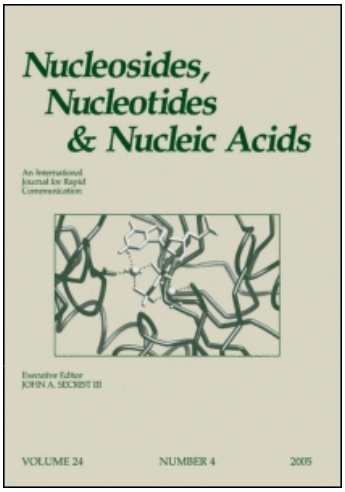

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Solid Phase Synthesis of DNA Under a Non-Depurinating Condition with a Base Labile 5'-Protecting Group (Fmoc) Using Phosphiteamidite Approach

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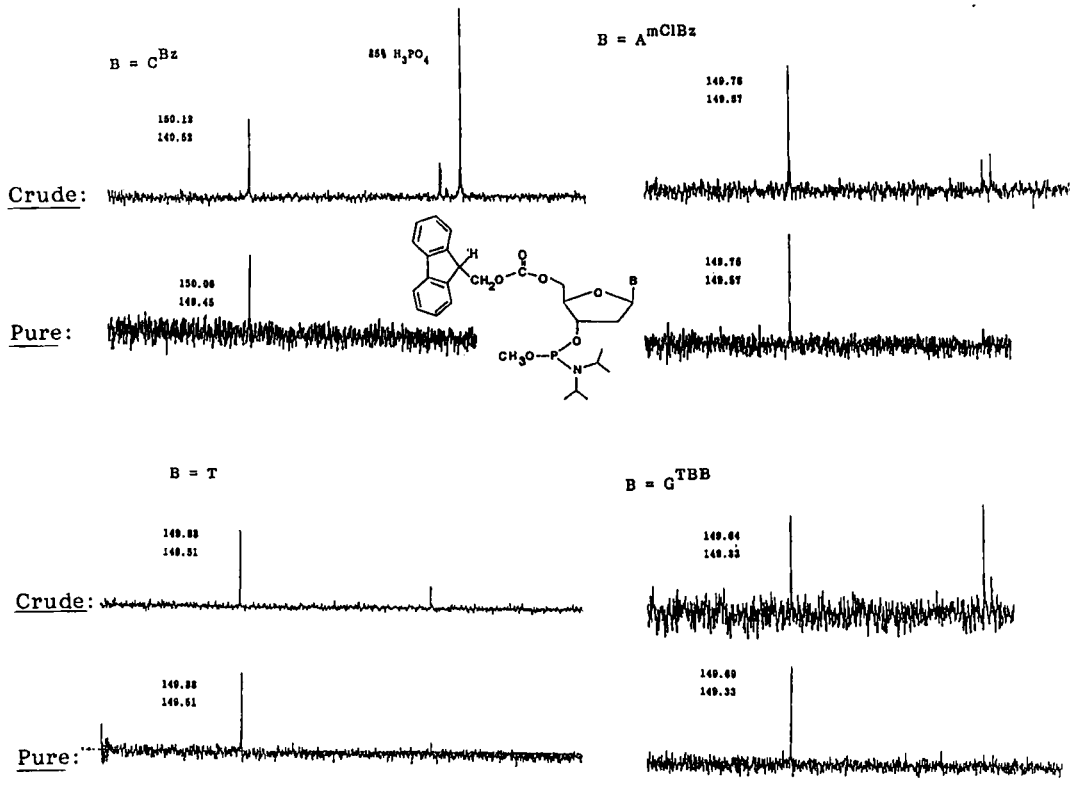
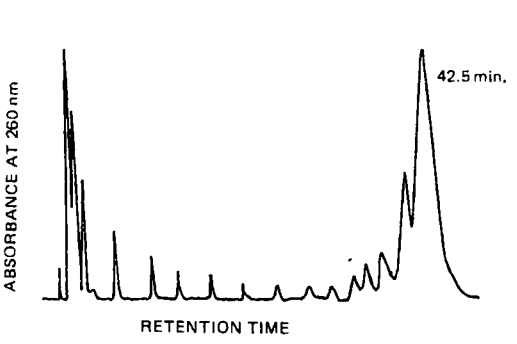
Summary: 5'-Fmoc protected 2'-deoxynucleoside building blocks have been employed in DNA synthesis in order to remedy the depurination problem.

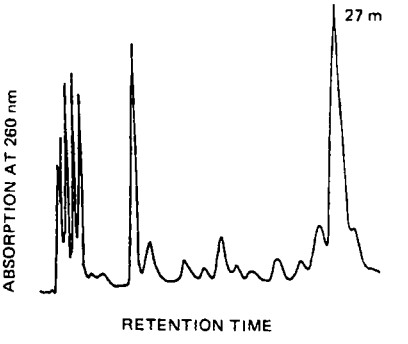
One disadvantage of the methods[1,2] used today in the synthesis of DNA is the depurination encountered during the removal of the 5'-DMTr group from N^6-protected deoxyadenosine blocks. Several attempts have been made to prevent this by either altering the acidic conditions employed[3] or by varying 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)

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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References

1. C.B. Reese, Tetrahedron (1978), 3143.
2. L.J. McBride and M.H. Caruthers, Tetrahedron Lett. (1982), 245.
3. T. Tanaka and R.L. Letsinger, Nucleic Acids Res. (1982), 3249.
4. L.J. McBride, R. Kierzelc, S.L. Beaucage and M.H. Caruthers, J. Am. Chem. Soc. (1986), 2040 and references therein.
5. M. Sekine and T. Hata, J. Org. Chem. (1983), 3011.
6. H. Seliger and U. Kotschi, Nucleosides and Nucleotides (1985), 153.
7. C. Gioeli and J. Chattopadhyaya, J.C.S. Chem. Comm. (1982), 672.
8. N. Balgobin and J. Chattopadhyaya, Acta Chem. Scand. B39 (1985), 883.
9. M. Kwiatkowski, A. Sandström, N. Balgobin and J. Chattopadhyaya, Acta Chem. Scand. B38 (1984), 721.