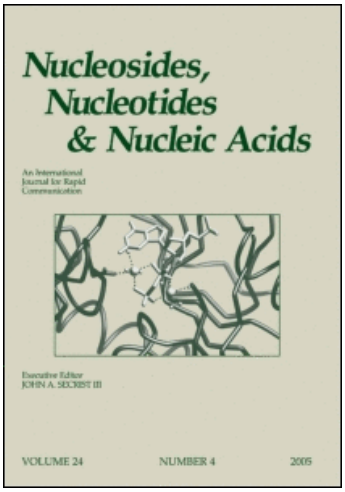

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Photochemistry of the *O*-Nitrobenzyl Protecting Group in RNA Synthesis

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PHOTOCHEMISTRY OF THE *o*-NITROBENZYL PROTECTING GROUP IN RNA SYNTHESIS

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Abstract: UV irradiation of 2'-*O*-(*o*-nitrobenzyl)adenylyl(3'-5')uridine in the presence of O_2 yields the corresponding nitrobenzoyl derivative in addition to the expected A-U. A mechanism proposed for this oxidation involves the successive removal of the two benzylic protons with a hydroperoxide as the intermediate between the two steps.

Effective use of *o*-nitrobenzyl for the protection of 2'-hydroxyls in RNA synthesis requires that the group be removed quantitatively by photolysis after chain construction. In an earlier study on RNA synthesis[1], we observed that substantial reductions in deblocking yields can sometimes occur, and that this effect is due to photo-oxidation at the benzylic carbon atom of the protecting group. We have now studied the problem in detail using the model compound 2'-*O*-(*o*-nitrobenzyl)-adenylyl(3'-5')uridine, A(NBzl)-U. Exposure of this material to long-wave UV light can give two products: the expected A-U and an A-U derivative that is resistant to further irradiation. The relative amounts of these depend on the presence of oxygen and on the pH of the solution, with higher pH values effecting the formation of increased amounts of the resistant derivative. The yields of A-U obtained under different conditions are 100% at pH 3.5; 81% (pH 5.5); 72% (pH 7.5); 63% (pH 8.5); 94% (pH 8.5 purged with N_2); 37% (pH 8.5 purged with O_2); and 81% (in 40% EtOH). The single side product formed in each case at pH values above 3.5 is assigned the structure 2'-*O*-(*o*-nitrobenzoyl)-adenylyl(3'-5')uridine. It has paper electrophoretic mobilities at neutral and acidic pH values similar to those of A-U and A(NBzl)-U, and is not hydrolyzed by spleen phosphodiesterase. Moreover, it gives a positive periodate test for the presence of a *cis*-diol grouping, and it is initially converted to A-U and *o*-nitrobenzoic acid upon exposure to concentrated ammonia.

The mechanism of the oxidation probably involves the removal of a proton from the benzylic methylene caused by a lowering of its pKa value in the activated state, and reaction with O_2 to generate a hydroperoxide at the methylene position; further absorption of UV light results in the dehydration of this intermediate to produce the nitrobenzoyl moiety. This is supported by a study on the photo-oxidation of ethyl *o*-nitrobenzyl ether. Brief irradiation of the ether in a dilute ammonia solution purged with O_2 gives two products that can be separated by TLC: ethyl *o*-nitrobenzoate and α-ethoxy-*o*-nitrobenzyl hydroperoxide. The latter is identified by its IR spectrum and by the fact that it produces *o*-nitrobenzaldehyde on treatment with aqueous KI. The hydroperoxide is stable in dilute ammonia solution but, upon further exposure to UV light, it is converted to ethyl *o*-nitrobenzoate.

The first part of this mechanism is analogous to that suggested by Wan[2] for the photo-oxidation of *meta* and *para* isomers of nitrobenzyl ethers. He found that irradiation of methyl *m*-nitrobenzyl ether yields α-methoxy-*m*-nitrobenzyl hydroperoxide while the *para* isomer is converted in alkaline solution to *p*-nitrobenzoic acid. On the assumption that the latter product is formed via the corresponding hydroperoxide, he proposed that the breakdown of this hydroperoxide proceeds by a dehydration-hydrolysis (non light-induced) or by photo-oxygenation. In contrast, our work on the *ortho* nitrobenzyl derivatives favors a light-induced dehydration mechanism for the hydroperoxide breakdown, involving the removal of the second benzylic proton from the activated state, followed by loss of hydroxyl to produce the ester.

When using *o*-nitrobenzyl protection in RNA synthesis, it is important to avoid these oxidative side reactions because the alkaline conditions necessary for removal of any nitrobenzoyl groups formed can cause some cleavage of internucleotide linkages. Accordingly, we recommend that the photolysis of nitrobenzyl-protected oligoribonucleotides should be carried out at pH 3.5 in solutions purged with N_2.

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