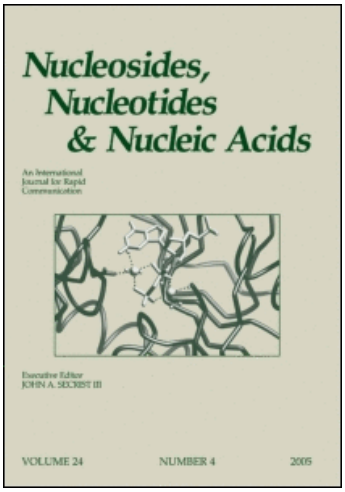

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Improved Solid Supports and Spacer/Linker Systems for the Synthesis of Spatially Addressable PNA-Libraries

S. Matysiak[a]; N. C. Hauser[a]; S. Würtz[a]; J. D. Hoheisel[a]
[a] Functional Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany

To cite this Article Matysiak, S. , Hauser, N. C. , Würtz, S. and Hoheisel, J. D.(1999) 'Improved Solid Supports and Spacer/Linker Systems for the Synthesis of Spatially Addressable PNA-Libraries', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1289 — 1291
To link to this Article: DOI: 10.1080/07328319908044694
URL: http://dx.doi.org/10.1080/07328319908044694

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1289–1291 (1999)

IMPROVED SOLID SUPPORTS AND SPACER/LINKER SYSTEMS FOR THE SYNTHESIS OF SPATIALLY ADDRESSABLE PNA-LIBRARIES

S. Matysiak, N. C. Hauser, S. Würtz, J. D. Hoheisel*
Functional Genome Analysis, Deutsches Krebsforschungszentrum,
Im Neuenheimer Feld 506, D-69120 Heidelberg, Germany

Abstract: Recently, consequential technologies[1-3] in molecular biology were developed using ordered arrays of DNA- and PNA-oligomers, which serve as analytic and diagnostic tools for DNA analyses We want to present our developments towards new spacer/linker systems for improved synthesis, cleavage and analysis of PNA-arrays, using new porous materials as solid support.

Introduction: Basis for the SPOT-synthesis[4] of PNA-arrays using the Fmoc-strategy is a specially manufactured membrane of inert porous material and appropriate pore size. We have developed such membranes with different spacer molecules for sterical and solubility reasons and tested their suitability for the synthesis of PNA libraries and subsequent hybridisation with DNA.

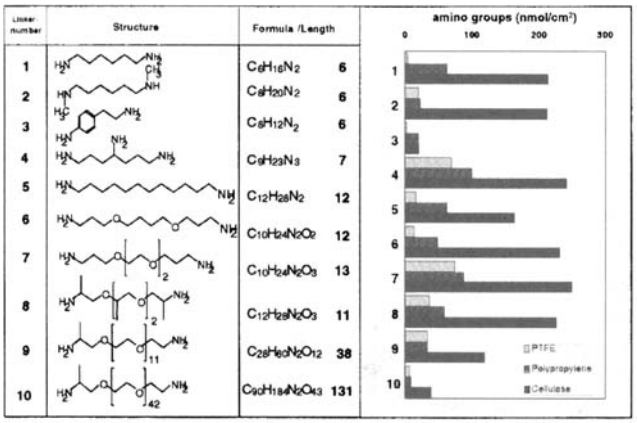

FIG.1: Spacer molecules and the corresponding concentration of amino groups (nmol per cm²) on different membrane materials.

Results: Three commercially available materials[5] (cellulose, polypropylene and PTFE) in terms of chemical resistance were tested. Different spacer molecules **1-10** (FIG.1) were then covalently attached using a very easy derivatisation step[6]. The concentration of amino groups was determined using the bromophenol blue assay[7]. In general, amino groups can be best introduced to cellulose material, followed by the polypropylene and the PTFE membrane. Beside the use of spacer molecules for steric reasons, the low solubility of PNA strands in aqueous systems due to the uncharged backbone is an important issue. We found that especially poly-G strands show high tendency to aggregate and then to precipitate[8]. To investigate the influence of hydrophilic moities at the N-terminus we synthesized

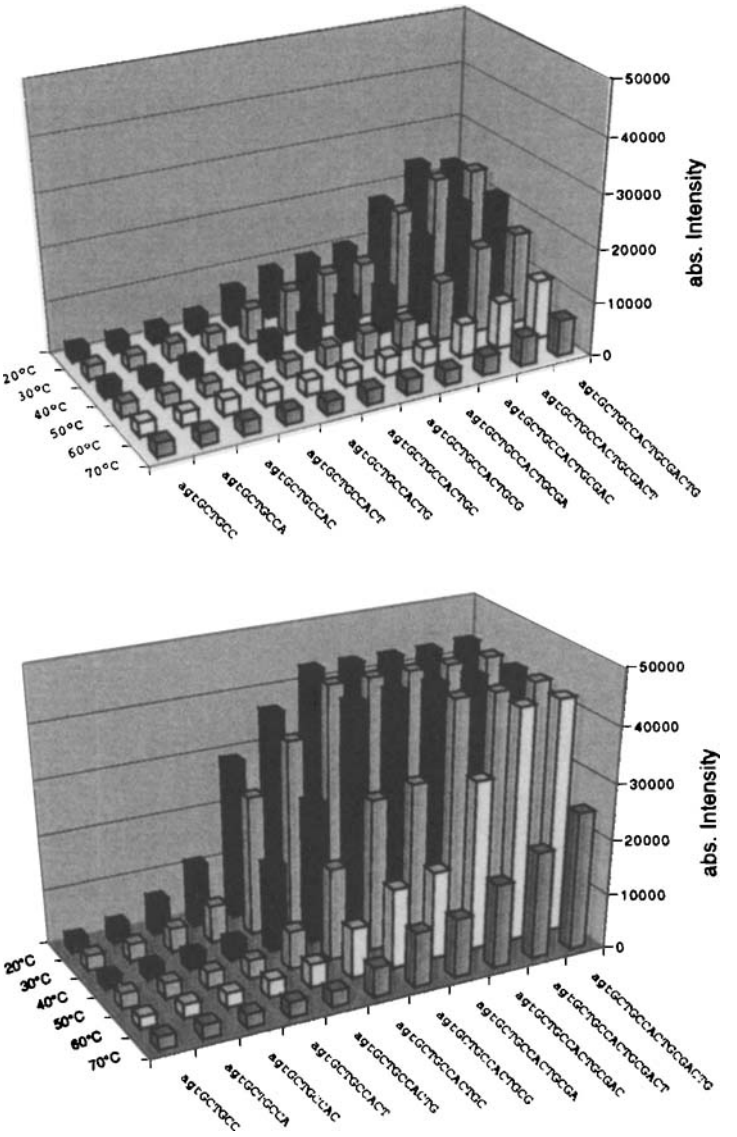

FIG. 2: Signal intensities of fluorescent labelled DNA oligonucleotide (dCAGTCGCAGTGGCAGC) matching PNA strands of different length (6-16mer, in capital letters) on polypropylene (left) and cellulose (right).

undecameric homologe A-, C-, G- and T-oligomers on a cellulose membrane with a hydrophilic spacer molecule **9** (FIG.1). After the standard PNA synthesis solubility enhancer like MeO-PEG-COOH or charged residues like lysine were introduced. The influence of these molecules is rather low (data not shown). Although the hybridisation of the complementary radioactive labelled dA_{16}, dC_{16}, dG_{16} and dT_{16} gave the expected pattern, the polyG (PNA) strand also showed a significant binding capacity towards the polyG(DNA) strand, which could only be removed by harsh stripping conditions (data not shown). Another important point are the hybridisation conditions applied. In consecutive hybridisation experiments using a fluorescent labelled 16mer DNA oligonucleotide as a probe, different conditions concerning buffer and temperature were tested. Various concentrations of Na^+-ions and detergent seemed to have minor influence within the investigated range (6-600 mM Na+, 0,07-7 % Na-lauroylsarcosine) (data not shown).

Significant and selective signal intensities can be obtained matching a fully complementary 10mer PNA even at high hybridisation temperatures[9] (FIG.2). These investigations clearly demonstrate that under specific conditions (hydrophilic membrane-spacer-system, high hybridisation temperatures) libraries of decamere PNA-strands show already sufficent selectivity and stability for DNA screening assays.

Acknowledgements: We would like to thank J. Krestyn and F. Reuthner for their excellent technical assistance. This work was funded by the European Commission under contract B104-CT97-2092.

References:

1 Hoheisel, J. D. *Trends Genet.*, **1994**, 10, 79-83.
2 Southern, E. M. *Electrophoresis*, **1995,** 16, 1539-1542.
3 Hoheisel, J. D. *Trends Biotechnol.*, **1997**, 15, 465-469.
4 Frank, R. *Tetrahedron*, **1992**, 48, 9217-9232.
5 AIMS Scientific Products, Mascheroder Weg 1, 38124 Braunschweig, Germany.
6 Matysiak, S., Frank, R. *German Patent Application* **1996** DE(1)9638085.5.
7 Matysiak, S.,Würtz, S., Hauser, N.C., Gausepohl, H., Hoheisel, J. D. in *PNA: Protocols and Application*, Nielsen, P. E. (ed.), Horizon Scientific Press, in press.
8 Weiler, J., Gausepohl, H., Hauser, N. C. , Jensen, O. N. , Hoheisel, J. D. *Nucleic Acids Res.*, **1997**, 25 (14), 2792-2799.
9 Griffin, T. J., Smith, L. M. *Anal. Biochem.*, **1998**, 260, 56-63.