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A Facile Route to 3'-Modified Oligonucleotides

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Abstract: We describe an easy method for the solid phase synthesis of 3'-modified oligonucleotides. The general synthetic scheme involves the immobilisation of 5'-DMTr-T to CPG *via* a sulfonate linker, oligonucleotide synthesis and subsequent basic treatment to afford 3'-modified oligonucleotides containing a 2,3'-anhydronucleoside moiety. These compounds can be readily transformed into 3'-substituted oligonucleotides such as 3'-deoxy-3'-azido species.

Modified nucleosides and oligonucleotides have attracted much interest as potential therapeutics[2]. 5'- and 3'- modified oligonucleotides bearing different reporter groups and lipophilic residues have been described[3]. It has also been shown that 3'-deoxy-3'-aminonucleoside-containing oligonucleotides possess higher T_m compared to unmodified oligonucleotides[4]. For a study of HIV transcription we needed large amounts of oligothymidilates bearing an AZT residue[5] at the 3'-end. The azido group is incompatible with phosphorus (III) chemistry and consequently we could not simply use 5'-phosphoramidite of AZT and 5'-phosphoramidites of 3'-DMTr-nucleosides[6] for the reversed (5' to 3') oligonucleotide synthesis. To avoid a preparation of AZT-triphosphate for small-scale-limited enzymatic synthesis or a synthesis of AZT-based synthon for the reversed (5' to 3') phosphotriester oligonucleotide chemistry, we set out to design a method compatible with the phosphoramidite approach.

The anhydrocycle of 2,3'-anhydro-thymidine is succeptible to nucleophilic displacement at the 3'-position by nucleophiles like the azide ion to give 3'-deoxy-3'-substituted thymidine derivatives[7]. The formation of 2,3'-anhydro-thymidine occurs with satisfactory yield when 3'-OTs or 3'-OMs derivatives of Thd

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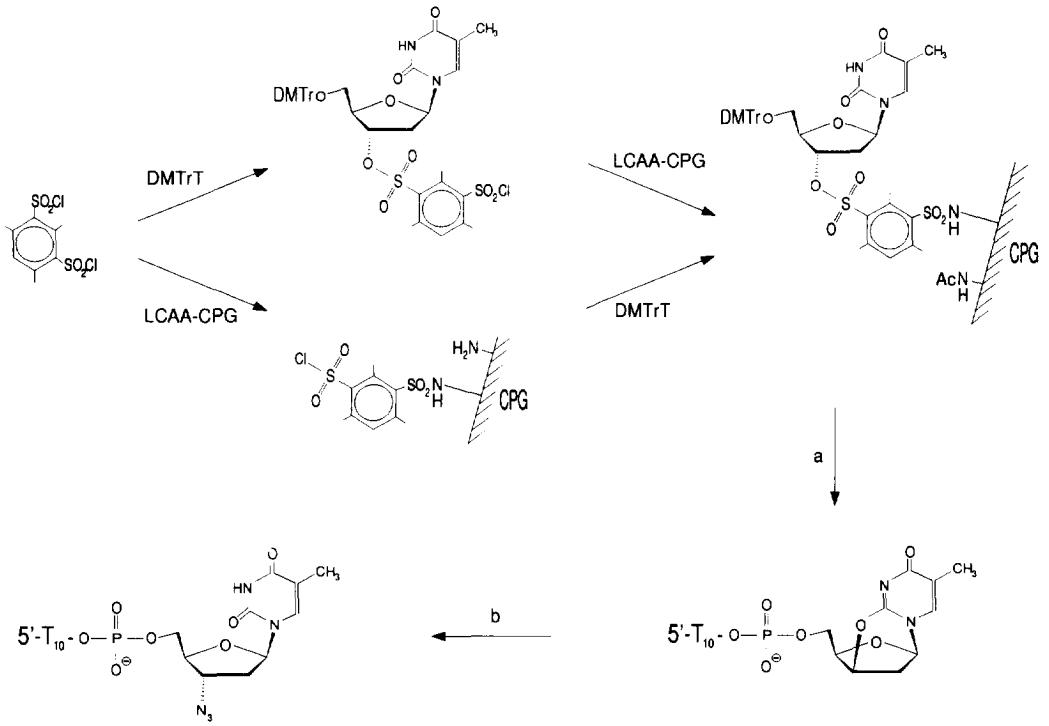
are treated with strong bases such as potassium phthalimide or DBU[7-9]. In our method, an appropriate solid support containing arenesulfonyl chloride moieties, which can play the role of good leaving groups, was prepared using several methods. In the first, LCAA-CPG (500 Å, Pierce) was successively treated with 5 equiv of diphenylcarbamoyl chloride in pyridine at rt for 5h and then with chlorosulfonic acid in 1,2-dichloroethane at 0°C. Subsequent reaction of this CPG with 5'-DMTrThd in Py led to the attachment of the nucleoside to the solid support through a sulfonate linkage formation with loading of ~ 15 μmol/g (determined by trityl assay[10]). However, as this CPG support was found to be sensitive to treatment with chlorosulfonic acid even at high dilution and at low temperatures, we developed efficient procedures according to which the LCAA-CPG is treated either with a large excess of bifunctional cross-anchoring reagent-arenedisulfonyl chloride (eg 1,5-naphtalendisulfonyl chloride, which can be easily prepared from freshly recrystallised 1,5-naphtyldisulfonic acid by treatment with PCl_5 in CCl_4 for 6h) with subsequent addition of 5'-DMTrThd or *in situ* formation of 3'- nucleoside arenesulfonate monosulfochloride which acts as an acylating



Scheme 1. a: oligonucleotide synthesis, then DBU in DMAA, 70°C, 1h; b: dry LiN_3 in DMAA, 100°C, 1.5h

reagent in the second step, giving the loading of nucleoside on the CPG support of about 10μmol/g. Higher loadings were obtained when mesitylene disulfonyl chloride (MDS, Aldrich) was employed as a cross-linking

reagent. In a typical experiment (Scheme 1), a suspension of LCAA- CPG beads in anhydrous Py was treated with 5 equiv of MDS at rt overnight, washed with CH_2Cl_2, dried *in vacuo* and then treated with a solution of 6 equiv of 5'-DMTr-Thd and 0.06 equiv of DMAP in anhydrous Py. The reaction mixture was left for 48h with occasional shaking, then anhydrous MeOH was added and the CPG was stirred for another 2h. After washing twice with Py and acetonitrile the CPG was capped (Ac_2O/Py) for another 2h, washed several times with acetonitrile and CH_2Cl_2 and dried *in vacuo*. The same support was obtained by mixing DMTrThd (dried by coevaporation with anhydrous Py) in Py with 0.01 equiv of DMAP and 1 equiv of MDS in 1,2-dichloroethane, stirring for 48h at rt and subsequent addition of the LCAA-CPG to the reaction mixture. After similar work-up the support with the loading ~ 30 μmol/g was obtained. In order to estimate the suitability of this modified CPG to act as a good leaving group we treated it with 0.5 M DBU in dimethylacetamide at 70°C for 1h. The product isolated by column chromatography with 62% yield was identical (^{1}H-NMR, TLC,MS) to 5'-DMT-2,3'-anhydro-Thd synthesized as described[11].

An automated oligonucleotide synthesis on this support was carried out using conventional phosphoramidite chemistry on ABI 380B DNA synthesizer. The sulphonate linkage was quite stable under the conditions of this chemistry, and an average yield for a condensation step was as high as that for commercial CPG supports with succinate linkages (more than 98%). Upon completion of the synthetic cycle decathymidilate-containing CPG support was treated with 0.5 M DBU in DMAA at 70°C for 1h, the solid was discarded and supernatant was treated with excess of LiN_3 (dried over P_2O_5) at 100°C for 1.5h. The product was then precipitated with cold acetone and purified by RP-HPLC to give ~45% of the 3'-azido-decathymidilate[12]. Some polypyrimidine oligonucleotides of mixed composition containing Cyd as well as Thd nucleosides were also synthesized using this method.

The method could only be applied to the synthesis of oligonucleotides bearing 3'-nucleosides capable of anhydronucleoside formation, therefore it cannot be used to synthesize, for example, 3'-deoxy-3'-azidoadenosine residues. Experiments to further extend this method by employing nucleophiles other than the azide ion and also by using anhydro-nucleosides of different bases (eg. Cyd, dCyd, Guo, dGuo and 2, 2'-deoxy Urd) are now in progress.

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12. The conditions of HPLC used: Beckman-314 HPLC system, Zorbax ODS column (4.6 x 250 mm), 5-30% grad. of MeCN in 0.1 M TEAA over 40 min, UV detection at 254 nm. Retention time for the control T_{10}: 15.5 min; retention time for $T_9T(N_3)$: 16.4 min. Mass-spectrum (MALDI-TOF) of $T_9T(N_3)$, desalted prior to running over NH_4OH- treated DOWEX 50WX8-200 resin: : 3005, 77 (calculated mass for $T_9T(N_3)$: 3004, 89). The mass-spectrum of the reaction mixture also showed the presense of about 10-15% of a compound with mass peak at 2981.29, which is probably the mixture of T_{10} and 5'-T_9(xylo-T) (calculated for T_{10} and 5'-T_9(xylo-T): 2980,23).

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