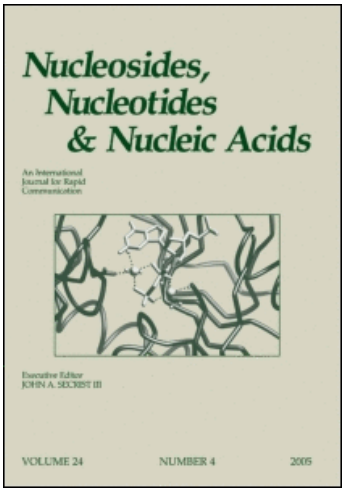

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Use of A Uridine Nucleotide as an Affinity Handle and 5' Phosphorylating Agent for Synthetic DNA

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USE OF A URIDINE NUCLEOTIDE AS AN AFFINITY HANDLE AND 5' PHOSPHORYLATING AGENT FOR SYNTHETIC DNA

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Abstract: The 5'-(*O*-cyanoethyl *N*,*N*-diisopropyl phosphoramidite) of 2',3'-*O*-bis(4,4'-dimethoxytrityl)uridine can be used to attach a uridine residue through a 5'-5' phosphodiester linkage to a synthetic oligodeoxyribonucleotide. This 5'-terminal structure allows the oligomer to be selectively retarded on a chromatographic support containing dihydroxyboryl substituents, and to be converted upon periodate oxidation and β-elimination to the form possessing a 5' phosphate group.

An appropriately derivatized uridine 5'-phosphoramidite has been devised for use in DNA synthesizers to generate oligonucleotides of the form rU5'-5'dN-dN_n-dN. Such oligomers have a free 2',3' *cis*-diol group at their 5' ends that can serve as an affinity handle in a chromatographic technique[1] employing column supports derivatized with dihydroxyborylphenyl groups. This application provides a simple strategy for isolating full-length synthetic oligomers, free from the failure sequences which invariably contaminate them. As an added benefit, the ribonucleoside carries a phosphate onto the terminus; the nucleoside can be readily removed by periodate oxidation/β-elimination to leave the molecule with a 5' phosphate group[2]. Moreover, in molecular biological manipulations, the uridine can serve as a temporary protecting group for this phosphate, preventing any unwanted participation of the 5' terminus in enzyme-mediated condensations.

The synthetic terminal unit was prepared by converting 2',3'-*O*-bis(4,4'-dimethoxytrityl)uridine[2] to its 5'-(*O*-cyanoethyl *N*,*N*-diisopropyl phosphoramidite) by the procedure of Sinha *et al.*[3], and was characterized by ^{31}P NMR. This monomer is designed to be used in a DNA synthesizer during the final addition cycle, and it was tested in the preparation of a number of oligonucleotides each bearing a uridine

residue attached by a terminal 5'-5' linkage. The crude products from these syntheses were taken through the normal deblocking procedures and then analyzed using an HPLC system consisting of a 25 X 0.5 cm Selecti-Spher-10 Boronate column (Pierce, Rockford, IL) with a solvent prepared by adjusting a solution of NH_4Cl (1 mol) to pH 8.5 with concentrated NH_4OH, adding 300 ml of acetonitrile, and bringing the final volume to 1 liter with water. Samples (1-5 AU_{260}) of the oligomers and three standards were separately applied to the column and chromatographed at 0.2 ml/min. Elution volumes (ml) were d(T-T_4-T), 3.5; r(U-U_4-U), 15.7; rU5'-5'd(T-T_4-T), 19.0; rU5'-5'd(TTTTTGCGAGTATGACGCCGAGTATGACG), 7.7; d(GCGAGTATGACGCCGAGTATGACG), 3.0; rU5'-5'd(GATCTCCTTGGCTGACGTCAGAGAGA), 9.0; rU5'-5'd(AAACAGAGAAGTCA), 11.4; rU5'-5'd(CTGACAGTCCTGTTT), 10.1; rU5'-5'd(GTAAAACGACGGCCAGT), 10.2; rU5'-5'd(GCGAGTATGACGCCGCGTATGACG), 5.8. The elution position of the thymidine hexamer is characteristic of molecules that do not interact with the chromatographic support; this contrasts with the position obtained for the same hexamer bearing a reverse-linked uridine where there is complex formation between its *cis*-diol group and the column's dihydroxyboryl functions. The latter behavior is matched by that of the uridine hexamer, where analogous complexing takes place at the 3' end of the molecule. Longer molecules containing the terminal uridine are also retarded, but to a lesser extent; however, all of these are well separated from oligomers having the normal 5'-terminal structure.

The phosphoramidite derived from N^6,N^6,$O^{2'}$,$O^{3'}$-tetrabenzoyl-adenosine has also been used in the synthesizer, to generate molecules equivalent to those listed above. Each of these has a stronger affinity for the column than its uridine-containing counterpart - for example, substitution of rA for rU in the case of rU5'-5'd(CTGACAGTCCTGTTT) increases the elution volume from 10.1 to 12.4 ml.

REFERENCES

1. H. L. Weith, J. L. Wiebers, and P. T. Gilham, *Biochemistry* **9**, 4396 (1970). M. Rosenberg, J. L. Wiebers, and P. T. Gilham, *Biochemistry* **11**, 3623 (1972).
2. J. G. Nadeau, C. K. Singleton, G. B. Kelly, H. L. Weith, and G. R. Gough, *Biochemistry* 23, 6153 (1984).
3. N. D. Sinha, J. Biernat, J. McManus, and H. Koster, *Nucleic Acids Res.* 12, 4539 (1984).