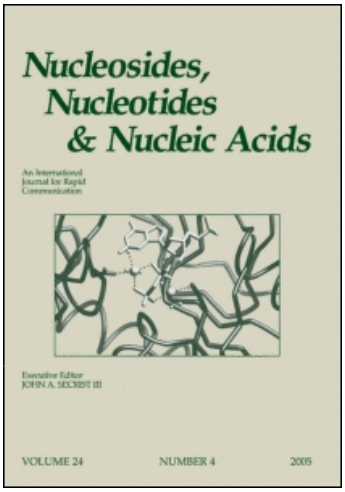

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Reversed(5'-3')Oligonucleotide Synthesis on Oxalyl-CPG Support

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To cite this Article Bogan, J. , Ignatovich, L. and Stankevic, E.(1999) 'Reversed(5'-3')Oligonucleotide Synthesis on Oxalyl-CPG Support', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1183 — 1185
To link to this Article: DOI: 10.1080/07328319908044657
URL: http://dx.doi.org/10.1080/07328319908044657

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1183–1185 (1999)

REVERSED(5'-3') OLIGONUCLEOTIDE SYNTHESIS ON OXALYL-CPG SUPPORT

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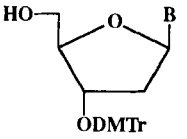
ABSTRACT : A solid-phase reversed(5'-3') oligodeoxyribonucleotide synthesis on oxalyl and succinyl aminopropyl controlled-pore glass (CPG) is presented. Oxalyl linked oligomers are deprotected without cleavage from the support.

There is an increasing interest in the solid-phase reversed(5'-3') chemical synthesis of oligonucleotides which after chain elongation and deprotection remain bound to the support. Such oligomers could, for instance, be used in the molecular diagnostic method based on primer extension.

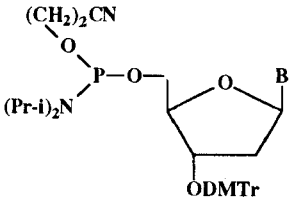
Up till now there are two methods of obtaining covalently 5'-3'linked deprotected DNA fragments or oligomers. By the first approach previously synthesized and modified (5'-amino or 5'-phosphate groups) oligomers are attached to carboxyl or amino groups containing supports using carbodiimides as the coupling agent [1,2]. Recently it was shown that on aminated polypropylene in the 5'-3' and 3'-5'direction built up oligomers after removing of protecting groups by standart procedure remain linked to the support [3,4]. In this case a base-stable phosphoramidate bond with the support was formed. By another approach a nucleoside was attached to a support via the 5'-OH comprising a photoremovable 3'-OH protecting group. In order to free it for the next coupling the support must be irradiated [5]. There are investigations about synthesis of oligomers directly on glass supports [6,7]. But these linkers are not stable enough during standart deprotection.

We succeeded in realizing a scheme of automated oligodeoxyribonucleotide synthesis in the 5'-3'direction on aminopropyl controlled-pore glass by means of succinyl and oxalyl anchors. As building blocks we used compounds I-VIII.

On succinyl aminopropyl CPG we realized reversed(5'-3') synthesis of the following oligomers: dTC_9; dTA_9; dT_{10}; d(5'-TACGAGATTCTCAGGAACCGA).



I B = T
II B = C^{Bz}
III B = A^{Bz}
IV B = G^{Dbf}



V B = T
VI B = C^{Bz}
VII B = A^{Bz}
VIII B = G^{Dbf}

Bz = benzoyl
Dbf = N, N-di-n-butylformamidine
DMTr = 4, 4'-dimethoxytrityl

Coupling was performed in acetonitrile in the presence of tetrazole. Detritylation was carried out with 1% CF_3COOH in methylene chloride. For capping of 3'-OH groups that failed to condense we used acetic anhydride and dimethylaminopyridine (DMAP).

In order to obtain oxalyl linked oligomers nucleoside-oxalyl-CPG (3'-O-DMTr-T-oxalyl-CPG and 3'-DMTr-G-oxalyl-CPG) were prepared from appropriate 3'-O-DMTr-nucleoside oxalyl chloride, triazole and aminopropyl CPG. On these supports using compounds V-VIII as building blocks we realized the reversed(5'-3') synthesis of 5'-d(TAGCATCTAGACCTCTCACT) and 5'-d(GGACAGAATCTTTCCACCAG). Coupling procedure was performed in acetonitrile in the presence of tetrazole. For detritylation we used 1% CF_3COOH in methylene chloride and capping was done with acetic anhydride and DMAP.

The oxalyl linked oligomer was dried in vacuum and treated with a solution of 0.5 M hydrazine hydrate in pyridine/acetic acid (4:1) [9] for 24 hours ar 25°C. Then the support was washed with ethanol, acetonitrile and dried. A mixture of dried pyridine and diisopropylanine (1:1) was added and the mixture left at 30°C for 20,5 h.

Then the support was washed with acetonitrile and dried in vacuum. Conc. ammonium hydroxide was added to the support for less than 2 min at room temperature and the solution passed through a NAP column.

All oligomers synthesized in 5'-3' direction were purified by RP chromatography on a Pharmacia FPLC system in the tritylated and detritylated forms. They were identical with authentic samples prepared on succinyl CPG in the 3'-5' direction and deprotected with conc. ammonium hydroxide by the standard procedure.

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