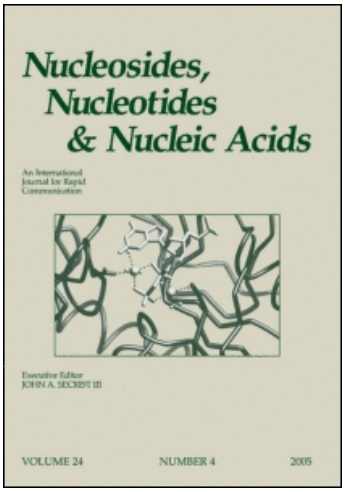

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Biotinylation of Oligonucleotides Using a Water Soluble Biotin Ester

M. Bengtström[a]; A. Jungell-nortamo[a]; A-C. Syvänen[a]
[a] Orion Corp., Orion Pharmaceutica, Genetic Engineering Laboratory, Helsinki, Finland

To cite this Article Bengtström, M. , Jungell-nortamo, A. and Syvänen, A-C.(1990) 'Biotinylation of Oligonucleotides Using a Water Soluble Biotin Ester', Nucleosides, Nucleotides and Nucleic Acids, 9: 1, 123 — 127
To link to this Article: DOI: 10.1080/07328319008045115
URL: http://dx.doi.org/10.1080/07328319008045115

NUCLEOSIDES & NUCLEOTIDES, 9(1), 123-127 (1990)

BIOTINYLATION OF OLIGONUCLEOTIDES USING A WATER SOLUBLE BIOTIN ESTER

M. Bengtström*, A. Jungell-Nortamo and A-C. Syvänen
Orion Corp., Orion Pharmaceutica, Genetic Engineering Laboratory, Valimotie 7, SF-00380 Helsinki, Finland.

ABSTRACT: Oligonucleotides aminated at the 5'-end were biotinylated with a water soluble N-hydroxysuccinimide ester of biotin in large scale. The biotinylated oligonucleotides were purified by reversed phase HPLC.

Biotin binds strongly and specifically to avidin or streptavidin, $K_d=10^{-15}$. Using biotin-labelled synthetic oligonucleotides this interaction can be utilized to capture specific DNA-sequences and DNA-binding proteins from complex mixtures[1,2].

Biotin can be attached to the 3'-end of oligonucleotides enzymatically with biotinylated dNTPs[3] or chemically to the 5'-end by the mediation of an amino group[4]. Conventionally amino groups are biotinylated with biotin-N-hydroxysuccinimidyl ester in water/dimethylformamide[5]. The disadvantage of this method is the insolubility of the biotin ester in water. In order to set up a more efficient and reproducible biotinylation method for oligonucleotides in large scale (100-600 nmoles) a water soluble sulphonated N-hydroxysuccinimide ester of biotin (sulfo-NHS-biotin, Pierce) was chosen[6]. The advantage of this aqueous reaction is that large amounts of oligonucleotides can be biotinylated in a small volume without any organic solvent. The conditions for this reaction were optimized in this study.

The aminated oligonucleotides were synthesized on a syntheziser using β-cyanoethyl phosphoramidates[7] and the reagent aminolink 2 (Applied Biosystems), FIG. 1. The aminated oligonucleotides were precipitated with ethanol and purified by HPLC, if necessary (as described below). For biotinylation

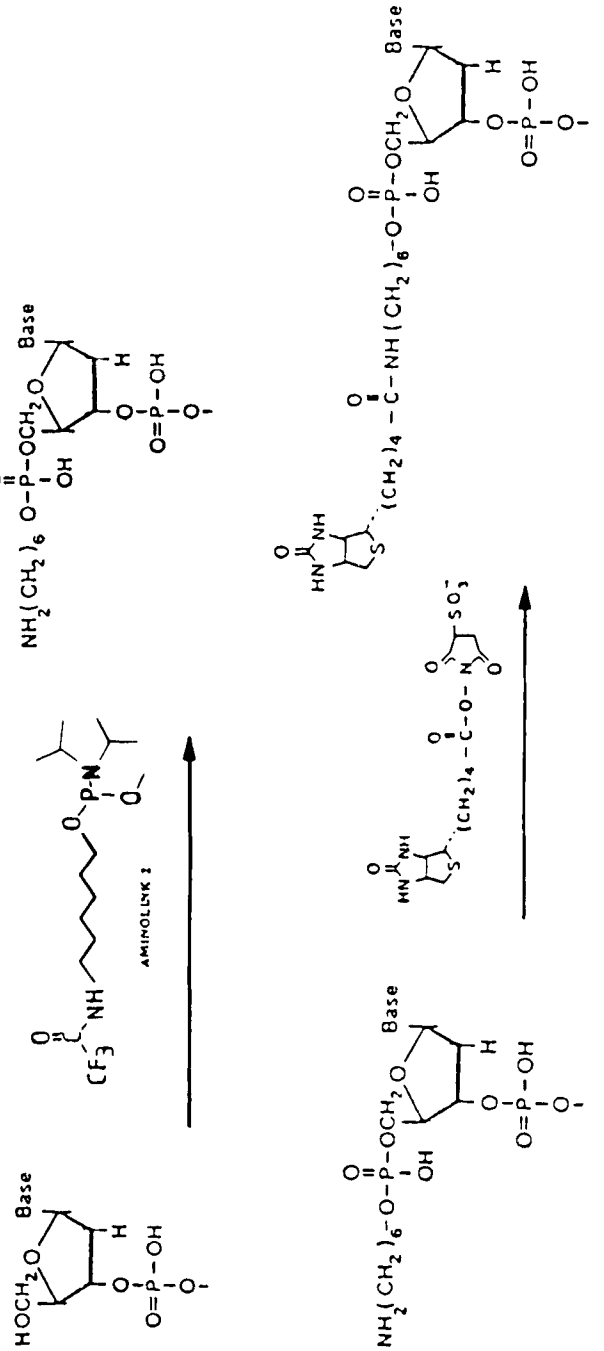

FIG. 1 The reactions for amination and biotinylation of synthetic oligonucleotides

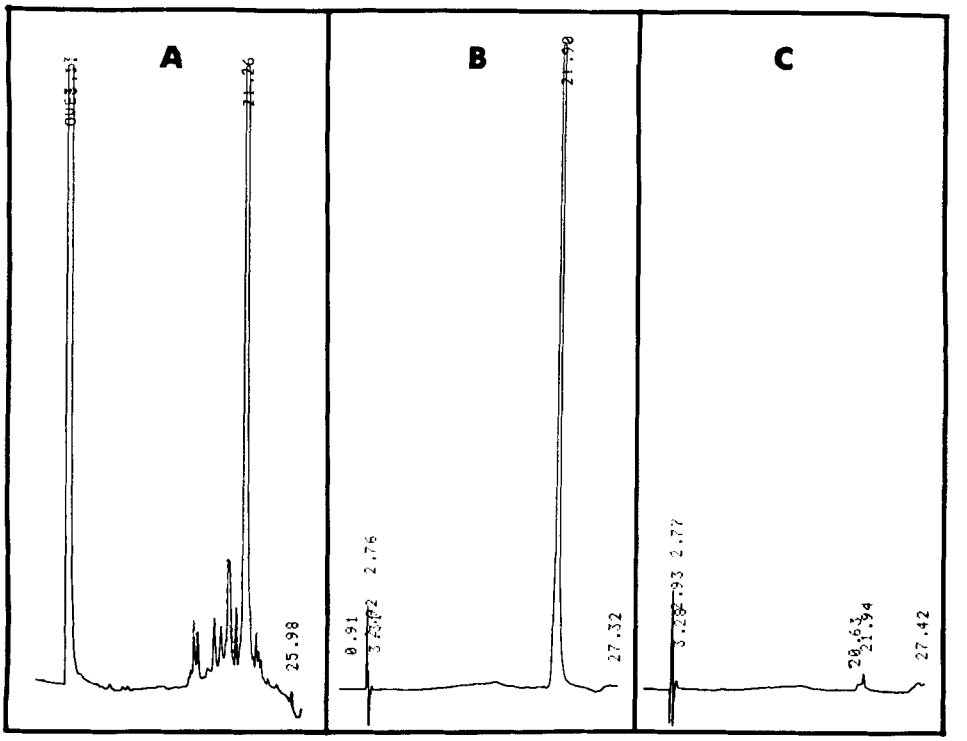

FIG. 2. HPLC-chromatogram of **A.** The biotinylation reaction mixture; aminated oligonucleotide (Rf 19,58) and biotinylated oligonucleotide (Rf 21,26) (Vydac 218TP510) **B.** The purified biotinylated oligonucleotide (Vydac 218TP54) **C.** The filtrate after incubating the biotinylated oligonucleotide 2B with streptavidine-agarose

TABLE 1
Conditions used for the optimization of the biotinylation reaction of oligonucleotides

Amount of oligo (nmole)	Excess biotin	Final phosphate conc.(mM)	Reaction time (min)	% Biotin-oligo obtained
5	100	5	120	20
5	100	25	60	74
5	100	100	60	85
30	100	125	120	99
550	100	250	30	76
630	100	250	30	80
140	40	150	30	97
420	40	100	30	95

the aminated oligonucleotides were dissolved in phosphate buffer and a freshly prepared water-solution of sulfo-NHS-biotin was added, FIG. 1.

The optimal conditions for the biotinylation reaction were determined using 5 nmole of an aminated 20-mer oligonucleotide. The biotin ester was used in 10-100 molar excess and 20 to 50 mM concentration, while the phosphate concentration was varied between 5 and 250 mM at pH 7,5 and 8,5. A 40 molar excess of sulfo-NHS-biotin was required as well as a high buffer concentration of 100 mM. No difference in biotinylation was observed when the sulfo-NHS-biotin concentration was varied between 20 and 50 mM with the pH of the phosphate buffer either 7,5 or 8,5. No improved biotinylation is achieved after 2 hours due to the hydrolysis of the sulfo-NHS-biotin in water. The best yields were obtained with 500 μM of oligonucleotide and 50 mM of sulfo-NHS-biotin in 100 μl of 100 mM phosphate buffer pH 7,5 or 8,5 and by allowing the reaction to proceed for 2 hours at 37°C, TABLE 1.

The extent of biotinylation was analyzed by HPLC. At the given reaction conditions a 80-100% yield of the biotinylated oligonucleotide was routinely achieved in large scale (up to 630 nmole) for 17-42-mer oligonucleotides. For 60-70-mer oligonucleotides peak broadening during HPLC purification complicated the interpretation of the chromatograms and lowered the yield.

The biotinylated oligonucleotides were purified on a reversed phase C-18 HPLC-column. A good separation was achieved in 20 minutes using a linear gradient of 5 to 20 % acetonitrile in 0,1 M triethylammonium acetate pH 6,9, FIG. 2A. The concentration was determined by UV spectroscopy and the purity was verified by incubating 50 pmole of biotinylated oligonucleotide with 25 μl of streptavidin-agarose particles (Bethesda Research Laboratories) in phosphate buffered saline for 30 minutes at 37°C and analyzing the filtrate with HPLC, FIG. 2B and 2C.

Oligonucleotides biotinylated by this procedure are routinely used in our laboratory as hybridization probes in the affinity-based hybrid collection procedure[1] and as primers in the polymerase chain reaction[8].

REFERENCES

1. Syvänen, A-C., Laaksonen, M. and Söderlund, H. Nucleic Acids Res. 14 (1986) 5037
2. Leblond-Francillard, M., Dreyfus, M. and Rougeon, F. Eur. J. Biochem. 166 (1987) 351
3. Riley, L., Marshall, E. and Coleman,M.S. DNA 5 (1986) 333
4. Chollet, A. and Kawashima, E. Nucleic Acids Res. 13 (1985) 1529
5. Guesdon, J-L., Ternynck, T. and Avrameas, S. J. Histochem. Cytochem. 27 (1979) 1131
6. Updyke, T. and Nicolson, G. Methods Enzymol. 121 (1986) 717
7. Beaucage, S. and Caruthers, M. Tetrahedron Lett. 22 (1981) 1859
8. Syvänen, A-C., Bengtström, M., Tenhunen, J. and Söderlund, H. Nucleic Acids Res. 16 (1988) 11327

Received April 25, 1989.