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A CHEMICAL METHOD OF LABELLING OLIGODEOXYRIBONUCLEOTIDES WITH BIOTIN: A SINGLE STEP PROCEDURE USING A SOLID PHASE METHODOLOGY

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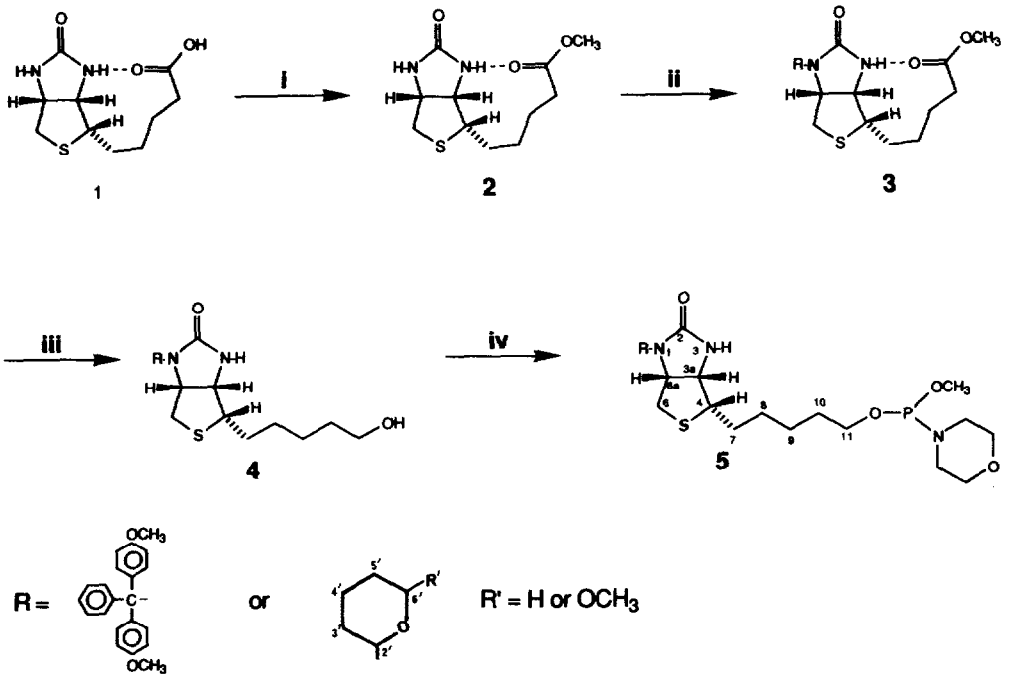
Abstract: Protected D(+)-biotin methylesters 3 were reduced to the corresponding alcohols 4 and phosphitylated with chloromethoxymorpholinophosphine. The resulting phosphoramidites 5 were used on a commercial DNA synthesiser to biotinylate synthetic DNA.

Chemical labelling of synthetic DNA for use as hybridisation probes continues to be of interest and a variety of methods have been reported.[1,2,3,4] Typically, biotin is coupled to an oligodeoxyribonucleotide in solution using a selection of cross-linking agents. We wish to report a one step procedure for biotinylation of an oligodeoxyribonucleotide which is compatible with a phosphoramidite solid phase methodology.

The low solubility of biotinol[5] in anhydrous organic solvents precluded the preparation of the corresponding phosphoramidite by reaction with chloromethoxymorpholinophosphine[6] in the presence of diisopropylethylamine. We reasoned that substitution of the ureido ring would have the dual purpose of increasing the lipophilicity of the biotin moiety and prevent phosphitylation at N-1 and N-3. A small number of N-substituted biotin derivatives have been described[7,8] previously. We found it possible to protect the N-1 position of the ureido ring by acid labile groups as shown in the Scheme. Substitution occurred only at the N-1 position, even with a 10 molar excess of DMTrCl/pyridine or toluenesulphonic acid/2,3-dihydro-4H-pyran or 2,3-dihydro-2-methoxy-4H-pyran. However, there was evidence in the proton n.m.r spectrum of 5 of phosphitylation at N-3 when more than the stoicheometric amount of chloromethoxymorpholino-phosphine was used.

Scheme



i, MeOH/$SOCl_2$; ii, DMTrCl/pyridine or toluenesulphonic acid/2,3-dihydro-4H-pyran or 2,3-dihydro-2-methoxy-4H-pyran; iii, $LiAlH_4$/THF; iv, $ClP(OCH_3)N\langle\rangle O$.

An oligodeoxyribonucleotide of sequence 5' AAGAAATACAGCCCG 3' (α2,55)[9] was synthesised on an Applied Biosystems 380A DNA Synthesiser (0.2 μmole scale) and biotinylated on the solid phase by 3x100s treatments with a 0.3M solution of 5 activated by tetrazole. The oligodeoxyribonucleotide was cleaved from the solid support and the protecting groups on the heterocyclic bases removed by treatment with concentrated ammonia solution (5h at 55°C). The N-1 protecting group was removed by treatment with acid (80% AcOH, 30 min at room temperature for DMTr; 0.1M HCl, 45 min at room temperature for THP derivatives). In a separate experiment, it was noted that no depurination with subsequent chain cleavage occurred when the parent oligodeoxyribonucleotide was exposed to conditions required to remove the THP protecting groups from the biotin moiety. The biotinylated synthetic DNA was readily separated from the faster migrating oligodeoxyribonucleotide by hplc (ion exchange and reverse phase columns) and further evidence for the presence of a biotin moiety on the slower migrating product was its almost complete retention (86%) by an avidin column. Finally, biotinylated (α2,55) was successfully used as a hybridisation probe for the α2 interferon gene with an alkaline phosphatase-based detection system.[10]

Representative experimental procedures.

1-N-(4,4'-Dimethoxytrityl)-D(+)-biotin methyl ester.
D(+)-Biotin methyl ester[5](5.0g,19.5mmole) was dried by azeotroping with pyridine and then dissolved in pyridine(125ml). 4,4'-Dimethoxytrityl chloride(7.24g,21.4mmole) was added and the solution stirred at room temperature for 60 min. Excess DMTrCl was destroyed by addition of MeOH(20ml) and the reaction mixture worked up. The residual oil was taken up in methylene chloride and purified by medium pressure chromatography(SiO_2,eluant A,CH_2Cl_2;eluant B, MeOH(8%) in CH_2Cl_2) to give the product(4.4g,40%). M/S M(560);δ($CDCl_3$,200MHz);7.22(9H,m,ArH),6.83(4H,d, ArH),4.94(1H,br.s.,NH),4.35(2H,m,H-3a,H-6a),3.78(3H,s,CH_3O-C),3.68(3H,s,CH_3OCO),3.11(1H,m,H-4), 2.47(1H,dd,H-6),2.29(2H,t,CH_2CO),2.27(1H,dd,H-6),1.5(6H,m,3CH_2).

1-N-(4,4'-Dimethoxytrityl)-D(+)-biotinol.
4,4'-Dimethoxytrityl-D(+)-biotin methyl ester(4.0g,7.1mmole) was reduced with $LiAlH_4$ (1.3g, 32.5mmole) in THF(130ml) at ambient temperature. Excess $LiAlH_4$ was destroyed (wet THF), butan-1-ol(500ml) was added and the Li/Al complex decomposed with Na_2SO_4aq (sat.),(100ml). The solvent was removed and the product(3.0g,80%) was obtained by medium pressure chromatography (SiO_2,eluant MeOH(10%) in CH_2Cl_2).M/S M(532).

[1-N-(4,4'-Dimethoxytrityl)-D(+)-biotinolyl]methoxymorpholinophosphine.
4,4'-DMTr-1-N-D(+)-biotinol(1.42g,2.67mmole), in a mixture of CH_2Cl_2(60ml) and diisopropylethylamine(1.3g,10.7mmole) under N_2 at -40°C was treated with chloromethoxymorpholinophosphine[6] (0.49g,2.67mmole). The reaction mixture attained ambient temperature during 30 min, and the solvent was removed in vacuo. The product (a mixture of diastereoisomers) was obtained by flash chromatography(SiO_2,eluant EtOAc(20%) in Et_3N). δ($CDCl_3$,200MHz);7.22(9H,m,ArH),4.92(1H,s, NH),4.33(2H,m,H-3a,H-6a),3.8(3H,s,CH_3O),3.7(2H,m,morpholino protons),3.68(1H,m,H-4),3.6(2H,t, 2CH_2-OP),2.47(1H,dd,H-6),2.26(1H,dd,H-6),1.52(8H,m,4CH_2).

1-N-(Tetrahydropyran-2-yl)-D(+)-biotin methyl ester.
Biotin methyl ester(3g,11.6mmole), in CH_2Cl_2(40ml) was added to freshly distilled 2,3-dihydro-4H-pyran(10.2g,121mmole) and the mixture cooled to 0°C. Toluenesulphonic acid monohydrate (11.4g,60mmole) was added and the stirred reaction mixture warmed to ambient temperature during 10 min and worked up. The product(1.8g,45%) was isolated as a white solid by crystallisation from ether/petrol(b.p.40-60°C). δ($(CD_3)_2$S=O,Bruker WH400);6.85(1H,s,H-3),4.80(1H,dd,H-2'),4.48 (1H,m,H-6a)4.10(1H,m,H-3a),3.87(1H,dd,H-6'),3.40(1H,dd,H-6'),3.61(3H,s,OCH_3),3.15(1H,m,H-4), 3.10(1H,d,H-6),2.83(1H,dd,H-6),2.33(2H,t,2H-11),1.9-1.3(12H,m,2H-7,2H-8,2H-9, 2H-3',2H-4', 2H-5').

Biotinylated oligodeoxyribonucleotides.

5 (R=DMTr; THP; MeOTHP) (150 μmole) in a mixture of $ClCH_2CH_2Cl$ (0.33ml) and CH_3CN (0.22ml) was used on an ABI 380A DNA Synthesiser with a modified 0.2 μmole scale cycle (coupling step, 3x100s and no capping step). The oligodeoxyribonucleotide was cleaved from the support and the base protecting groups removed by treatment with concentrated NH_3aq and then the protecting group on the biotin moiety was removed (80% AcOH, 20 min at R.T. for DMTr; 0.1M HCl, 45 min for THP derivatives). The product (4–11%) was obtained by hplc (a) SAX, eluant A, 60% formamide; eluant B, 0.3M KH_2PO_4 in 60% formamide with gradient 0–100% in 50 min^{11}; retention times, (α2,55) 16 min and (α2,55) (biotin) 17 min, and (b) C–18 reverse phase, eluant A, 0.1M NH_4OAc; eluant B,0.1M NH_4OAc/50% CH_3CN, retention times (α2,55) 28.8 min, and (α2,55) (biotin) 31 min.

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Processes for the preparation of biotinylated nucleotides and analogues thereof, as well as protected intermediates for use in such processes, are the subject of pending applications in a number of countries including Europe (European Patent Application Number 88307934.5), Japan and USA.

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