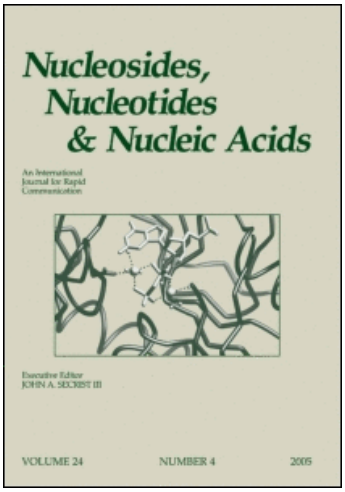

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Controlled Chemical Cleavage of Synthetic DNA at Specific Sites

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CONTROLLED CHEMICAL CLEAVAGE OF SYNTHETIC DNA AT SPECIFIC SITES.

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ABSTRACT: In this communication we report the synthesis of a protected abasic molecule, 1'-O-(2-nitrobenzyl)-2'-deoxyriboside, and a special N-4-(6-hydroxyhexyl)-ribocytidine derivative as light- and periodate-sensitive selectable cleavage moieties, respectively, and their use in the characterization of linear and branched single-stranded DNA molecules.

To assist in the structural analysis of chemically synthesized branched DNA oligomers (see Chang et al. this journal), we have incorporated special treatment-dependent cleavable moieties into the oligomers at predetermined positions. After purification these moieties can be selectively cleaved in order to segment the DNA molecule into a set of smaller oligomers for further analysis. We report here two compounds that can be employed for selectable cleavage of DNA: a deoxyribose derivative that can be employed to introduce an abasic site at any position and a ribocytidine analog that can be hydrolysed in mild base after periodate oxidation.

Abasic sites in DNA molecules are readily hydrolyzed under mild basic conditions to give the 3'- and 5'-phosphates derived from the 5'- and 3'-side of the abasic site, respectively (1a,1b). Introduction of abasic sites into a synthetic DNA oligomer has been reported. For our purposes none of the reported routes were attractive since it would have been either necessary to use an esoteric enzyme (2) or to use acidic conditions that could introduce additional abasic sites through depurination (3,4).

We have developed an alternative chemical route for abasic site introduction using a protected 2'-deoxyribose derivative that can be photolytically deprotected to generate an abasic site (5). The protected abasic moiety, 1'-O-(2-nitrobenzyl)-2'-deoxyriboside [dr(NBn)], is readily synthesized from deoxyribose and 2-nitrobenzyl alcohol. The corresponding 5'-DMT-3'-phosphoramidite can be incorporated into DNA at predetermined positions during standard chemical solid-phase synthesis. After purification of the product, photolysis generates an abasic site at the predetermined positions in the DNA oligomer, and these can subsequently be cleaved under mild basic conditions to yield the component oligomers.

To demonstrate the utility of the cleavage scheme the protected abasic nucleoside phosphoramidite was incorporated under standard conditions into an oligomer 5'-T_{10}-dr(NBn)-T_{20}-3' on a solid support. The fragment was deprotected with DCA (to remove 5'-DMT), thiophenol (to remove methyl) and ammonium hydroxide (to cleave the 3'-succinate linkage). Purified samples of the product (Fig 1., lane 1) were subjected to a series of chemical treatments and analyzed by PAGE. On exposure to ammonium hydroxide at 60° C for 18 hours no cleavage of the product was observed demonstrating the stability of the

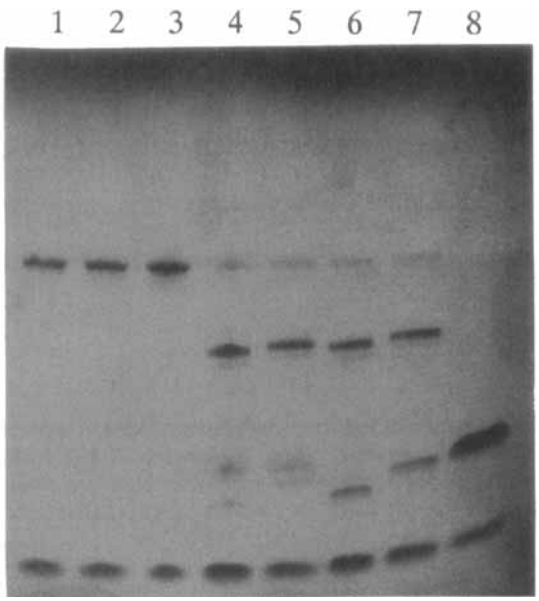
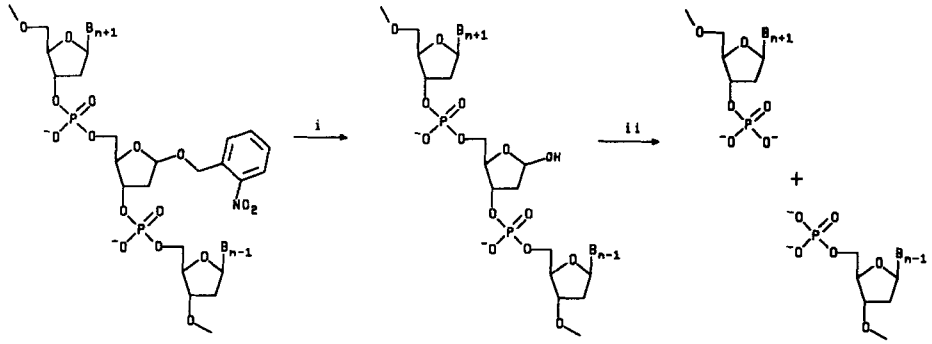


Fig.1. PAGE analysis of 5'-T_{10}-dr(NBn)-T_{20}-3' subjected to chemical treatments.

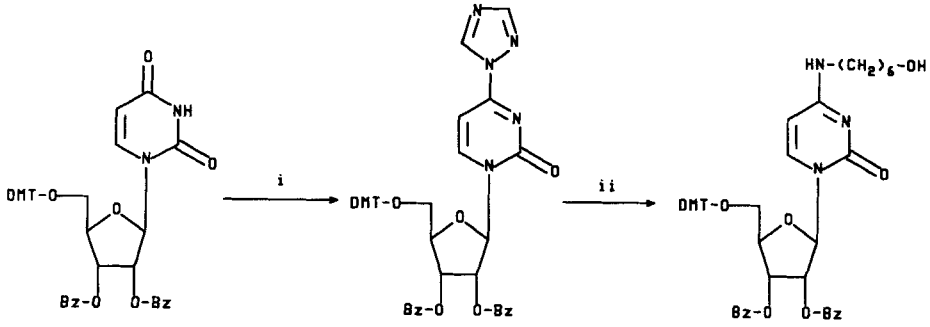
2-nitrobenzyl protected abasic moiety to the conditions used to remove the protecting groups on the exo-cyclic amines of the nucleobases (lane 2). A sample in water was subjected to photolysis for 20 minutes using a high intensity Hg lamp to remove the 2-nitrobenzyl group. No cleavage of the oligomer occured during the photolysis step (lane 3). Samples of the oligomer which had been subjected to photolysis were incubated in ammonium hydroxide for two hours at 20°C and at 60°C. Insignificant strand cleavage occured at 20°C, whereas treatment at 60°C for 1 hour resulted in complete cleavage of the oligomer into the two component oligomers 5'-p-T_{20} and T_{10}-3'-p-dr, where dr= deoxyribose residue (lane 4). Only 5'-p-T_{20} was dephosphorylated by alkaline phosphatase (lane 5). Treatment with 1M NaOH at 60°C for 1 hour also resulted in complete strand cleavage to give the phosphorylated component oligomers, 5'-p-T_{10} and T_{20}-3'-p (lane 6). Both cleavage products from lane 6 were dephosphorylated by alkaline phosphatase (lane 7). The oligomer T_{10} was used as a reference (lane 8). Scheme 1 outlines the cleavage of 1'-O-(2-nitrobenzyl)-2'deoxyriboside linked DNA fragments when using NaOH.

Oxidative cleavage of the cis-diol system with sodium periodate readily occurs in the terminal ribonucleoside of RNA molecules. In the presence of amines the resulting di-aldehyde eliminates both the base moiety and the phosphate at the 5'-carbon (6). We have used this concept in the design of a selectable cleavage molecule where two DNA oligomers are linked via the 5'- and the side-chain hydroxyl groups of a N-4-(6-hydroxyhexyl)-cytidine molecule (abbreviated R).

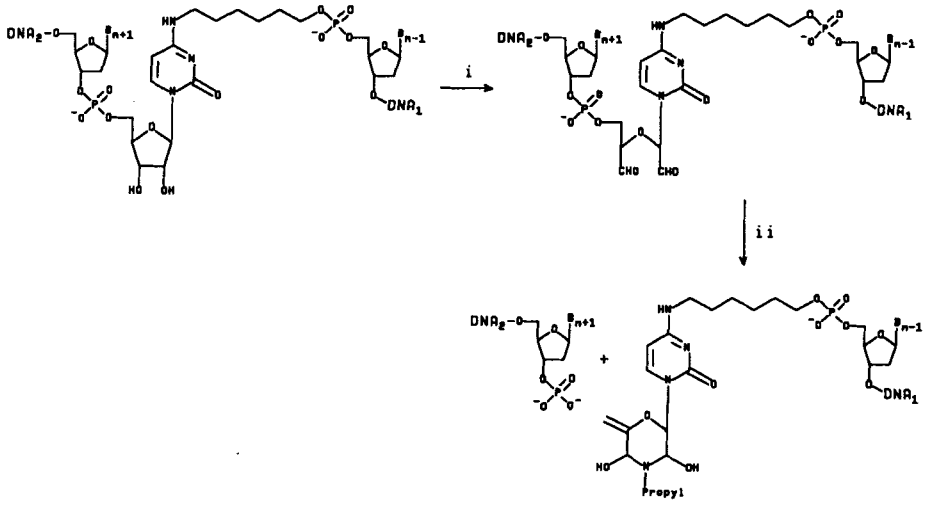
The modified ribonucleoside R containing an exo-cyclic alkyl hydroxyl group was synthesized from uridine via published procedures (7) as outlined in scheme 2. The *protected* R ribonucleoside phosphoramidite was incorporated under standard conditions



Scheme 1. Abasic site formation and cleavage.
i. light >350 nm; ii. 1M NaOH @ 60°C for 1 hour



Scheme 2. Synthesis of modified ribonucleoside R
i. $POCl_3$/triazole/triethylamine; ii. 6-Aminohexanol



Scheme 3. Periodate/n-propylamine cleavage of R ribonucleoside linked DNA fragments.
i. Sodium periodate @ 4 C for 30 min.; ii. n-Propylamine / TEAA @ 60 C for 90 min.

into an oligomer 5'-T_{10}-R-T_{15}-3' on a solid support. Purified samples of the product were subjected to a series of chemical treatments and the samples analyzed by PAGE. No cleavage of the oligomer was observed after treatment with ammonium hydroxide at 60°C for 18 hours. Treatment with sodium periodate in water at 4°C for 30 minutes resulted in partial cleavage. Further exposure of periodate treated oligomer to n-propylamine in triethylammonium acetate at 60°C for 90 minutes resulted in complete cleavage of the oligomer into T_{10}-3'-p and a T_{15} species modified at the 5' end. Scheme 3 outlines the cleavage of R ribonucleoside linked DNA fragments.

The cleavage scheme has been applied to several branched DNA (b-DNA) oligomers, where the protected R ribonucleoside phosphoramidite was incorporated during the first cycle of the secondary synthesis of solid-supported linear oligomers containing 10, 20, and 30 comb branching monomers (BM2), respectively (7). In each case the secondary synthesis was a T_{10} oligomer resulting in branched oligomers of the following structure:

3'-T_{20}-$(BM2)_n$-5' , where n= 10, 20, 30
|
(R-T_{10}-5')$_n$

These molecules were subjected to the cleavage conditions. PAGE analysis indicated that all the side arm oligomers were cleaved, and T_{10}-3'-p was the main product in all cases.

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