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p-Nitrobenzyloxymethyl: A New Fluoride-Removable Protecting Group for Ribonucleoside 2′-Hydroxyls

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Abstract: A novel protecting group for the 2′-hydroxyl of ribonucleosides, *p*-nitrobenzyloxymethyl, is used in the rapid solid phase synthesis of the oligonucleotide U-$(U)_{11}$-U; it can be readily removed from the oligomer product by treatment with tetrabutylammonium fluoride.

The most widely used system for automated solid-phase construction of RNA employs ribonucleoside phosphoramidite monomers bearing *t*-butyldimethylsilyl (TBDMS) groups for protection of their 2′-hydroxyls.[1-4] However, the synthesis of RNA is slow with these monomers; coupling times of up to 15 min. are required in each nucleotide addition cycle, compared with the two minute reactions commonly used for DNA. The relatively long coupling times are presumably a consequence of steric interference by the bulky, lipophilic TBDMS group with the activated phosphoramidite moiety.

In the course of studies aimed at developing less hindered systems,[5] we have discovered a new hydroxyl protecting group, *p*-nitrobenzyloxymethyl. This function is stable to acid and base, but is easily removed by treatment with fluoride ion.[6,7] Moreover, in its special application as a 2′-hydroxyl protecting group in oligoribonucleotide synthesis,[8] it leads to fast (*ca.* 2 min.) coupling times, allowing RNA to be synthesized as rapidly as DNA.

For attaching the *p*-nitrobenzyloxymethyl group to ribonucleosides, an alkylating agent was prepared from *p*-nitrobenzyl methylthiomethyl ether,[9] by first converting it into the corresponding chloromethyl ether.[10] This, in turn, was allowed to react with an excess of pyridine and, after removal of solvent, the resulting quaternary salt, 1-(4-nitrobenzyloxymethyl)pyridinium chloride,[11] was stored as a stock solution in anhydrous DMF.

2′,3′-*O*-Dibutylstannylene uridine[12] was treated with this reagent at 75° C in the presence of a catalyst, tetrabutylammonium bromide,[13] resulting in an approximately 1:1 mixture of 2′- and 3′-*O*-(*p*-nitrobenzyloxymethyl)uridine. After dimethoxytritylation, chromatographic separation of the isomers yielded pure 5′-*O*-dimethoxytrityl-2′-*O*-(*p*-nitrobenzyloxymethyl)uridine (37% overall).[14] This was converted into its 3′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite)[15] derivative ready for use in automated RNA synthesis.

The phosphoramidite was dissolved in anhydrous acetonitrile at a concentration of 0.15 M and used in a MilliGen 7500 Nucleic Acid Synthesizer to construct the oligoribonucleotide U-$(U)_{11}$-U. With standard DNA protocols (a coupling time of 2 min.), the stepwise yields for the synthesis averaged 99%.

The oligonucleotide was released from the support and deprotected, by treatment first with anhydrous NH_3/EtOH at 55° C for 24 h, then with sieve-dried 1.0 M tetrabutylammonium fluoride in THF for 24 h at 25° C. The product was purified by HPLC on a polyethyleneimine-silica column[16] to give U-$(U)_{11}$-U (28% isolated yield).

Thus, *p*-nitrobenzyloxymethyl appears to have considerable potential as a 2′-hydroxyl protecting group in automated RNA synthesis, and its application to the other ribonucleosides will be reported in the near future. Additionally, we are exploring use of the fluoride lability of nitrobenzyl functions in schemes for general hydroxyl protection.

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References and Notes

1. Usman, N.; Ogilvie, K. K.; Jiang, M. Y.; Cedergren, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854.
2. Ogilvie, K. K.; Usman, N.; Nicoghosian, K.; Cedergren, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5764-5768.
3. Scaringe, S. A.; Francklyn, C.; Usman, N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441.
4. Lyttle, M. H.; Wright, P. B.; Sinha, N. D.; Bain, J. D.; Chamberlain, A. R. *J. Org. Chem.* **1991**, *56*, 4608-4615.
5. Schwartz, M. E.; Breaker, R. R.; Asteriadis, G. T.; deBear, J. S.; Gough, G. R. *BioMed. Chem. Lett.* **1992**, *9*, 1019-1024.
6. We find that this remarkable lability to fluoride ion is shared by ortho- and para- (but not meta-) nitrobenzyl ethers and acetals. Similar cleavage of *p*-nitrobenzyl esters of carboxylic acids has been noted previously.[7]
7. Ueki, M.; Kai, K.; Amemiya, M.; Horino, H; Oyamada, H. *J. Chem. Soc. Chem. Commun.* **1988**, 414-415.
8. We have already successfully employed the *ortho* nitrobenzyloxymethyl 2′ protecting group for rapid RNA synthesis;[5] it is readily cleaved from completed oligomers by irradiation with long-wave UV light. While, as noted above, it is also labile to fluoride ion, its slower rate of removal relative to the para isomer makes fluoride ion deblocking of *o*-nitrobenzyloxymethyl-protected oligomers inefficient.
9. The methylthiomethyl ether was prepared in 40% yield using a modification of the general method of Pojer and Angyal.[17] Its structure was confirmed by IR, ^1H and ^{13}C NMR, and elemental analysis.
10. Benneche, T.; Strande, P.; Undheim, K. *Synthesis* **1983**, 762-763.
11. A related compound, 1-(3-nitrobenzyloxymethyl)pyridinium chloride, has been described as a derivatizing agent in the preparation of modified cellulose for use as an immunosorbent.[18]
12. Wagner, D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Org. Chem.* **1974**, *39*, 24-30.
13. David, S.; Thieffrey, A.; Veyrieres, A. *J. Chem. Soc. Perkin I* **1981**, 1796-1801.
14. More detailed descriptions of the chemical manipulations involved in protecting and purifying nucleosides of this type are given in reference 5.
15. Sinha, N. D.; Biernat, J.; McManus, J.; Koster, H. *Nucleic Acids Res.* **1984**, *12*, 4539-4557.
16. Lawson, T. G.; Regnier, F. E.; Weith, H. L. *Anal. Biochem.* **1983**, *133*, 85-93.
17. Pojer, P. M.; Angyal, S. J. *Aust. J. Chem.* **1978**, *31*, 1031-1040.
18. Teichmann, T. B. *J. Prakt. Chem.* **1974**, 821-831.

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