

Synthesis of a Novel Photolabile Group for Selective Protection of Exocyclic Amino Function of Deoxynucleosides

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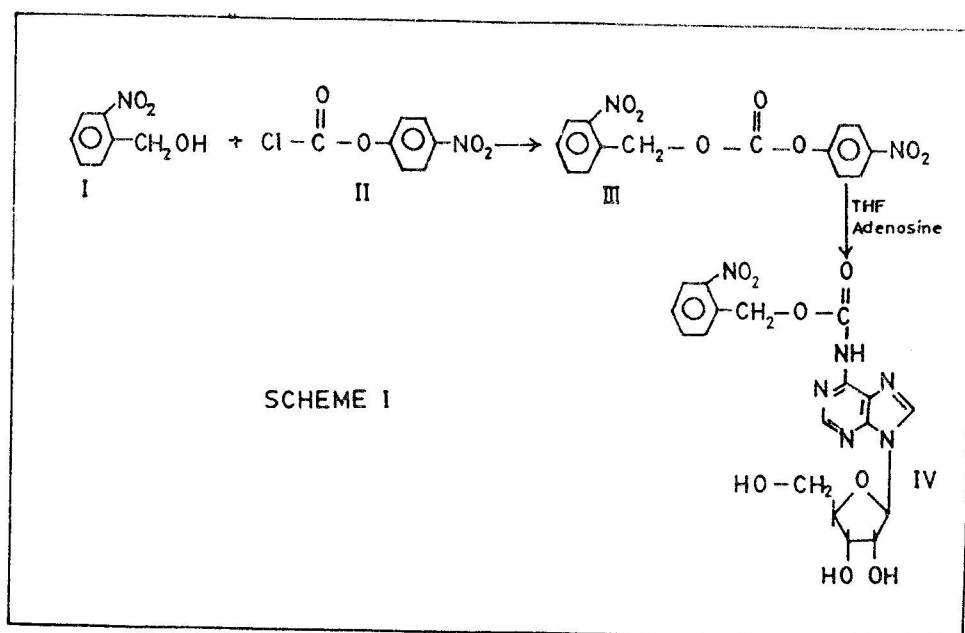
Abstract. 2-Nitrobenzyloxycarbonyl (NBOC) group has been used as a light sensitive urethane for blocking exocyclic amino function of the nucleoside viz. 2'-deoxyadenosine. The easy introduction, quick photolabile removal, depurination resistivity, higher yields and stability throughout the course of synthesis are the advantages of this group.

Introduction

The synthesis of the sequence defined oligonucleotides requires fully protected monomers in order to avoid side reactions during coupling. Therefore, protection of different nucleophilic functions in different bases is essential for chemical synthesis. Since, the amino protecting groups¹⁻⁶ are necessary throughout the course of synthesis, hence they are extremely important. The protection is usually accomplished directly by converting the particular group into a derivative which is known to be stable under the experimental conditions to be used and from which the original group is regenerated without affecting the synthesized molecule. The removal of these protecting groups involves, in general, either treatment with acid or base or chemical reduction or a combination of these methods.

In this communication, the use of 2-nitrobenzyloxycarbonyl (NBOC) group as a light sensitive urethane for blocking the exocyclic amino function of the nucleosides has been reported. This group was first proposed for protection of amino group in case of amino acids by Patchornik et al⁷ in 1970. However, so far no work regarding protection of the amino function by this group has been reported in case of nucleotides. This group is not only reactive but is also selective as well and due to its photolytically removable characteristics is expected to prevent depurination. Thus, the advantages envisaged in the use of NBOC protected monomers are that they are stable under synthetic conditions, depurination resistant, neatly and quickly removable and can be easily purified. A prerequisite for a photochemically removable protecting group is a chromophore which is sensitive to light, but relatively stable to most of the wide variety of chemical reagents. Therefore, it would be a distinct advantage to have a protecting group with a short lived excited state. Most commonly employed photolabile group is 2-nitrobenzyl group⁸ for which the internal photoredox reaction characteristic of o-nitro aromatic compounds containing a carbon-hydrogen bond in the ortho position is exploited. The o-nitrobenzyl function finds promising application in oligonucleotide synthesis as a light sensitive protecting group⁹.

In our earlier publications, we have reported a number of new groups for amino protection viz. 3-methoxy-4-phenoxybenzoyl², phenoxyacetal³, naphthaloyl⁴, α -phenyl cinnamoyl⁵ and 2-nitrophenyl sulphenyl⁶ as depurination resistant groups. Some other groups have also been reported to be less susceptible to the protic acid depurination¹⁰⁻¹¹. Moreover, the deprotection of NBOC group is very neat and quick as it takes much lesser time than other conventional amino protecting groups. The N-protected derivative of the 2'-deoxy nucleoside with exocyclic amino group protected with NBOC viz. 2'-dA^{NBOC} was prepared by the reaction of 2-nitrobenzyl-4-nitrophenyl carbonate with



2'-deoxyadenosine in molar proportion in THF in presence of 2N NaOH (Fig. 1).

The reagent used for the introduction of this group, i.e. 2-nitrobenzyl-4-nitrophenyl-carbonate was prepared by the reaction of 2-nitrobenzyl alcohol with 4-nitrophenyl chloroformate in acetone in presence of pyridine in 68% yield. The reagent after usual workup was purified on silica gel column and its purity was checked by melting point determination and spectral studies. Its homogeneity was confirmed by tlc.

The removal condition for NBOC group in case of the 2'- nucleoside was studied by irradiating the N-protected derivative by irradiation with a high pressure mercury lamp under nitrogen atmosphere for 1.5 hrs. After removal, the mixture was analyzed for deprotection on tlc and subsequent estimation by UV spectroscopy (λ_{\max} 262). The deprotection is quantitative.

2. Experimental Procedure

2'-dA was purchased from Fluka, Buchs, Switzerland. 2-Nitrobenzyl alcohol and 4-nitrophenyl chloroformate were purchased from Merck, Germany. Solvents were purified and dried prior to use. Pyridine was refluxed with ninhydrin for 5-6 hours and distilled over KOH. Acetone was purified by refluxing with pot. permanganate for 3-4 hours and then distilled. TLC was performed on silica gel plates and visualization of the spots was done by iodine spray or charring after acidifying the plate with 20% sulfuric acid. Spectral studies were done by Hitachi UV-spectrophotometer and Perkin Elmer 881.

Preparation and characterization of 2-Nitrobenzyl 4-nitrophenyl carbonate (III): 4-Nitrophenyl chloroformate (II) (1.400 gms; 0.007 mol) was added in small portions with stirring to an ice cold solution of 2-nitrobenzyl alcohol (I) (0.900 gm, 0.006 mol) in pyridine (1.45 ml) and acetone (3.35 ml). The product began to precipitate immediately after the addition of the last portion. After stirring for additional 15 min. the mixture was added to water (22.5ml). The product was collected by filtration and washed with water. Recrystallization from methanol affords the pure product (1.39g) in 68% yield. M.pt. 108°C, Rf: 0.78 (DCM:MeOH::8.5:1.5); λ_{\max} 267 nm. Anal. found: C, 52.20; N, 8.83; H, 3.06. $C_{14}N_2O_7H_{10}$ requires C, 52.83; N, 8.81; H, 3.14.

N-(4-Nitrobenzyloxycarbonyl) 2'-deoxyadenosine (IV): A mixture of 2'-Deoxy adenosine (0.500 gm, 0.002 mol), 2-nitrobenzyl 4-nitrophenyl carbonate (0.400 gm, 0.0033 mol), 2N NaOH solution (1 ml) and THF (2 ml) was stirred at room temp. for 21 hours. The precipitated solid was dissolved in 1.0 molar $NaHCO_3$ solution. pH of the combined filtrate was adjusted at 5.8 and the resulting mixture was extracted with ether to remove 4-nitrophenol and any unreacted 4-nitrobenzyl 4-nitrophenyl carbonate. Ether was added to the acidified aqueous phase and the pH was maintained at 2.0. The aqueous layer was repeatedly washed with ether. The combined ether extracts were washed with water followed by sat. NaCl solution and then dried with anhyd. sodium sulphate. Removal of the solvent and crystallization of the residue from methanol affords the protected compound (0.47g in 61% yield) λ_{\max} (methanol) 390 nm; IR (KBr) ν_{\max} (cm^{-1}); 3600 (sec -OH); 1550 ($ArNO_2$); 1340 (tert-N); 1700 (urethane). Conditions for removal of NBOC group: A solution of protected dA (0.40 g) in 1:1 dioxan/water (100 ml) was irradiated with a Hanovia 654 A-36 high-pressure mercury lamp housed in a water-cooled quartz immersion well and fitted with a vycor glass filter. Nitrogen gas was passed through the solution during the course of the reaction which was completed in 1.5 hr. The solution was evaporated to dryness and the residue was treated with acetone. The insoluble part was filtered to give free dA.

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