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Photolabile Linker for the Solid-Phase Synthesis of Base-Sensitive Oligonucleotides

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Abstract: *o*-Nitrophenyl-1,3-propanediol was covalently linked to long chain alkyl amine controlled pore glass (LCAA-CPG) beads through a stable phosphoramidate diester link. The resulting solid support was found suitable for the automated synthesis of 3'-phosphate unmodified as well as base-sensitive oligonucleotides. Efficient cleavage from the solid support was achieved by photolysis.
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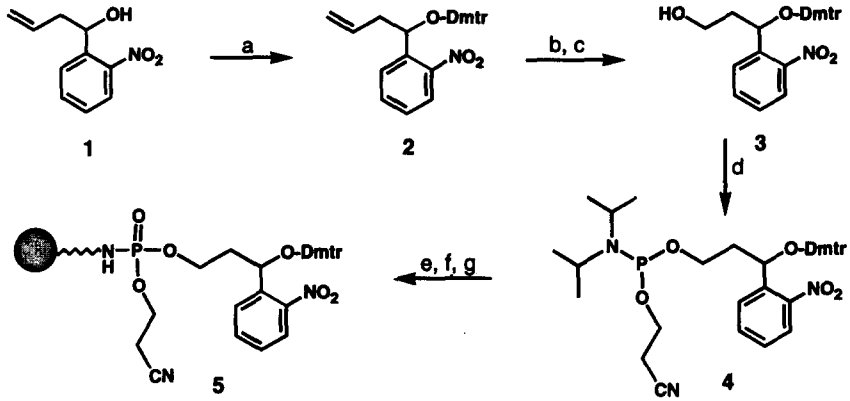
Modified oligonucleotides have been receiving increased attention over the past few years as diagnostic and mechanistic probes, as well as pharmacologically active compounds. Several methods have been developped that allowed the incorporation of base-sensitive nucleobases, functional groups or internucleoside links thanks to the use of nucleobase protecting groups removable under mild alkaline[1], reductive[2] or oxidative[3] conditions. Parallely, a variety of linkers between the oligonucleotides and the solid support that exhibit enhanced base lability[4,5] or are cleaved under acidic[6] or reductive[7-10] conditions have also been developped. Additionaly, Greenberg *et al.* reported the use of photolabile linkers based on the photochemistry of *o*-nitrobenzyl group and which were successfully applied to the solid-support synthesis by the phosphoramidite method of an oligodeoxynucleotide containing the fragile (5R)-5,6-dihydro-5-hydroxythimidine[11,12]. The versatility of photolabile linkers was evidenced by the synthesis of oligonucleotides with a 3'-amino or carboxylic acid function[13,14]. Cleavage from the support was achieved using either a transilluminator (λ_{max} 365 nm) or the 400 nm band pass filtered output of a high-pressure Hg/Xe lamp. Typically, after 1.5 to 2 hr irradiation, the yield of isolated oligonucleotides (up to 20-mer) relative to yield obtained using conventional aqueous ammonia mediated cleavage vary between 67 and 82%[15]. Under these conditions, formation of thymidine-thymidine photodimers was found less than 3%. Reduction of irradiation time should lessen the effects of this undesirable proccess specially in thymidine such oligonucleotides.

In this respect, we investigated a new photolabile solid support the linker of which is constituted of 1-*o*-nitrophenyl-1,3-propanediol. The corresponding 3-O-dimethoxytrityl-1-O-phosphoramidite derivative has been previously used as a photocleavable DNA building block and applied to phototriggered hybridization[16]. Upon irradiation at 355nm, oligonucleotide break was observed at position of the photocleavable block producing a 5'-fragment bearing a 1-*o*-nitrophenyl-propan-1-one-3-yl group at its 3'-end and a 3'-fragment bearing a phosphate at its 5'-end. We reasoned that reacting the amino function of solid support with isomeric building block **4** (Scheme) having a phosphoramidite group on position 3 and a dimethoxytrityl protecting group on the secondary hydroxyl function on position 1 would afford useful photolabile solid support **5** for the



Scheme: a) 4,4'-dimethoxytrityl chloride, 4-dimethylaminopyridine, Et_3N, pyridine; b) OsO_4, $NaIO_4$, dioxan-water; c) $NaBH_4$, water; d) bis (diisopropylamino)-(2-cyanoethyl)-phosphine, diisopropylammonium tetrazolide, CH_2Cl_2; e) LCAA-CPG, tetrazole, acetonitrile; f) I_2, water, pyridine, g) Ac_2O, N-methylimidazole, tetrahydrofuran.

synthesis of 3'-phosphate oligonucleotides. The expected stability of the phosphoramidate diester link[6] located between solid support and the photolabile linker in **5** under the conditions applied during the assembling of oligonucleotides represented an additional favorable feature.

Building block **4** was synthesized in a 24% overall yield in four steps from 1-*o*-nitrophenyl-3-butenol **1**[16]. Selectively protected diol **3** was obtained following dimethoxytritylation of **1** with a 3 fold molar excess of dimethoxytritylchloride in pyridine and subsequent oxidation with osmium tetraoxide in presence of sodium periodate[17]. The resulting aldehyde was not isolated but reduced with sodium borohydride. The modest yield of these one-pot redox reactions was mainly due to partial loss of dimethoxytrityl group likely induced by rather acidic sodium periodate[18] during the oxidation step as evidenced by TLC analysis. After purification by silica-gel column chromatography, primary alcohol **3**[19] was converted to phosphoramidite **4**[20] according to a standard procedure[21]. Then, 0.2 M solution of building block **4** in anhydrous acetonitrile was used on a DNA synthesizer (ABI model 381A) in a same conditions as regular nucleoside building blocks[22]. Synthesis of photolabile solid-support **5** was carried out in a "10 µmol" column filled with LCAA-CPG (0.39 g, 70 $\mu mol.g^{-1}$ amine, Sigma) using a modified "10 µmol" regular cycle. Modifications concern essentially the extention of coupling (180 s) capping (300 s) and oxidation (60 s) times. Loading based on dimethoxytrityl cations release was 64 $\mu mol.g^{-1}$ indicating that 7 molar equivalents of phosphoramidite **4** were sufficient to react virtually with every amino function present of the CPG support. The usefulness of this photolabile solid-support was evidenced by the efficient synthesis of base- and nucleophile-sensitive dodecathymidylate analog bearing eleven methylphosphotriester internucleoside linkages. Synthesis was carried out on 2 µmol scale using commercialy available thymidine methylphosphoramidite. An extended coupling time (180 s) was applied during the first two cycles in order to achieve high yield and 1.1 M *ter*-butylhydroperoxide was substituted for 2% iodine as oxidizer at every cycle to avoid demethylation of the internucleoside linkages[4]. Cleavage from the solid support was achieved by exposing a mechanically stirred suspension of supported oligonucleotide (2 µmol in H_2O-MeOH 1/1, 1 ml) to the Pyrex (2 mm thick) filtered output of a 125 W high-pressure Hg lamp for 15 min at 20°C. Fig. 1A shows the HPLC chromatogram from the analysis of the crude material (100 A_{260} units) obtained after removal of the glass beads by filtration. This material was virtually homogeneous before

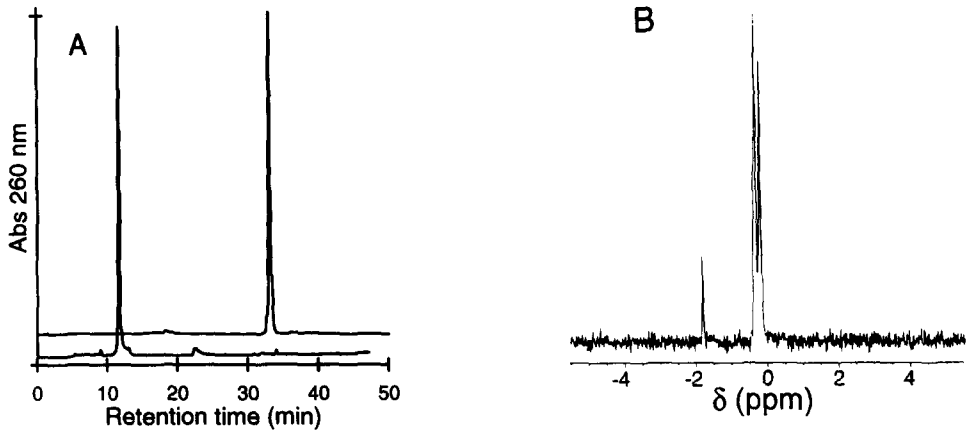


Fig.1: analysis of crude 5'-methylphosphodiester dodecathymidine methylphosphotriester. A: reverse-phase HPLC before (upper profile) and after treament with thiophenolate and then concentrated aqueous ammonia (lower profile); B: ^{31}P-NMR spectrum.

and after removal of internucleoside methyl groups by treatment with thiophenol-triethylamine-dioxane (1/1/2, v/v/v) for 1.5 hr at room temperature and then 30% aqueous ammonia for 2 hr at 55°C which suggests that thymidine-thymidine photodimerisation occured at very low extent during photolysis[23]. Its purity was further confirmed by ^{31}P-NMR analysis (Fig. 1B). Two sets of signals corresponding to internucleoside methyl phosphate triesters (multiplet centered at δ 0.25 ppm) and to methyl phosphate diester (δ 1.85 ppm) were found in a ratio of 1/11.8. Moreover, the electro-spray ionisation mass spectrum obtained in positive mode revealed the presence of three species at m/z values 1279, 1918 and 1928 corresponding to $[M+3H]^{3+}$, $[M+2H]^{2+}$ and $[M+1H+Na]^{2+}$ respectively. The observed maximum charge is identical to that obtained previously for unmodified dodecathymidylate[24] and experimental molecular weight is in good agreement with calculated average one (3834 and 3836.7 respectively).

Finally, the efficiency of this photolabile solid support for the synthesis of unmodified 3'-phosphate oligonucleotides was evaluated. 16-mer d(CAGTCGGTCAAGTAGT)p was prepared according to cyanoethylphosphoramidite chemistry on 1 μmol scale. Based on trityl responses, our results indicate that increased coupling times (180 s instead of 15 s) should be applied to maintain the average coupling yield comparable to that (98.6 %) obtained with regular succinyl solid support. We assigned this lower reactivity to the high loading (64 as compared to 35 μmol g^{-1} for regular support) rather than to the nature of the linker present between CPG and the growing olgonucleotide chain. Indeed, when loading of photolabile support was deliberately decreased to 35 μmol g^{-1} by the use of a lower amount (7 instead of 18 molar eq.) of the first incoming nucleoside phosphoramidite and then applying standard conditions during the following cycles, an average coupling yield of 99.6 % was obtained. These results were corroborated by the reverse-phase HPLC profiles of crude material (110 A_{260} units) obtained after deprotection with 30 % aqueous ammonia (5h at 55°C) and cleavage from the solid support by photolysis.

In conclusion, we described a photolabile linker which was introduced on amino-derivatized solid support by the phosphoramidite chemistry on a DNA synthesizer. The resulting solid support was found fully compatible with the conditions used during phosphoramidite-based oligonucleotide synthesis. High loading was obtained which renders this support appropriate for the large-scale synthesis of 3'-phosphate unmodified

as well as base-sensitive oligonucleotides. Cleavage from the solid support was acheived by photolysis with negligeable formation of photo-induced damages.

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19. ^{1}H-NMR ($CDCl_3$): δ 7.7 (broad d, 1H, J 7.5 Hz, H_3 or H_6 on nitrobenzyl ring); 7.60 (dd, 1H, J 8.2 and 1.2 Hz, H_3 or H_6 on nitrobenzyl ring); 7.42-7.08 (m, 11H, H_4 and H_5 on nitrobenzyl ring and trityl); 6.67-6.56 (m, 4H, trityl); 5.44 (dd, 1H, J 6.2 and 4.2 Hz, Ar-C<u>H</u>-CH_2-); 3.80-3.58 (m, 2H, -C<u>H_2</u>-OH); 3.72 and 3.7 (2s, $2CH_3O$-); 2.37-2.07 (m, 3H, -C<u>H_2</u>-CH_2-O<u>H</u>). ESI-MS positive mode: $[M+Na]^+$ 522, $[2M+Na]^+$ 1021.6.
20. ^{31}P-NMR (CD_3CN): δ 147.56 and 147.45. ESI-MS positive mode: $[M+H]^+$ 700.1, $[M+Na]^+$ 722.3, $[2M+Na]^+$ 1421.9.
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22. Handling and reactions with photosensitive compounds including oligonucleotide synthesis were carried out in attenuated light.
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