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A simple procedure for constructing 5′-amino-terminated oligodeoxynucleotides in aqueous solution

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ABSTRACT

A rapid method for the synthesis of oligodeoxynucleotides (ODNs) terminated by 5′-amino-5′-deoxythymidine is described. A 3′-phosphorylated ODN (the donor) is incubated in aqueous solution with 5′-amino-5′-deoxythymidine in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), extending the donor by one residue via a phosphoramidate bond. Template- directed ligation of the extended donor and an acceptor ODN, followed by acid hydrolysis, yields the acceptor ODN extended by a single 5′-amino-5′-deoxythymidine residue at its 5′ terminus.

Oligodeoxynucleotides (ODNs) containing a terminal amino functionality are of considerable interest for the construction of bioconjugates with novel properties. The presence of the amine allows selective attachment of various ligands, including reporter groups (1,2) and peptide epitopes (3,4). A terminal amine is readily attached to an ODN via a non-nucleotide linker. In some instances, however, it is preferable that the ODN itself contains a terminal 5′-amino-5′-deoxynucleoside. The requisite 5′-amino-5′-deoxynucleoside phosphoramidites are not commercially available. Preparation of these compounds (1,2,5) involves a difficult multi-step synthesis that is not easily carried out in most molecular biology laboratories. This prompted us to develop a simple procedure for the preparation of 5′-amino-terminated ODNs that can be conducted in any laboratory without the use of sophisticated chemical equipment (Fig. 1).

A commercially available 3′-phosphorylated 'donor' ODN (DNA-1) was converted to a phosphoramidate-linked ODN one residue greater in length (Fig. 2, lanes 1 and 2). In a typical reaction, 0.01 M donor ODN was treated with 0.1 M 5′-amino-5′-deoxythymidine (Sigma) and 0.15 M EDC in 0.1 M 2-methylimidazole (2-MeIm) buffer (pH 7.0) (6). The reaction was complete after 2–4 h at 50°C, furnishing the modified donor ODN in 60–90% yield. 5′-amino-5′-deoxyadenosine is also available for use in this reaction.

The donor ODN was extended on the 3′ side of the phosphoramidate linkage either by template-directed ligation to an 'acceptor' ODN (DNA-2) (Fig. 2, lanes 3–5) or by extension using a DNA-dependent DNA polymerase (7). When a ^{32}P label was desired, 0.1 μM [5′-^{32}P]DNA-2, 2.5 μM extended DNA-1 and 2.5 μM of the corresponding DNA template (TEM) were incubated in the presence of 1 U/μl T4 DNA ligase, 20 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT and 1 mM ATP at 23°C for 2 h. The ligation of very short ODNs (e.g. a 5mer) was performed at 16°C for 24 h. When ^{32}P labeling was not required,

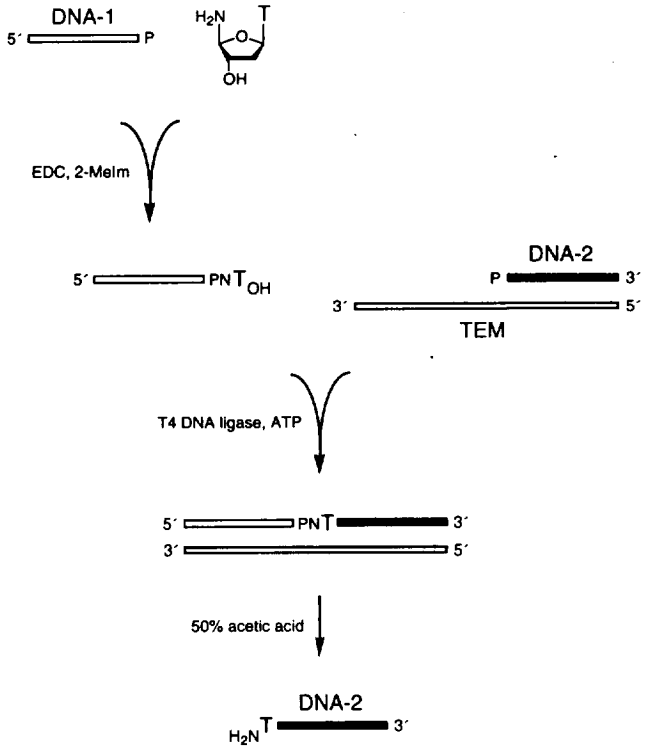

Figure 1. Scheme for the synthesis of 5′-amino-terminated ODNs. First, condensation of a 3′ phosphorylated 'donor' ODN (DNA-1) and 5′-amino-5′-deoxythymidine in the presence of EDC and 2-MeIm yields a phosphoramidate-linked ODN intermediate. Next, enzymatic ligation of this intermediate and a 5′-phosphorylated 'acceptor' ODN (DNA-2) in the presence of a complementary template (TEM) places the 5′-amino-5′-deoxythymidine residue immediately upstream of DNA-2. Finally, cleavage of the phosphoramidate bond under acidic conditions furnishes the 5′-amino-5′-deoxythymidine-terminated product.

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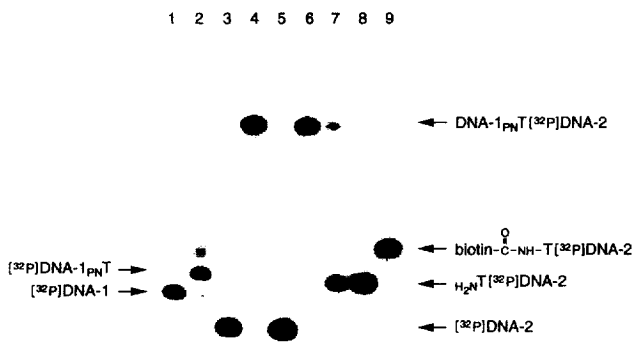

Figure 2. Analysis by denaturing polyacrylamide gel electrophoresis of reaction intermediates and products depicted in Figure 1. Lane 1, 5′-^{32}P-labeled DNA-1 having the sequence 5′-GGACTGATGCTATGp-3′. Lane 2, condensation of DNA-1 and 5′-amino-5′-deoxythymidine, followed by labeling of the reaction mixture with T4 polynucleotide kinase and [γ-^{32}P]ATP to yield [5′-^{32}P]DNA–1$_{PN}$T. Lane 3, 5′-^{32}P-labeled DNA-2 having the sequence 5′-pCACGAGCGAGTCT-3′. Lane 4, enzymatic ligation of unlabeled DNA–1$_{PN}$T and 5′-^{32}P-labeled DNA-2 in the presence of a complementary template having the sequence 5′-AGACTCGCTCGTGACATAGCAT-CAGTCC-3′. Lane 5, ligation reaction as in lane 4, but in the absence of the template. Lane 6, gel-purified ligation product DNA–1$_{PN}$T[^{32}P]DNA-2. Lane 7, acid cleavage of the ligation product to yield $_{H2N}$T[^{32}P]DNA-2. Lane 8, gel-purified $_{H2N}$T[^{32}P]DNA-2. Lane 9, derivitization of the 5′-amine of $_{H2N}$T[^{32}P]DNA-2 using sulfosuccinimidyl-6-(biotinamido) hexanoate.

the concentration of each ODN was increased to 10 μM and the amount of ligase was increased to 4 U/μl. The presence of the phosphoramidate linkage upstream of the ligation junction did not inhibit the activity of T4 DNA ligase.

The phosphoramidate linkage is labile under acidic conditions (8). Incubation of the ligation product in 50% acetic acid at 23°C for ≥3 h resulted in the release of the 5′-amino-5′-deoxythymidine-derivitized acceptor ODN (Fig. 2, lanes 6 and 7). The ODN product may be purified by HPLC or polyacrylamide gel electrophoresis. Purification is facilitated by designing the donor and template ODNs such that they differ in size compared to the desired product. The identity of the phosphoramidate-linked ODN intermediate and the 5′-amino-terminated ODN product were confirmed by matrix-assisted laser desorption ionization (MALDI) mass spectrometry (data not shown). The 5′-amino-terminated ODN product was biotinylated upon incubation with 25 mg/ml sulfosuccinimidyl-6-(biotinamido) hexanoate (Pierce Chemical) in 70 mM $NaHCO_3$ (pH 8.5) at 23°C for 1 h, confirming the availability of the primary amine (Fig. 2, lanes 8 and 9).

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