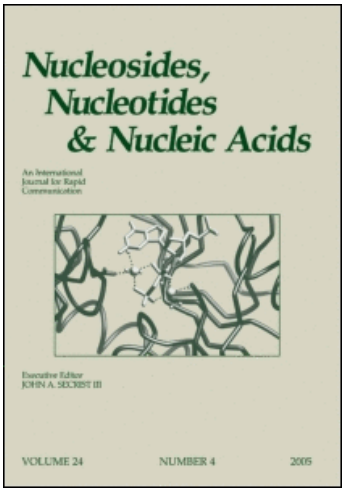

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Derivatization of Oligonucleotides Through Abasic Site Formation

J.-J. Vasseur[a]; D. Peoc'h[a]; B. Rayner[a]; J.-L. Imbach[a]
[a] Laboratoire de Chimie Bio-Organique, U.R.A. 488 du CNRS, affilié à l'INSERM, Université de Montpellier II, Sciences et Techniques du Languedoc, Montpellier Cédex 5, France

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DERIVATIZATION OF OLIGONUCLEOTIDES THROUGH ABASIC SITE FORMATION

J.-J. Vasseur, D. Peoc'h, B. Rayner and J.-L. Imbach*

Laboratoire de Chimie Bio-Organique, U.R.A. 488 du CNRS, affilié à l'INSERM, Université de Montpellier II, Sciences et Techniques du Languedoc, case courrier 008, Place Eugène-Bataillon, 34095 Montpellier Cédex 5, France.

ABSTRACT: Various approaches to create an abasic site in an oligonucleotide are described. A general method for oligonucleotide derivatization based on transient abasic site formation is then proposed.

Abasic (AB) sites in DNA are created by loss of purine or pyrimidine residue. Such DNA lesions, which can arise spontaneously under physiological conditions, after base modifications with electrophilic species, or through the action of N-glycosylases during the repair process, are considered to be mutagenic because of the lack of information during the DNA replication [1]. As a result AB sites have been the subject of intensive research during the last decade [2]. Structurally, an AB site in DNA exists as a tautomeric equilibrium between the cyclic α and β-anomeric hemiacetals and the open-chain aldehydic structure of the 2-deoxyribose residue (*Figure 1*).

As a consequence of the formation of such species, treatment of AB-DNA with various amino reagents (ranging

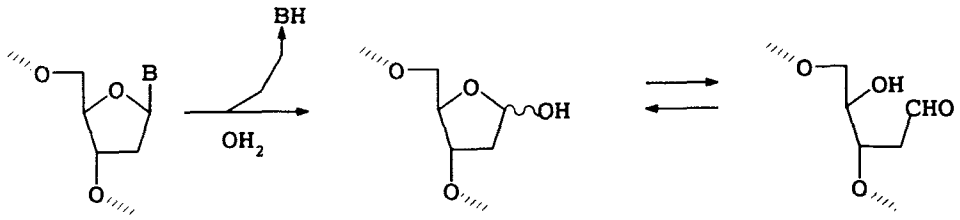


Fig. 1 - Abasic Site Structure.

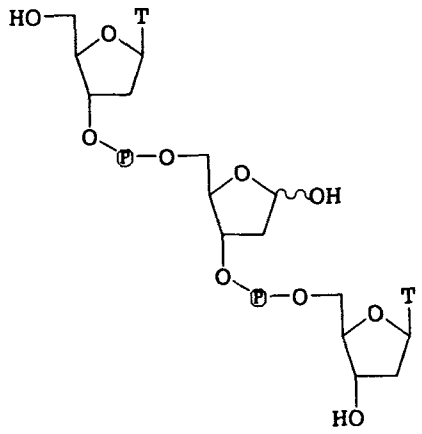
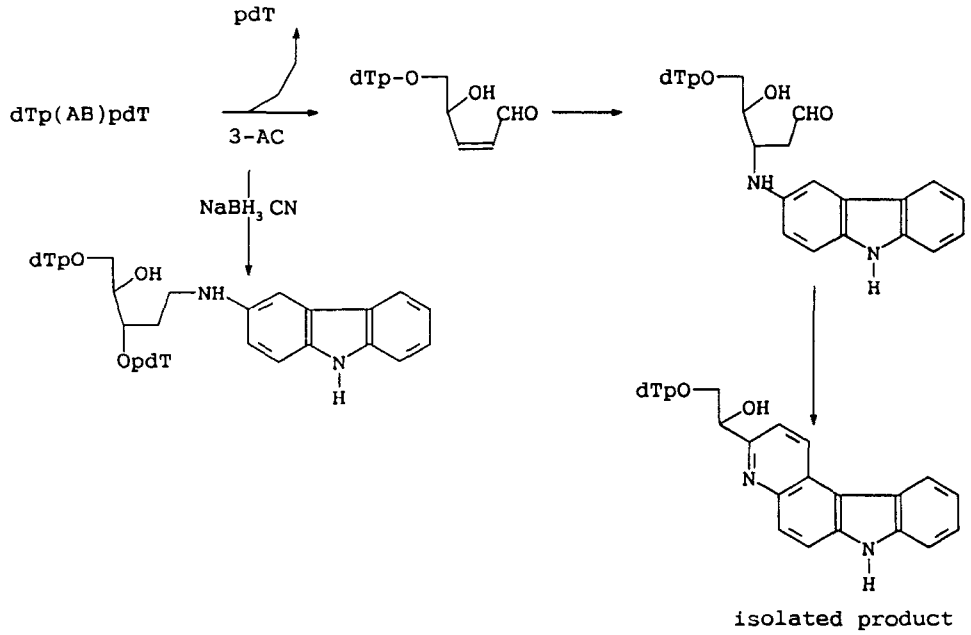


Fig. 2 - First AB-DNA model.

from phenylhydrazine [3] to the tripeptide Lys-Trp-Lys [4,5]) gives rise to DNA cleavage. Among those amino reagents, one of the most effective is the 9-aminoellipticine (9-AE) which gives a complete breakage of the AB-DNA chain at 1 μM [6]. However the exact breakage mecanism was not firmly established but it was postulated that the cleavage arises through a β elimination process induced by Schiff base formation [3].

Owing to its instability, the chemical synthesis of a DNA fragment containing an AB site was not reported since our leading report in 1986 [7]. However some mimicking



- *Fig. 3* - Pathway of the reaction between the AB site model and 3-AC.

models have been previously proposed but they do not reflect the reactivity of an AB site [8].

Based on the fact that a purine is more readily removed from DNA than a pyrimidine on acid treatment , we synthesize first a very simple AB-DNA model namely, dTp(AB)pdT (*Figure 2*). The exact mecanism of breakage of this AB oligonucleotide with various amino reagents was then determined [9,10,11]. Using 3-amino carbazole (3-AC) as a model, and monitoring the course of the reaction by HPLC, two detected intermediates were identified and the final isolated product fully caracterized [9] (*Figure 3*).
Therefore the first step of this reaction leads to the DNA chain breakage but the expected Schiff base was not detected. However as upon reaction with other amines, the same AB-DNA model shows formation of the corresponding Schiff

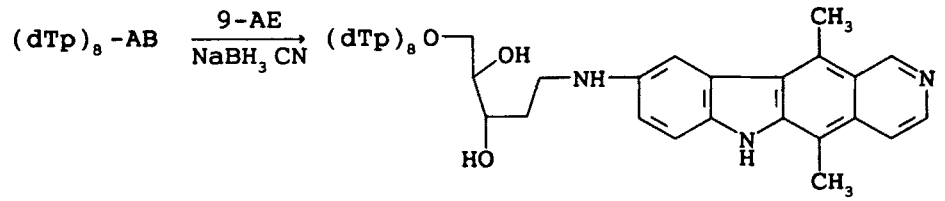


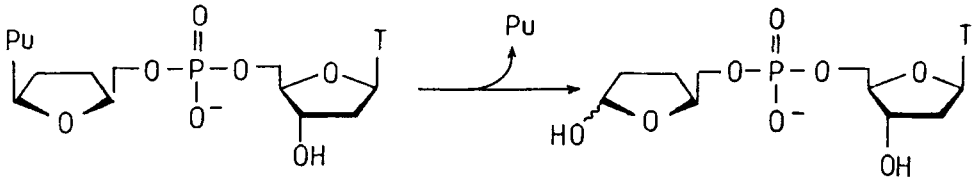
Fig. 4 - 3'End functionalization of an oligodeoxynucleotide with 9-AE.

base [7,10], we suspected its formation, followed by a fast β elimination process giving rise then to the detected α,β unsaturated aldehyde. Further experiments having shown that the breakage step was a second order reaction [12], we performed the same experiment in presence of $NaBH_3CN$ at pH 5 and isolated the corresponding reduced Schiff base (*Figure 3*).

Under the same experimental conditions, the dTp(AB)pdT model and 9-AE gave rise to the expected derivative which was isolated and fully characterized [11]. Extension of this approach to longer AB-oligodeoxynucleotides was then performed as shown on *figure 4*.

This last finding opened the way to an original method for the functionalization in a selected position of an oligonucleotide with various amino reagents. In addition the intercalating group is covalently linked to the oligonucleotide through a C_5 linker (coming from the ribose ring) and it has be shown that it is the optimum length for interaction with complementary "ss" or "ds" nucleic acids [13,14].

However this approach was limited to all-pyrimidines sequences, as we formed AB site through a selective dA acid hydrolysis. Some other pyrimidines oligodeoxynucleotides functionalized with various amines (*i.e.* spermidine, proflavine ...) were thus obtained [15].



	T 1/2 (min)	k ($10^{-2}min^{-1}$)
Purine	**2.9**	**23.7**
Adenine	**9.5**	**7.3**
dTpdApdT	**850**	**0.082**

Elementary calculation predict a quite quantitative depurination for ddNeb and less than 1% of dA hydrolysis in 13-15 min.

Fig. 5 - Apparent first order rate constants for the acid hydrolysis (30 mM HCl at 37°C).

We were then looking to extend this AB derivatization approach to any oligonucleotide bearing all the four usual bases. For that purpose we thought to incorporate a more acid-labile nucleoside than dA in the oligomer chain and removed it selectively through controlled acid hydrolysis. Following litterature reports on the rate of acidic catalyzed hydrolysis [16,17,18] we decided to evaluate the use of 2',3'-dideoxynucleosides and among them the one corresponding to nebularine (ddNeb), as it was reported to be more sensible to acid treatment than the corresponding adenine nucleoside [19]. Starting from the 5'phosphoroamidite derivative corresponding to ddNeb we synthesized first two model compounds namely ddNebpdT and ddApdA and performed some hydrolysis studies (HPLC monitoring) using dTpdApdT as a reference.

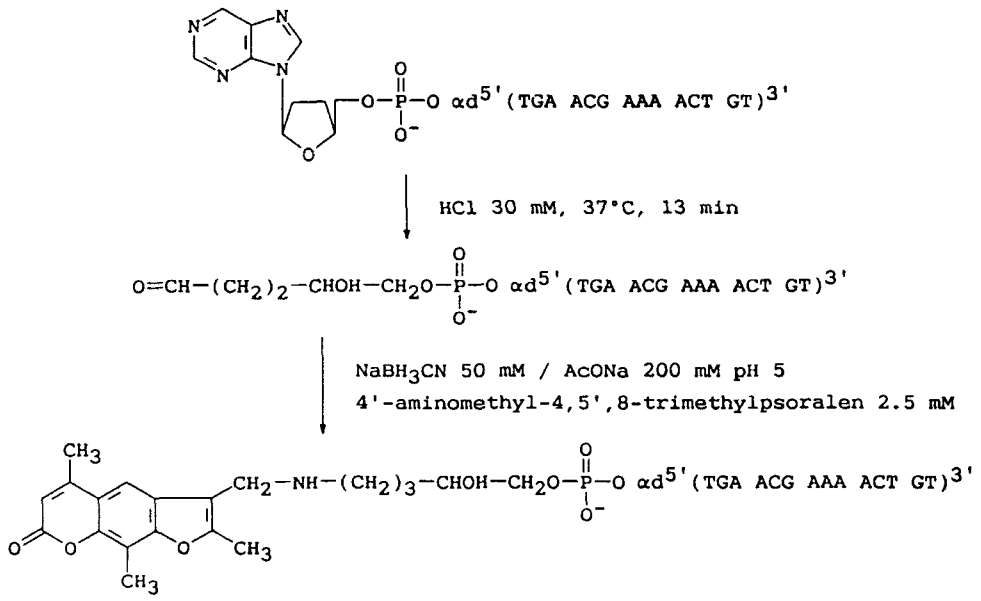


Fig. 6 - 5' End functionalization of an α-DNA with psoralen.

As shown on *figure 5* ddNeb is, as expected, much more sensible to hydrolysis than dA and on the basis of elementary calculations it could be used for selective formation of an AB site but only at the 5' end of an oligonucleotide.

Using such approach some oligonucleotides of natural or unnatural configuration (*i.e.* α-DNA, α-RNA ...) but bearing various effectors have been easily obtained.
As an example to demonstrate the validity of this method we will rapidly comment on the synthesis of a psoralen derivatized α-DNA. The 5'nebularine substituted α-oligodeoxynucleotide (*Figure 6*) was obtained using already described solid phase method [20,21] with incorporation of the nebularine phosphoroamidite at the last step. After usual deprotection work-up, this oligomer was hydrolyzed.
Formation of the corresponding AB-α-DNA was monitored by HPLC and after completion of the reaction, the 4'-aminome-

thyl-psoralen [22] derivative was added under reducing conditions as previously established.
As shown on *figure 6* the expected psoralen derivatized α-deoxyoligonucleotide is readily obtained and was isolated after purification. The structure of this product was ascertained by enzymatic digestion with Snake venon phosphodiesterase and alkaline phosphatase followed by HPLC analysis which shows the expected nucleoside ratio and the aminopsoralen bearing moeity (λ_{max}: 249 and 397 nm).

As can be seen, this method for functionalization of oligonucleotide is very easy to perform as, from the crude oligomer obtained from the DNA synthesizer and after the usual deprotection work up, a one pot reaction monitored by HPLC can give rise to various 5' substituted oligomers with excellent yields. This derivatization approach, through AB site formation, is therefore of a great interest but the use of ddNeb synthon allows only a 5' end functionalization of the oligonucleotide.

Therefore to further improve this functionalization strategy, we were looking for a more general method for creating an AB site at any given position of an oligomer. In addition, as an acid treatment is used for AB site formation, which could in some cases gives rise to unwanted side reactions, we were then interested on the design of a neutral method to generate specifically one (or more) abasic site in an oligonucleotide.
Obviously such strategy implies the use of a specific synthon as shown on *figure 7*, where **R** being a transient protecting group which should remain intact during the synthetic process and during the aqueous ammonia treatment (cleavage from the support and base deprotection).
In addition, **R** should be removed under conditions which leave intact the reactive abasic oligonucleotide. In fact we first thought to look at some classical protecting group already used for the 2'OH protection during the RNA synthe-

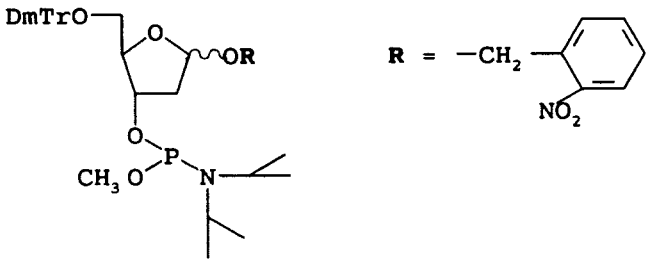


Fig. 7 - Abasic synthon.

sis (*i.e.* TBDMS, THP...) but most of them are acido labile [23]. Therefore we selected the photolabile ortho-nitrobenzyl group already used by Ikehara for 2'-OH protection in RNA synthesis [24].

After obtention of the corresponding phosphoroamidite synthon (*Figure* 7) using the usual methodology, we first synthesized on a machine the very simple model d(TpXpT), X being 1-o-nitrobenzyl-deoxyribofuranoside, in order to evaluate its ability to be specifically removed upon irradiation under non destructive conditions. It is noteworthy that this trimeric compound should then give the same dTp(AB)pdT model as previously obtained through specific acid hydrolysis of dA (see before). After 10 min of irradiation (Hg lamp/pyrex filter) of a solution of this compound in aqueous acetic acid (pH 4) we observed a quantitative formation of the expected AB trimer without any side reaction (HPLC monitored).

In order to further evaluate this approach we turned on longer sequences such as CTT(AB)TCC (previously synthesized through dA acid hydrolysis) and then a 30 mer containing the four nucleobases namely AACGTGAGTGCCGTG(AB)GTGCCGTGAGTGCA. In both cases we observed a complete removal of the 1'protecting group as shown by HPLC analysis. Basic hydrolysis of final product followed by alkaline phosphatase treatment gave rise to the two residual oligonucleotides of

expected length as shown by gel electrophoresis comparaison with authentical samples of the same length.

In conclusion one can say that we have for the first time synthetized a true abasic site in an oligonucleotide chain and elucidate the exact cleavage mechanism of AB-DNA through amino reagent action.
During the course of this study we have shown that abasic oligonucleotides could be used to reach functionalized probes, through an amino reductive reaction. This approach is very simple to handle as it gives directly with a one pot reaction from the oligonucleotide, the final derivatized probe. Furthermore the effector is always link to the oligo with a C_5 linker coming from the ribose ring.

As the important step of this approach is the AB-oligonucleotide synthesis, we designed a very convenient approach to reach on a machine various kind of oligonucleotides with an AB site at any selected position.

We are confident that this method of oligonucleotide functionalization will be of great interest as it is very simple to use and as it allows the introduction of numerous kind of effectors on various series of oligonucleotides.

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REFERENCES

[1] L.A. Loeb, *Cell* (1985) **40**, 483-484.

[2] L.A. Loeb, B.D. Preston, *Annu. Rev. Genet.* (1986) **20**, 201.

[3] D.C. Livingston, *Biochim. Biophys. Acta* (1964) **87**, 538-540.

[4] J. Pierre and J. Laval, *J. Biol. Chem.* (1981) **256**, 10217-10220.

[5] T. Behmoaras, J.J. Toulmé and C. Hélène, *Nature* (1981) **292**, 858-859.

[6] C. Malvy, P. Prevost, C. Gansser, C. Viel and C. Paoletti, *Chem. Biol. Interact.* (1986) **57**, 41-53.

[7] J.J. Vasseur, B. Rayner and J.L. Imbach, *Biochem. Biophys. Res. Commun.* (1986) **134**, 1204-1208.

[8] J.R. Bertrand, J.J. Vasseur, B. Rayner, J.L. Imbach, J. Paoletti, C. Paoletti and C. Malvy, *Nucleic Acids Res.* (1989) **17**, 10307-10319.

[9] J.J. Vasseur, B. Rayner, J.L. Imbach, S. Verma, J.A. Mc Closkey, M. Lee, D.K. Chang and J.W. Lown, *J. Org. Chem.* (1987) **52**, 4994-4998.

[10] J.J. Vasseur, B. Rayner and J.L. Imbach, *J. Heterocyclic. Chem.* (1988) **25**, 389-392.

[11] J.R. Bertrand, J.J. Vasseur, A. Gouyette, B. Rayner, J.L. Imbach, C. Paoletti and C. Malvy, *J. Biol. Chem.* (1989) **264**, 14172-14178.

[12] J.J. Vasseur, B. Rayner, J.L. Imbach, J.R. Bertrand, C. Malvy and C. Paoletti, *Nucleosides & Nucleotides* (1989) **8**, 863-866.

[13] C. Cazenave, N. Loreau, N.T. Thuong, J.J. Toulmé and C. Hélène, *Nucleic Acids Res.* (1987) **15**, 4717-4736.

[14] J.J. Toulmé, H.M. Krisch, N. Loreau, N.T. Thuong and C. Hélène, *Proc. Natl. Acad. Sci.* (1986) **83**, 1227--1231.

[15] J.J. Vasseur, B. Rayner, J.L. Imbach, unpublished results.

[16] J. Lyndal-York, *J. Org. Chem.* (1981) **46**, 2171-2173.

[17] J. Arpalahti, R. Käppi, J. Hovinen, H. Lönnberg and J. Chattopadhyaya, *Tetrahedron* (1989) **45**, 3945-3954.

[18] E. Zielonacka-Lis, *Nucleosides & Nucleotides* (1989) **8**, 383-405.

[19] V. Nair, *Nucleosides & Nucleotides* (1989) **8**, 699-708.

[20] F. Morvan, B. Rayner, J.L. Imbach, D.K. Chang and J.W. Lown, *Nucleic. Acid. Res.* (1986) **14**, 5019-5035.

[21] F. Morvan, B. Rayner, J.P. Leonetti and J.L. Imbach, *Nucleic Acids Res.* (1988) **16**, 833-847.

[22] We thank Dr. G. Gosselin for the generous gift of this reagent.

[23] During the course of this work, two approaches dealing with acid treatments for AB site formation in oligonucleotides just appeared

J.A. Iocono, B. Gildea and L.W. McLaughlin, *Tetrahedron Lett.* (1990) **31**, 175-178.

K. Groebke and C. Leumann, *Helv. Chim. Acta* (1990) **73**, 608-617.

[24] T. Tanaka, S. Tamatsukuri and M. Ikehara, *Nucleic Acids Res.* (1987) **15**, 7235-7248.