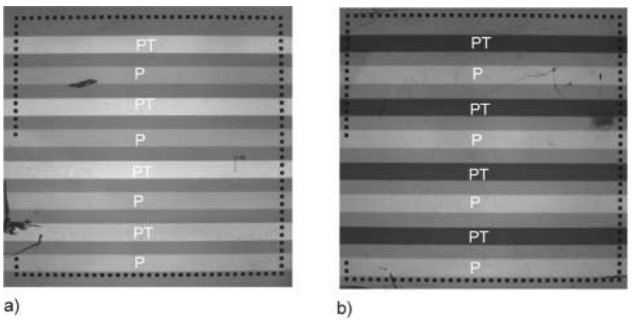

Figure 3. Shows the fluorescence images of arrays after hybridization of Cy3-labeled match oligodeoxynucleotides (P = Phosphate, PT = Phosphothioate modified ***T***-amidite) a) reference probe (no cleavage), b) hybridization after cleavage by silver nitrate.

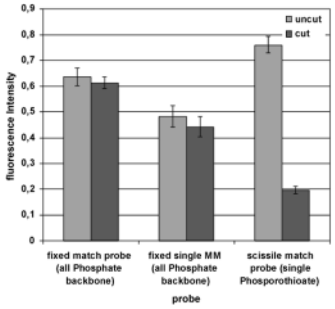

Figure 4. Shows the fluorescence intensity before and after treatment with silver nitrate.

oligonucleotide can hold the oligonucleotide with the fluorescence label, for the subsequent detection. There is not a distinction between a double-strand or single-strand domain during the cleavage.

Chip Technology

Array synthesis. Carried out with Clondiag® micro wet printing technology (16 stripes)

a. 4 stripes of fully matched sequence with phosphate backbone (P) 5′-AGC CCT TAC TTT GAC GGT ATA TCT-3′.
b. 4 stripes of fully matched sequence with modified phosphothioate backbone (PT) 5′-AGC CCT TAC T*T*T GAC GGT ATA TCT-3′.
c. 8 stripes of one base deletion sequence with phosphate backbone (on array between P and PT as control).

Selective cleavage of 5′-phosphorothioate linkage: ⇒ 40 mM aqueous silver nitrate solution, 30 minutes at room temperature (Fig. 4).

Hybridization conditions: ⇒ 10 nM 5′-AGA TAT ACC GTC AAA GTA AGG GCT-3′(5′-Cy3 labeled) in 6 × SSPE, 0.1% SDS buffer, 1 h at 50°C (Fig. 3).

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