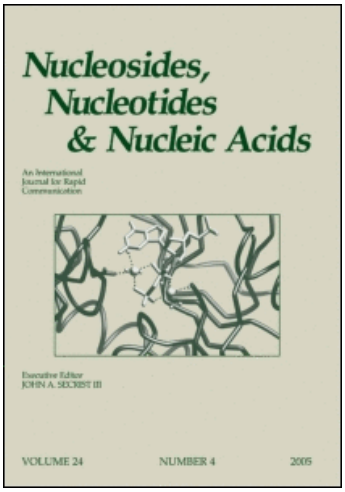

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PHTHALOYL STRATEGY - A NEW APPROACH TOWARDS OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

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ABSTRACT. A new rapid strategy for machine-aided oligodeoxyribonucleotide synthesis has been established utilizing phthaloyl groups for protection of the heterocyclic amino functions within the phosphoramidite approach. A large number of DNA-sequences has been synthesized. Due to high purity and speed of performance this strategy offers an excellent alternative to the common used acyl approach.

Among the various methods proposed for automated oligonucleotide synthesis the acyl approach[1] is the most common one and the appropriate building blocks are commercially avaible. Though improved chemistries allow faster deprotection procedures it usually takes not less than 5-6 hours to remove the widely used benzoyl and isobutyryl groups[2]. Simultaniously to the ammonia treatment the oligomer is cleaved off from the solid support and therefore due to contamination with protecting group residues an additional purification step is needed.
On the other hand aiming for crude products of high purity it is advisable to use a different approach e.g. the npe/npeoc-strategy proposed by Pfleiderer[3,4] or the allyl-approach by Noyori[5]. Both methods provide the advantage of removing all base- and phosphate protecting groups while the oligomer is still attached to the solid support. But rather long deprotection procedures and synthetic expense prevented that these methods became widespread used.

Our investigations towards new synthetic concepts in order to allow fast deprotection procedures resulting in crude products of high purity have led to the development of the phthaloyl strategy[6]. Within this approach the amino functions are blocked by phthaloyl groups (pth) that are found to be rapidly removed by DBU within 10-15 sec (FIG.1). The

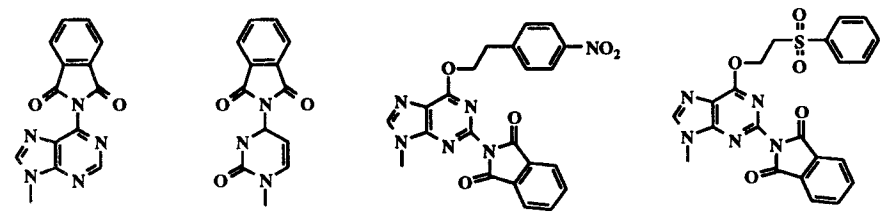
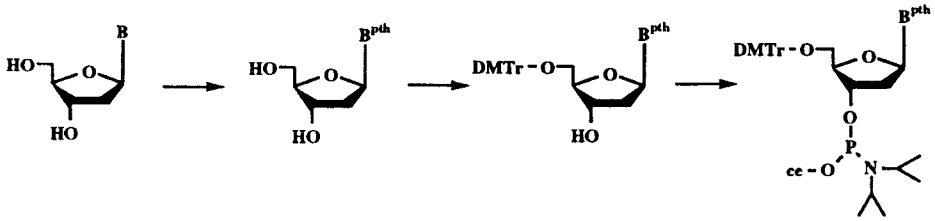


FIG.1 : Base protection within the phthaloyl strategy



B^{pth}	%
A^{pth}	96 %
C^{pth}	64 %
$G^{pth/npe}$	51 %
$G^{pth/pse}$	40 %

B^{pth}	%
A^{pth}	59 %
C^{pth}	61 %
$G^{pth/npe}$	67 %
$G^{pth/pse}$	66 %

B^{pth}	%
A^{pth}	72 %
C^{pth}	72 %
$G^{pth/npe}$	87 %
$G^{pth/pse}$	73 %

FIG.2 : Synthesis of the monomeric building blocks

lactam-function of 2'-deoxy-guanosine is protected either by an additional 2-(4-nitrophenyl)ethyl- (npe) or a 2-(phenylsulfonyl)ethyl group (pse) that can be deblocked with DBU as well. The straightforward synthesis of the monomeric building blocks implies the use of dimethoxytrityl- and ß-cyanoethyl-groups (ce) for 5'-OH and phosphate protection on a DBU-stable LCMAA-CPG support[7] (FIG.2).

The introduction of the phthaloyl groups into the heterocyclic bases was accomplished utilizing the transient protection method[8] as shown by Hata[9] for 2'-O-deoxyadenosine. Persilylation with trimethylsilylchloride and subsequent treatment with phthaloylchloride followed by removal of the tms-groups with pyridine/H_2O led to the phthaloyl protected nucleodsides .The synthesis of the tritylated compounds as well as the phosphoramidites

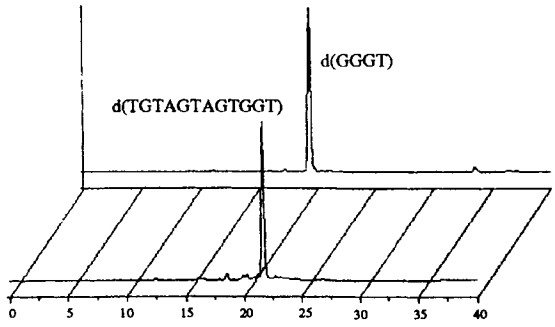


FIG.3 : RP-18-HPLC

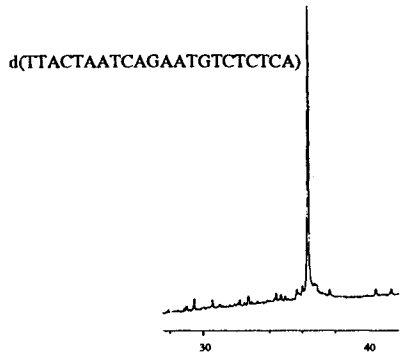


FIG.4 : Capillary electrophoresis

was accieved in the usual manner. Replacing tetrazole by the more versatile pyridine hydrochloride as the acidic catalyst during phosphitylation reaction resulted in our hands in slightly higher yields of the appropriate phosphoramidites. The coupling of the starting nucleoside to the DBU-stable LCMAA-CPG was realized either by synthesizing the 3'-O-succinate or via direct succinylation[10] of the LCMAA-CPG.

Performing oligonucleotide synthesis on a DNA-synthesizer using standard protocolls a large number of DNA-sequences up to a chain length of 22 nucleotides were obtained in excellent yield and purity. It was shown that this approach is orthogonal to iodine as well as to tert-butylhydroperoxide oxidation[11]. Furthermore tetrazole as the acidic catalyst could be easily replaced by pyridine hydrochloride to speed up condensation[12] time up to 5 times compared to tetrazole. After synthesis the final deprotection of all base and phosphate protecting groups was achieved by treatment with 2 M DBU in N-methylimidazole at

ambient temperature. After cleaving from the solid support the resulting crude oligonucleotides showed high purity as demonstrated by HPLC, CE, PAGE and MALDI-MS (FIG. 3, 4).

REFERENCES :

1 Barone, A.; Caruthers, M. *Nucleic Acid Res.* **1984**, 12, 4051

2 Khorana, H.G. *Bioorg. Chem.* **1978**, 1, 351

3 Stengele, K.P.; Pfleiderer, W. *Tetrahedron Lett.* **1990**, *31*, 2549

4 Himmelsbach, F.; Schulz, B.S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, 40, 59

5 Hayakawa, Y.; Hirose, M.; Noyori, R. *J.Org.Chem.* **1993**, 58, 5551

6 Beier, M; *Thesis*, University Konstanz **1996**

7 Stengele, K.P.; Pfleiderer, W. *Nucleic Acid Res.Symp.Ser* **1989**, 21, 101

8 Ti, G.S.; Gaffney, B.L.; Jones, R. *J.Am.Chem.Soc.* **1982**, 104, 1316

9 Kume, A.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1982**, 23, 4365

10 Pon, R.T. *Protocols for Oligonucleotides and Analogs*, Humanan Press **1993**, 465

11 Weiler, J.; Pfleiderer, W. *Nucleosides Nucleotides* **1995**, 14, 917

12 Beier, M.; Pfleiderer, W. paper in preparation