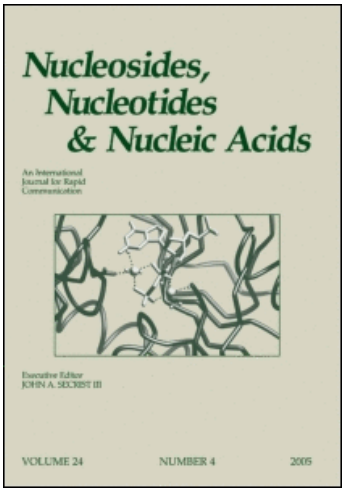

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Phosphoramidite Reagents for the Easy Preparation of Polylabelled Oligonucleotide Probes

Bazin Hervé[a]; Roget André[a]; Téoule Robert[a]
[a] CIS bioindustries; Département de Recherche Fondamentale/Laboratoires de chimie, Centre d'Etudes Nucléaires de Grenoble 85 X, GRENOBLE Cédex, France

To cite this Article Hervé, Bazin , André, Roget and Robert, Téoule(1991) 'Phosphoramidite Reagents for the Easy Preparation of Polylabelled Oligonucleotide Probes', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 363 — 366
To link to this Article: DOI: 10.1080/07328319108046480
URL: http://dx.doi.org/10.1080/07328319108046480

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 363-366 (1991)

PHOSPHORAMIDITE REAGENTS FOR THE EASY PREPARATION OF POLYLABELLED OLIGONUCLEOTIDE PROBES

Bazin Hervé, Roget André & Téoule* Robert
CIS bioindustries; Département de Recherche Fondamentale/
Laboratoires de chimie, Centre d'Etudes Nucléaires de Grenoble.
85 X, F-38041 GRENOBLE Cédex France.

Abstract: Di-O-dimethoxytritylated 1,4,7-heptanetriol and a di-o-dimetho xytritylated nucleoside derivative were converted to the respective phos phoramidites (4)& (7) used for the solid-phase synthesis of polylabelled branched chain oligonucleotides.

Oligonucleotides (ca.20 to 30 mers) are now widely used as probes (1) for specific detection or capture of nucleic acids sequences. Furthermore synthetic oligonucleotides are used as sequencing primers (2) and for PCR mediated DNA amplification (3). The main trend is the search for a sensitive, simple and safe methodology able to compete with the traditional ^{32}P labelling. Among the reported detection systems, biotin took a unique place due to its remarquable specificity and high association constant ($K_a = 10^{15}$) for the binding with avidin. It should be emphasized that linking a biotin residue to an aminoalkyl chain decreases the K_a by about two orders of magnitude(4). The preparation of polylabelled oligonucleotides has been suggested (5,6) as a way to increase the number or the strength of binding with avidin. Several biotin residues enzymatically incorporated on the 3'-end of an oligonucleotide ("tailing" with biotin-11-dUTP) showed a clear cooperative effect, the signal being proportional to the number of biotin residues (5). The same effect was observed using 5'-polybiotinyl oligonucleotides prepared chemically with a biotinyl-phosphoramidite (7). The conclusion is that the accessibility of the biotin toward the detection complex is a limiting factor. We anticipated that giving a 3-dimensional tree-structure to the labelled end of an oligonucleotide should allow a better access towards each biotin residue. The use of a number n of branching points in the sequence allows the simultaneous

incorporation of 2^n labels. These two remarks gave rise to the design of the compounds (4) and (7). Some results came from the use of water soluble polymer bearing biotin or lanthanide-chelating groups (8). A simple procedure allowing the preparation of labelled polymer directly in the course of the automated oligonucleotide synthesis was needed. The reagents have to fulfill the following requirements:

a) Compatibility with the phosphoramidite chemistry on commercially available automated synthesizer.

b) Straightforward synthesis from an easily accessible material.

c) To be a symmetrical molecule (4) or an optically pure molecule (7) then avoiding any problem due to diastereoisomery.

As starting material for the reagent (4) we chose the furylacrylic acid which was converted into ethyl 3-oxopimelate (1) (9). This compound was isolated by distillation (Bp 124-132°C/ 0.35 mbar) . The reduction of (1) in diethyl-ether with 2 molar equivalents of lithium aluminium hydride gave the 1,4,7-trihydroxy-heptane (2) in 90% yield. The triol (2) was treated with 2 equivalents of 4,4'-dimethoxytrityl chloride in pyridine solution (20°C,3h) to afford the di-tritylated derivative (3) in 72% isolated yield. The dried compound (3) was treated with diisopropylammonium tetrazolate and bis-diisopropylaminocyanoethoxyphosphine (1.3 eq) (10,11) in dichloromethane solution (20°C,1.5h). Standard work-up and lyophilization from benzene gave (4) in 70% yield. This compound showed a coupling yield in the range of 94 to 97% (determined by trityl assay). The nucleosidic derivative (7) was prepared as followed. The 5'-O-dimethoxytrityl-4-thiothymidine (7) was treated with 4 molar equivalents of 6-aminohexanol in ethanol solution (60°C, 16h) to give (5) in 95% yield. Further reaction with dimethoxytrityl chloride produced the di-O-protected derivative (6)in 83% yield. Phosphitylation gave the phosphoramidite (7) . A similar compound has been recently described for the synthesis of "multimers" which were tested for signal amplification in hybridization assay (12). The phosphoramidite reagents (4) and (7) were used in conjunction with the labelled nucleoside phosphoramidites (7) designed for the preparation of probes avoiding any post labelling operation or supplementary purification step. The branched-chain oligonucleotides were deprotected with concentrated ammonia solution and purified either on PAGE or RP-HPLC. The derivative (4) has been described recently in a patent application (13).

Experimental procedures : 1,4,7-Heptanetriol (2): Ethyl 3-oxopimelate (1) (0.5g,2.17mmol) in diethylether (10ml) was added to lithium aluminium hydride (0.16g,4.2mmol) in the same solvent. After 15 minutes refluxing, 20ml of a 10% water-THF mixture were added and the mixture was filtered through celite. The filtrate was adsorbed on silica (ca.5g, 0.2-0.5mm) and the dried silica loaded on a short silica column (5% MeOH/$CHCl_3$). The elution with 10-15% MeOH gave a syrup. Yield: 0.3g (92%). Rf=0.4 ($CHCl_3$/MeOH, 80:20). ^{1}H-NMR(CD_3OD):δ(ppm)= 3.56(pst,5H) H-1,4,7; 1.56(m,8H) H-2,3,5,6. ^{13}C-NMR(CD_3OD):δ(ppm)= 72.1(C-4); 63.0(C-1,7); 34.7(C-2,6); 29.9(C-4,5). FAB^+:$(M+H)^+$= 149.

1,7-Di-O-dimethoxytrityl-heptane-4-ol (3): The triol (2) (0.3g,2mmol) in pyridine (20ml) was treated with dimethoxytrityl chloride (1.4g). The compound was isolated on short column (silica G 60, stepwise gradient from 50% CH_2Cl_2-hexane to 2% acetone-CH_2Cl_2. Yield: 1.08g(72%). Rf= 0.6 (hexane/ethyl acetate, 80:20). ^{1}H-NMR:(CD_3COCD_3):δ(ppm)= 7.49-7.15 (m,18 H) and 6.82(d,8H) DMT; 3.70(s,12H)CH_3O; 3.50(m,1H)H-4; 3.07 (t,J= 6Hz, 4H)H-1,7; 1.85 (m,4H)H-2,6; 1.45(m,4H)H-3,5. FAB^+:$(M+H)^+$= 752.

1,7-Di-O-dimethoxytrityl Heptane-4-O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (4): A dichloromethane solution of (3) (1.3 g, 1.8 mmol) was treated with bis-diisopropylcyanoethoxyphosphine (0.65 ml, 2.1 mmol) and diisopropylammonium tetrazolate (150 mg, 0.9 mmol). The compound was purified over short silicagel column with 20% CH_2Cl_2 in hexane containing 1% triethylamine. The resulting product was lyophilized from benzene. Yield: 1.2 g (70%). R_f= 0.75 (hexane/ ethyl acetate, 80:20). ^{31}P-NMR(CD_3CN):δ(ppm)= 148.4. FAB+:$(M+Na)^+$= 975

5'-O-dimethoxytrityl-4-N-(6-O-dimethoxytrityl hexyl)-5-methyl-2'-deoxycytidine-3'-O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (7):Compound (5) was tritylated and purified as above to yield (6) which was in turn phosphitylated to give (7) as precipitate from hexane. Yield 0.33 g(92%) Rf 0.5 & 0.65 (dichloromethane/ethyl acetate/triethylamine, 50:45 :5). ^{31}P-NMR(CD_3CN):δ(ppm)= 149.4. FAB^+:$(M+Na)^+$= 1168.

Branched DNP-labelled oligonucleotides: The oligonucleotide synthesis was performed using Pac-amidites monomers (14). Phenoxyacetic anhydride was used for the capping. The reagents (4) or (7) were used as 0.2 M acetonitrile solution on an ABI 381A DNA synthesizer using the 0.2 μM cycle. The branched chain oligonucleotide was then labelled with a DNP-phosphoramidite (7). The reagent (7) was similarly used for the preparation of polybiotinylated probes.

Acklnoledgements: The authors are indebted to the Ministère de la Recherche et de la Technologie financial support. They thank B.Fouqué, T.Livache, S.Sauvaigo and B.Alpha for their work on biological aspects.

REFERENCES:

1. Matthews, J.A. and Kricka, L.J. Anal.Biochem. **1988**, 169, 1-25.
2. Ansorge, W.; Rosenthal, A.; Sproat, B.; Schwager, C.; Stegemann, J. and Voss, H. ; Nucleic Acids Res. **1988**, 16, 2203-2206.
3. Saiki, R.K.; Scharf, S.; Faloona, F, ; Mullis, K.B.; Horn, G.T.; Erlich, H.A.; Arnheim, N. Science **1985**, 230, 1350-1354.
4. Greene, N.M.; Biochemical J. **1963**, 89, 599.
5. Cook, A.F.; Vuocolo, E. and Brakel,C.; Nucleic Acids Res. **1988**, 16, 4077-4095.
6. Sproat, B.S.; Lamond. A.I.; Beijer, B.; Neuner, P. and Ryder, U. ; Nucleic acids Res. **1989**, 17, 3373-3386.
7. Roget, A.; Bazin, H. and Teoule, R.; Nucleic Acids Res. **1989**, 17, 7643-7651.
8. Sund, C.; Ylikoski, J.; Hurskainen, P. and Kwiatkowski, M. ; Nucleosides & Nucleotides **1988**, 7, 655-659.
9. Emerson, W.S. and Longley, R.I. Organic Syntheses Vol.IV, 302-303.
10. Barone, A.D.. Tang, J.Y. and Caruthers, M.H.; Nucleic Acids Res. **1984**, 12, 4051-4061.
11. Sinha, N.D.; Biernat, J.; Mac Manus, J. and Koster, H.; Nucleic Acids Res. **1984**, 12, 4539-4557.
12. Horn, T. and Urdea, S.M. ; Nucleic Acids Res. **1989**, 17, 6959-6967.
13. Kwiatkowski, M.; Sund, C. and Hurskainen, P. Patent n° WO 90/00622.
14. Schulhof, J.C.; Molko, D. and Teoule, R.; Nucleic Acids Res. **1987**, 15, 397-416.