A self-cleaving DNA nucleoside

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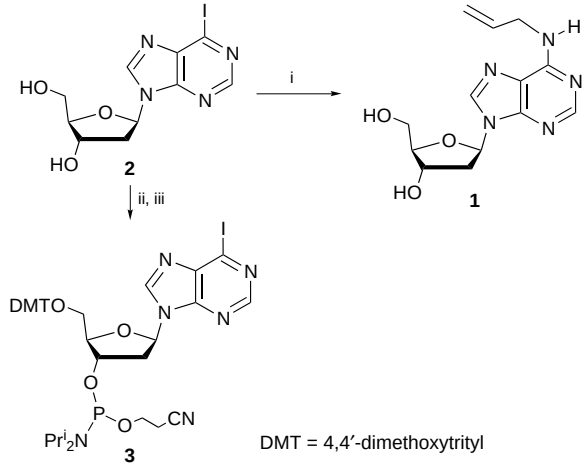
An allyl-modified nucleoside has been shown to self-alkylate and depurinate on treatment with iodine and heat; placement in DNA strands yields site-directed cleavage of the DNA.

Much attention has been focused on the development of methods for cleavage of DNA or RNA strands at specific sites. One common approach taken is the use of a reactive group which is delivered to specific sequences in a nucleic acid target by attachment of the reactive group to a DNA-binding ligand with known sequence preference.[1] One goal of such work has been the hybridization-directed cleavage of biologically harmful nucleic acid sequences, resulting in possible therapeutic applications.[2] Another application is the cutting of chemically- or enzymatically-synthesized DNA at positions which cannot be processed by known restriction endonuclease enzymes,[3] which can also be useful as a tool in the study of nucleic acid structure and function.

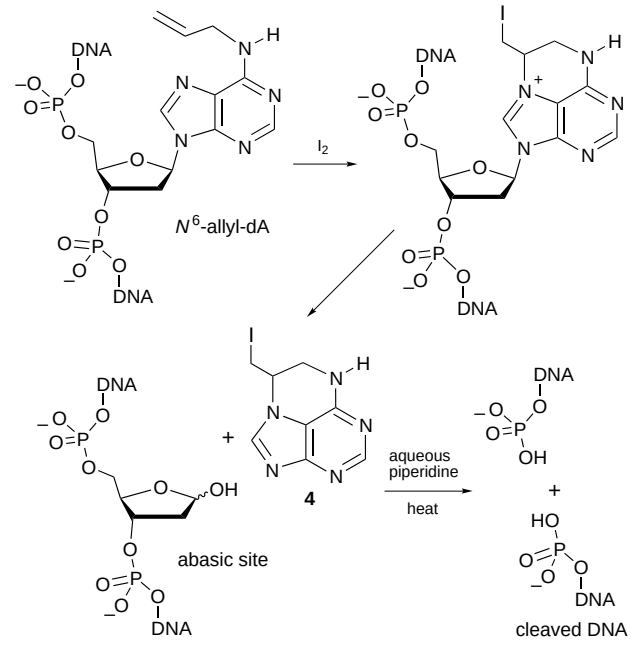
Our design of a self-cleaving DNA base was inspired by Maxam–Gilbert alkylation of purines. Alkylation at N^7 (or other ring nitrogens) by dimethyl sulfate results in depurination with formation of an abasic deoxyribose, and subsequent heating in aqueous base gives strand cleavage with loss of the sugar residue. In principle, direction of an alkylating group to a specific purine under mild conditions could enable facile and specific chain cleavage. We envisioned that placement of an allyl function near nucleophilic ring nitrogens might allow for such alkylation if a mild electrophilic activating reagent could be found. The reagent must be reactive enough to activate the alkylation functionality, but not so reactive that it modifies natural DNA.

We synthesized the nucleoside N^6-allyl-dA **1** to test this possibility (Scheme 1). This compound was obtained from iodopurinedeoxyriboside **2** by substitution with allylamine. After some experimentation we arrived at iodine as a mild electrophilic reagent for activating the double bond.[4] We found that I_2 reacts rapidly with N^6-allyl-dA in neutral aqueous solution or in DMSO at room temperature. This reaction was monitored by 300 MHz proton NMR in [$^2H_6$]DMSO. In addition, nuclear Overhauser enhancements were observed between H-8 and protons on methyl substituents added to the terminus of the allyl group (data not shown), establishing that the cyclization occurs on the N^7 nitrogen rather than the N^1 position. The ^{13}C NMR spectrum confirms the structure having the 6-membered-ring product. After 24 h at room temperature, or after 30 min heating (90 °C), a second new adenine-derived compound is observed in the NMR spectrum. We surmised that this is the cyclized base **4** which had been eliminated from the sugar; to test this we independently synthesized this compound by treating allyladenine with iodine. NMR and HPLC studies confirmed its identity. The second byproduct of the reaction in DMSO is hydroxymethylfuran, also identified by comparison to an authentic sample. The four natural DNA nucleosides did not react under these conditions as monitored by NMR spectroscopy; this is not unexpected, since iodine is a commonly-used reagent in the oxidative step of DNA synthesis.

We then incorporated N^6-allyl-dA into synthetic DNA strands to test whether this chemistry could be carried out in the polymer. Since iodine is used in the standard synthesis cycle, we added the allyl functionality immediately after synthesis of the DNA chain, by first incorporating the iodopurine phosphoramidite **3**.[5] We treated the resulting DNA attached to the solid CPG-support with neat allylamine (65 °C, 18 h) and followed this with a standard 12 h ammonia treatment to remove any remaining protecting groups. Two different sequences, containing one and two allyl-modified dAs respectively, were synthesized. Enzymatic digestion of these strands to the nucleosides followed by HPLC analysis showed that the N^6-allyl-dA was present in these strands in the expected ratio, with no evidence



Scheme 1 *Reagents and conditions*: i, allylamine (neat), room temp.; ii, 4,4′-dimethoxytrityl chloride, Pr^i_2EtN, CH_2Cl_2; iii, *N*,*N*-diisopropyl-*O*-cyanoethylphosphoramidic chloride, Pr^i_2EtN, CH_2Cl_2



Scheme 2

of any other derivatives such as deoxyadenosine or deoxyinosine (data not shown). This establishes that the postsynthetic addition of the amine proceeds with high (≥95%) conversion. The iodopurine **3** is thus an efficient vehicle for incorporation of amine nucleophiles into DNA.[6]

The expected products of self-cleavage of the sequence 5′-dTTTTTTA*TTTTT are 5′-dTTTTTTp and 5′-pdTTTTT (Scheme 2). We synthesized these fragments as authentic samples for comparison on high-resolution gels. The second modified strand had the sequence 5′-dTTTTTTTA*TTTTTTA*TTTTT, containing two possible cleavage sites. The result of iodine treatment with ABI oxidizing solution followed by heating with piperidine was efficient and clean chain cleavage for both sequences (Scheme 2). Experimentation with solvents and reaction conditions established that most selective cleavage occurred using 20 mM I_2 and 66 μM DNA strand concentration.† High-resolution polyacrylamide gels show the cleavage (Fig. 1), and separate gels show that the products co-migrate with the authentically synthesized fragments. Reversed-phase HPLC analysis also confirmed this finding. Control reactions show (Fig. 1) that the presence of the allyl function in the DNA is necessary for cleavage, as is iodine treatment of the DNA. Examination of the reaction products indicates that cleavage is not completely selective for the allyl-substituted base, with some background cleavage also occurring at other bases. Experiments with a third oligonucleotide of mixed sequence (5′-pdGTCAGTCAA*GTCAGTCAp) (data not shown) showed that this was more pronounced for purine-containing DNA. This nonselective iodine reaction was somewhat surprising because of the common use of iodine in DNA synthesis; however, it seems possible that altering conditions beyond those we examined, or using a more selective electrophile, might lead to increased specificity for allyladenine.

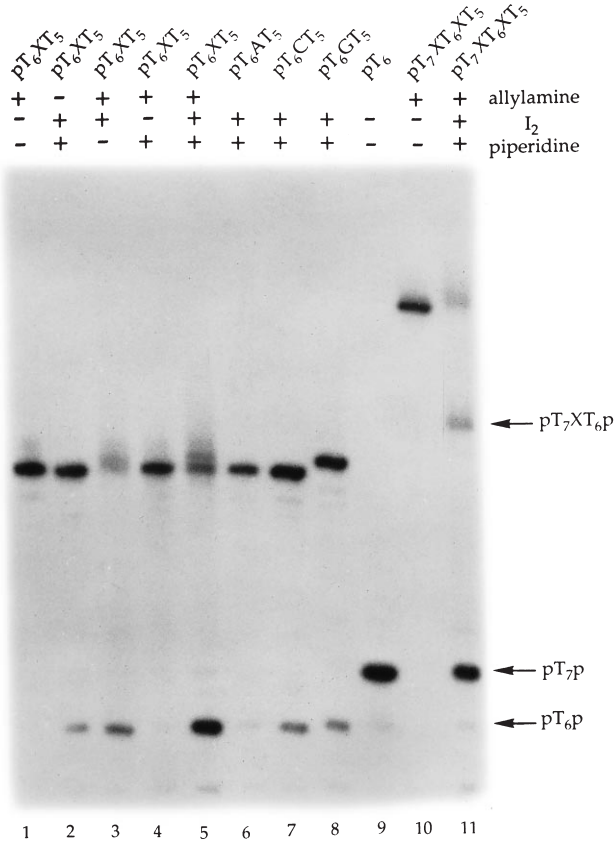


Fig. 1 Cleavage assay of two DNA sequences containing **1** by denaturing gel electrophoresis. All the sequences were 5′-^{32}P labelled. Lanes 1–5 show the reactions with the sequence 5′-$T_6A^*T_5$, lanes 6–8 the reactions with control DNA molecules, lane 9 the product marker and lanes 10–11 the reactions with DNA sequence 5′-$T_7A^*T_6A^*T_5$. Lane 1: no reaction. Lane 2: reaction of allyl untreated 5′-$T_6A^*T_5$ with both I_2 and piperidine. Lane 3: reaction of 5′-$T_6A^*T_5$ only with I_2. Lane 4: reaction of 5′-$T_6A^*T_5$ only with piperidine. Lane 5: reaction of 5′-$T_6A^*T_5$ with I_2 as well as piperidine. Lanes 6–8: reaction of 5′-T_6AT_5, 5′-T_6CT_5 and 5′-T_6GT_5 respectively with both I_2 and piperidine. Lane 10: no reaction. Lane 11: reaction of 5′-$T_7A^*T_6A^*T_5$ with both I_2 and piperidine. Lanes 3, 5 and 11 clearly show the cleavage of modified DNA to the expected products. Some non-specific cleavage can be seen in lanes 2, 6, 7 and 8.

A few other reports of methods for generating abasic (depurinated or depyrimidinated) sites or for site-directed cleavage in DNA exist in the literature.[7] The present method represents an alternative cleavage strategy which, unlike most of the previous approaches, allows essentially normal base pairing prior to the cleavage. Studies aimed at finding strategies for decreasing associated nonspecific cleavage are currently underway.

Footnotes

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† Conditions for reactions on DNA: (1) Allylamine treatment—1 ml of allylamine (neat) was added to CPG bound DNA (1 μmol synthesis) and the mixture was kept at 65 °C for 18 h. It was lyophilised and treated with NH_4OH at 55 °C for 12 h, lyophilised and gel purified. (2) Iodine treatment—5′-^{32}P labelled DNA sequence (200 pmol, 66 μM) was dissolved in 3 μl of 20 mM I_2 solution (20% v/v pyridine, 10% v/v water, 70% v/v THF), and incubated at 4 °C for 10 min. The mixture was then diluted with 150 μl H_2O and heated at 90 °C for 6 h. (3) Piperidine treatment—100 μl of a 1.0 M aq. piperidine solution in H_2O was added and the mixture heated at 90 °C for 30 min, and lyophilised.

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