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SOLID PHASE SYNTHESIS OF DNA UNDER A NON-DEPURINATING CONDITION WITH A BASE LABILE 5'-PROTECTING GROUP (Fmoc) USING PHOSPHITEAMIDITE APPROACH

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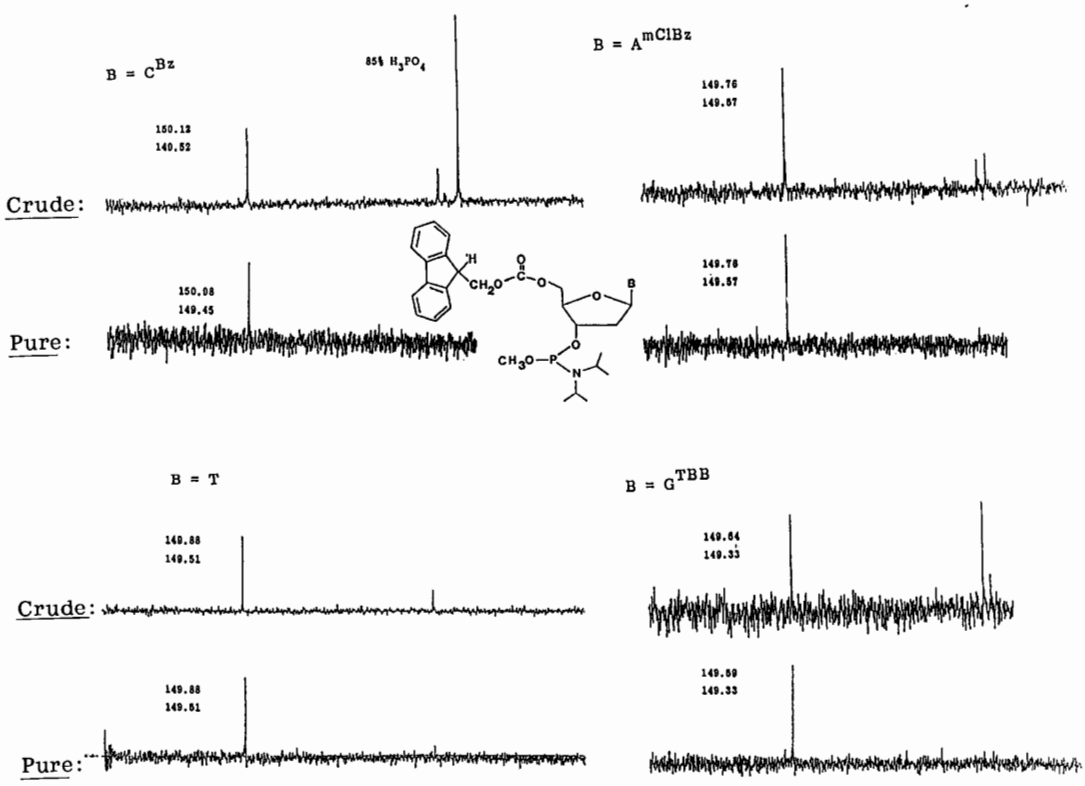
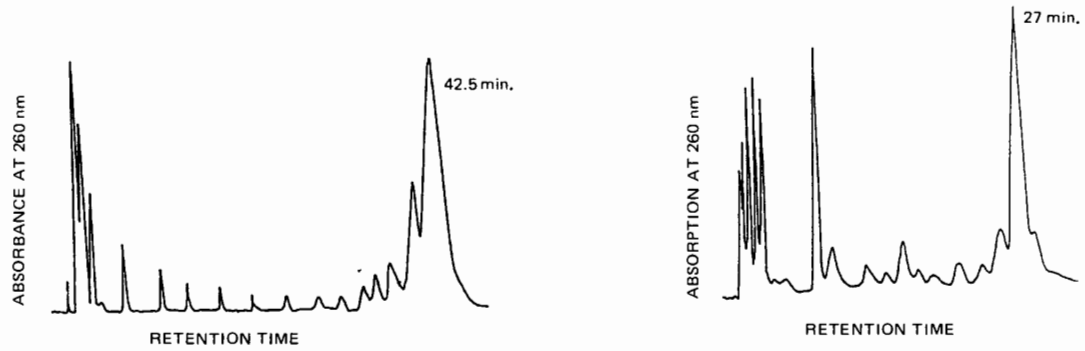
Summary: 5'-Fmoc protected 2'-deoxynucleoside building blocks have been employed in DNA synthesis in order to remedy the depurination problem.

One disadvantage of the methods[1,2] used today in the synthesis of DNA is the depurination encountered during the removal of the 5'-DMTr group from N^6-protected deoxyadenosine blocks. Several attempts have been made to prevent this by either altering the acidic conditions employed[3] or by varying the N^6 protecting group[4]. Other authors have modified the trityl group[5] or used p-phenylazophenyloxycarbonyl[6] which could be removed under basic hydrolytic conditions.

We have previously shown that the 9-fluorenylmethoxycarbonyl (Fmoc)[7] group could be used in oligodeoxynucleotide synthesis, by synthesizing a T_{24} fragment. We now wish to report that the Fmoc group can also be employed in the phosphiteamidite approach[2] on solid phase constituting a DNA synthesis strategy fully based on non-acidic reaction conditions. The 5'-Fmoc protected nucleosides were prepared in 60-80% yields by treatment with Fmoc-Cl (1.3 equiv. dissolved in dry acetonitrile 10 ml/mmol) of the nucleosides in dry pyridine (10 ml/mmol). These 5'-Fmoc protected nucleosides were then converted to their corresponding phosphiteamidites following standard methods[2], except that THF was used as solvent and that only two equiv. of base were used. The reacion was worked up as usual[8], and the phosphiteamidites were purified by silica gel chromatography using CH_2Cl_2:EtOAc:pyridine (2:2:1, v/v/v) for separation. The ^{31}P-NMR spectra of the crude and purified amidites are shown in Fig. 1. It was found that the 5'-Fmoc group could be cleaved by treatment with DBU (30 equiv.) in





HPLC of crude T_{16}

HPLC of crude $^{5'}d(TTAACGGATTCAACC)^{3}$

Fig. 2

dry acetonitrile within 18 seconds at 20 °C. Under these conditions there was approximately 1% cleavage of the support. To demonstrate the usefulness of this technique we have synthesized[8] T_{16} and a mixed sequence $^{5'}d(TTAACGGATTCAACC)^{3'}$. Fig. 2 shows the Hplc[9] elution profiles of the crude mixtures after deprotection[2]. They were also subsequently characterized by ^{32}P-labelling and electrophoresis. We believe that this method offers a potential solution to the problem of depurination although the stability of the support requires some improvement.

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