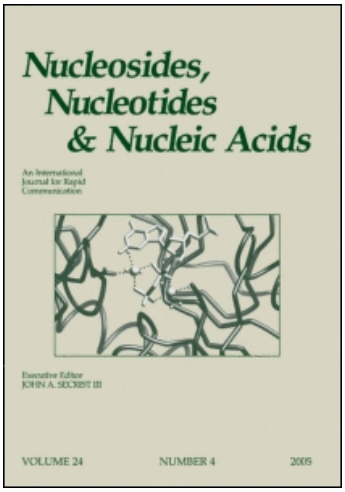

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New Developments in Light-Controlled Synthesis of DNA-Arrays

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NEW DEVELOPMENTS IN LIGHT-CONTROLLED SYNTHESIS OF DNA-ARRAYS

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ABSTRACT : Based on a photolithographic approach, DNA-arrays were synthesized utilizing phosphoramidite chemistry on a custom-built DNA-chip synthesizer. For temporary protection of the 5'-positions of the monomeric units, 2-(2-Nitrophenyl)propoxycarbonyl-groups (NPPOC) were employed that are easily removed by irradiation with light.

INTRODUCTION : Proposed in the late 1980's SBH[1] (sequencing by hybridisation) has attracted many research groups for its potential as a research tool in ongoing and forthcoming sequencing projects. In principle an unknown DNA-fragment, preferentially labeled with a fluorescent dye, is hybridized to a special DNA-array, that consists of a comprehensive set of all 65 536 8-mers, for example. From the hybridisation pattern, the sequence of the unknown probe will be reconstructed (*FIG.1*). Such DNA-arrays can either be fabricated by immobilisation of pre-synthesized oligomers[2] or by *in situ* synthesis of the oligomers[3].

RESULTS : For DNA-array synthesis, we have focussed on a photolithographic approach based on a 2-(2-nitrophenyl)ethoxycarbonyl-groups[4], a family of new photolabile protecting groups, recently introduced by Pfleiderer and Giegrich[5]. Besides the MeNPOC- and DMBOC-groups[6,7], both

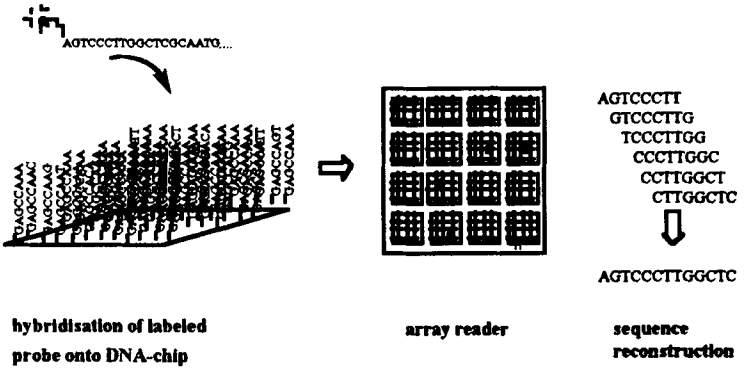


FIG.1 : Schematic representation of SBH

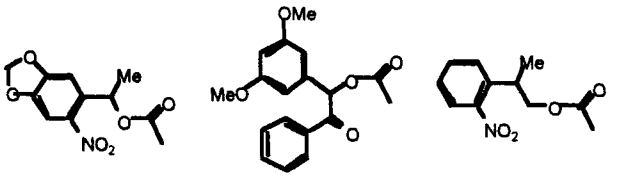


FIG.2 :Photolabile protecting groups employed for photolithographic synthesis of dna-arrays

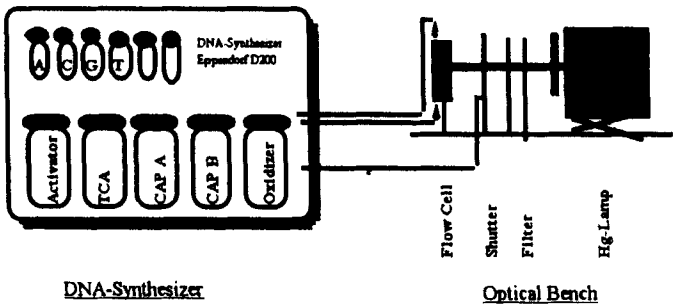


FIG.3 : DNA-Chip synthesizer

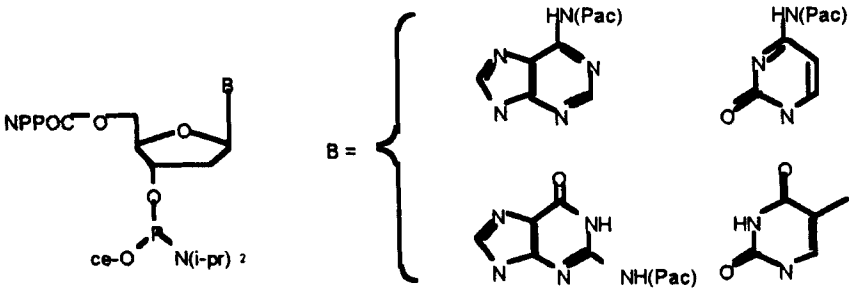
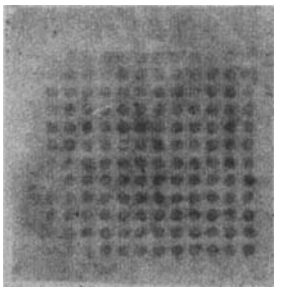


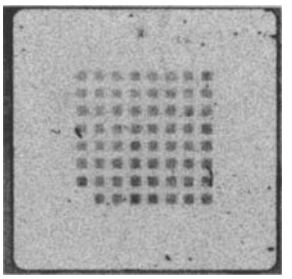
FIG.4 : NPPOC-protected phosporamidites

developed by Affymetrix, the 2-(2-Nitrophenyl)propoxycarbonyl-group (NPPOC) (*FIG.2*) represents a first promising member of this new family of photolabile groups that will enable the photolithographic synthesis on DNA-arrays of high-quality oligomers needed for SBH. The DNA-array synthesis was performed on a custom-built DNA-chip synthesizer (*FIG.3*). This experimental set-up consists of an optical bench for irradiation that is controlled by the DNA-synthesizer. Utilizing a special flow-cell reactor, the array-synthesis can be performed quasi-automatically. The NPPOC-phosphoramidites[8] (*FIG.4*) were phenoxyacetyl-protected at the heterocyclic bases, therefore permitting a quick deprotection step. Although giving a distinct lower loading, glass was favoured over polypropylene as solid support for DNA-array synthesis due to better mechanical properties.



array : 12 x 12 (3 x 3 cm)
support : glass
sequence : $d(T_7-T_{18})$
chemistry : DMTr & MeNPOC
irradiation : 6 min / cycle
hybridisation : 20ÞC ; 200 nM
probe : 5'-Cy^5-$d(A_{16})$

FIG.5 : DNA-array synthesized with MeNPOC-groups



array : 8 x 8 (1.5 x 1.5 cm)
support : glass
sequence : $d(T_{10})$
chemistry : NPPOC
irradiation : 8 min / cycle
hybridisation : 20ÞC ; 200 nM
probe : 5'-Cy^5-$d(A_{16})$

FIG.6 : DNA-array synthesized with NPPOC-groups

The DNA-synthesizer set-up was tested for its performance by synthesis of DNA-arrays from MeNPOC-protected phosphoramidites, resulting in DNA-arrays of comparable quality to conventional DMTr-protected phosporamidites *(FIG.5)*. Subsequently the NPPOC-protected phosphoramidites were employed for photolithographic DNA-array synthesis. Utilizing a 50 W mercury-lamp for irradiation, an 8 min exposure of the DNA-chip to the light source within each cycle proved to be suitable for generating high-quality DNA-arrays *(FIG.6)*. All DNA-arrays were quality-checked by hybridisation with the comlementary Cy^5-labeled strand and analyzed with a MD Storm 860 scanner at 50 µm resolution.

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