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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)<sub>11</sub>-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.<sup>1-4</sup> 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,<sup>5</sup> 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.<sup>6,7</sup> Moreover, in its special application as a 2'-hydroxyl protecting group in oligoribonucleotide synthesis,<sup>8</sup> 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,<sup>9</sup> by first converting it into the corresponding chloromethyl ether.<sup>10</sup> 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,<sup>11</sup> was stored as a stock solution in anhydrous DMF.

2',3'-*O*-Dibutylstannylene uridine<sup>12</sup> was treated with this reagent at 75° C in the presence of a catalyst, tetrabutylammonium bromide,<sup>13</sup> 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).<sup>14</sup> This was converted into its 3'-(2-cyanoethyl *N,N*-diisopropylphosphoramidite)<sup>15</sup> 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)<sub>11</sub>-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  $\text{NH}_3/\text{EtOH}$  at  $55^\circ\text{C}$  for 24 h, then with sieve-dried 1.0 M tetrabutylammonium fluoride in THF for 24 h at  $25^\circ\text{C}$ . The product was purified by HPLC on a polyethyleneimine-silica column<sup>16</sup> to give U-(U)<sub>11</sub>-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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