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Use of an Aminooxy Linker for the Functionalization of Oligodeoxyribonucleotides

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Abstract—We describe the preparation of oligonucleotides containing a 5′-linker bearing an aminooxy group. Use of the trityl protecting group for the aminooxy moiety allows purification of the modified oligonucleotide by reverse phase HPLC and cleavage in mild acidic conditions. Derivatization with an aldehydic reporter group is efficient and rapid.

Introduction

Modified oligonucleotides represent new tools in molecular biology for use as linkers, probes, primers, etc., and in therapy for gene expression inhibition. Their potential use for analytical and therapeutic applications has strongly spurred the development of new methods for linking covalently reporter groups on oligonucleotides. For these purposes, conjugation of oligonucleotides with fluorescent, lipophilic, intercalating, crosslinking, alkylating or DNA cleaving entities has been performed.[1] Most strategies involve generation of an aliphatic amino group at the 5′-terminus of the oligonucleotide and subsequent reaction with an electrophilic reporter molecule.[1–3] However, this approach generally requires large excess of the electrophilic reporter molecule (activated ester, isothiocyanate, etc.) to achieve complete conjugation.

In recent papers,[4,5] we described an efficient method for derivatization of oligonucleotides by use of the aminooxy–aldehyde coupling reaction. The oligonucleotide was functionalized with an aldehydic group located either on a base at any preselected position inside the sequence or at the 5′ extremity, and the reporter molecule carried an aminooxy group. We then considered the 'reverse strategy' in which the aminooxy moiety is supported by the oligonucleotide at the 5′-end. Aminooxy linkers have already been anchored on oligonucleotides by transamination[6] or by use of a phosphoramidite bearing a phthaloyl protected aminooxy group.[7] In the latter case, the phthaloyl group was cleaved during the ammonia treatment required for deprotection of the exocyclic amines of the nucleobases. The intermediate aminooxy-modified oligonucleotide was not isolated and the crude deprotected oligonucleotide had to be used without purification as a consequence of the high reactivity of the aminooxy group. We thus considered the use of a phosphoramidite bearing the aminooxy moiety protected by a group that could be selectively removed. The trityl protection was selected as it is stable to the basic deprotection conditions and the hydrophobicity of the trityl group allows the use of reverse phase HPLC for purification of the oligonucleotide.

In this paper, we report on the preparation of the phosphoramidite **1** (Fig. 1) and its incorporation into oligodeoxyribonucleotides. We describe the efficient conjugation of such modified oligonucleotides with the aldehydic fluorescein derivative **2** taken as an example.

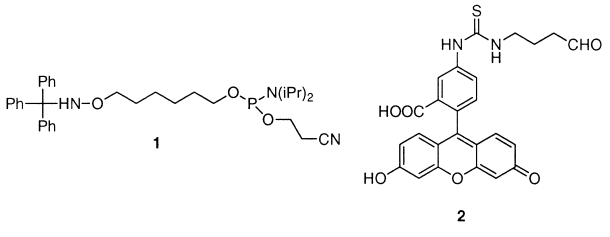

Figure 1.

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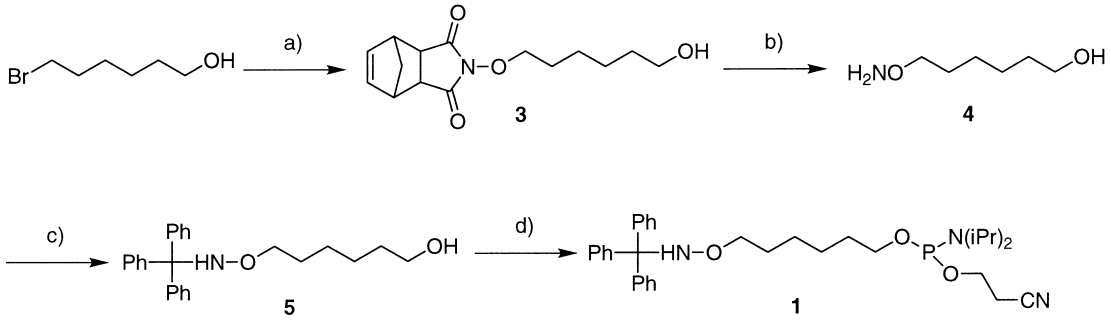
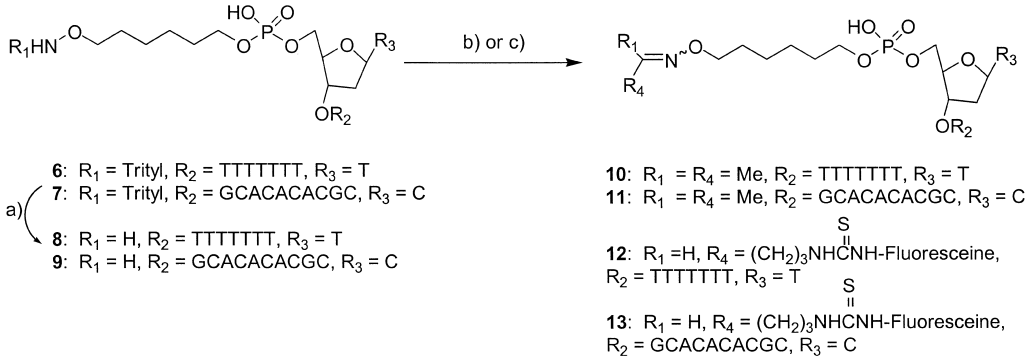


Scheme 1. (a) *N*-Hydroxy-5-norbornene-2,3-dicarboximide, K_2CO_3, DMF; (b) N_2H_4, EtOH; (c) Tr–Cl, pyridine; (d) *N,N,N′,N′*-tetraisopropyl-2-cyanoethylphosphorodiamidite, *N,N′*-diisopropylammonium tetrazolide, CH_2Cl_2.



Scheme 2. (a) 80% aqueous AcOH; (b) acetone; (c) **2**, DMF.

Results and Discussion

Preparation of the phosphoramidite 1 (Scheme 1)

Preparation of the phosphoramidite **1** was accomplished by the route depicted in Scheme 1. The aminooxy moiety was introduced using *N*-hydroxy-5-norbornene-2,3-dicarboximide as a precursor instead of the usual *N*-hydroxyphthalimide as the yields of coupling and subsequent cleavage were higher.[8] The desired *O*-protected oxyamine **3** was obtained in 80% yield by heating commercial 6-bromohexanol with the dicarboximide derivative at 50 °C in DMF in the presence of potassium carbonate. The aminooxy group was then deprotected using hydrazine in EtOH to afford the linker **4** in 92% yield. We then protected selectively the aminooxy function in **4** with the trityl group. Reaction of **4** with monomethoxytrityl chloride gave the corresponding aminooxy derivative that proved to be highly unstable. The more stable trityl protection was thus introduced by reacting **4** with trityl chloride in dry pyridine. Phosphitylation with *N,N,N′,N′*-tetraisopropyl-2-cyanoethyl-phosphorodiamidite afforded the phosphoramidite synthon **1**.[9]

Oligonucleotides synthesis (Scheme 2)

Two oligonucleotides containing the 5′-modification were synthesized according to standard β-cyanoethyl-phosphoramidite chemistry: the homopyrimidine d(XTTTTTTTT) and the undecamer d(XCGCACACACGC) in which X represents the 5′-aminooxy linker. After deprotection with concentrated ammonia for 24 h at 50 °C, the oligonucleotides **6** and **7** containing the trityl group at the 5′ extremity were purified by reverse

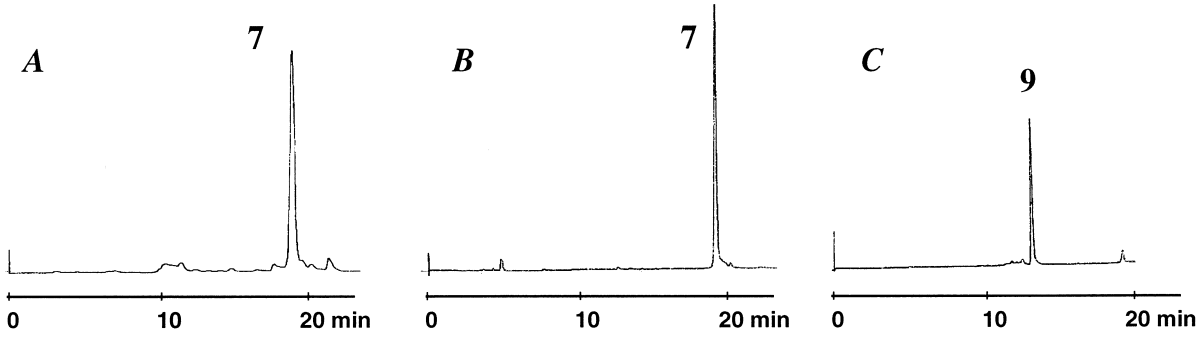


Figure 2. HPLC profiles of (A) crude mixture of undecamer **7**; (B) purified undecamer **7**; (C) crude detritylation mixture of **7**.

phase HPLC. Figure 2 shows the HPLC profile of the crude (A) and purified (B) oligonucleotide **7**. Both oligonucleotides **6** and **7** were characterized by ES-MS.[10] Cleavage of the trityl protection in the homopyrimidine oligonucleotide **6** was performed in 80% aqueous AcOH at room temperature for 6 h leading to selective formation of the deprotected oligonucleotide **8**. The same conditions were used for the purines containing oligonucleotide **7**. Even in this case no side reaction (depurination) occurred during the acidic treatment (Fig. 2C). Taking advantage of the high reactivity of the aminooxy function with carbonyl derivatives, the structures of **8** and **9** were confirmed by formation of the corresponding oxime ethers **10** and **11** by reacting the aminooxy oligonucleotides with acetone.[10]

The coupling reaction of the aminooxy-derivatized oligonucleotides with the fluorescein derivative **2** taken as an example of an aldehydic reporter group was examined. Reaction of **2** with oligonucleotides **8** and **9** was carried out at room temperature in water at pH 5 for 5 h using a 2-fold excess of the aldehydic fluorophore. In both cases the reaction was highly selective affording the corresponding oxime ethers **12** and **13**. The structure of the conjugated oligonucleotides **12** and **13** was confirmed by ES-MS.[10]

In conclusion, introduction of an aminooxy moiety at the 5′-end of oligonucleotides using the phosphoramidite **1** proved very efficient. This approach has the major advantage of an easy purification of well-characterized intermediates. Mild acidic cleavage of the trityl protection affords the aminooxy oligonucleotide without by-product formation. Derivatization with an aldehydic reporter group is rapid and quantitative as previously reported for that kind of aminooxy–carbonyl coupling reaction.[4,5,11,12]

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9. Phosphoramidite **1** was obtained as an oil; ^{1}H NMR ($CDCl_3$) δ 7.33–7.26 (15H, m, H–Ar trityl), 6.26 (1H, s, NH), 3.82 (2H, m, CH_2O), 3.68–3.58 (4H, m, CH_2O), 2.62 (2H, t, CH_2CN), 1.60–1.44 (5H, m, $2CH_2$ and CH), 1.21–1.17 (17H, m, $4CH_3$, $2CH_2$ and CH). ^{31}P NMR ($CDCl_3$) δ 145.4. MS (FAB, NBA matrix) *m*/*z* 575 [$M^{+\cdot}$].
10. ES-MS: calcd for **6**: 2808.6, found 2808.0; calcd for **7**: 3708.2, found 3710.9; calcd for **10**: 2606.6, found 2605.7; calcd for **11**: 3506.6, found 3505.7; calcd for **12**: 3024.6, found 3023.6; calcd for **13**: 3924.2, found 3925.7.
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