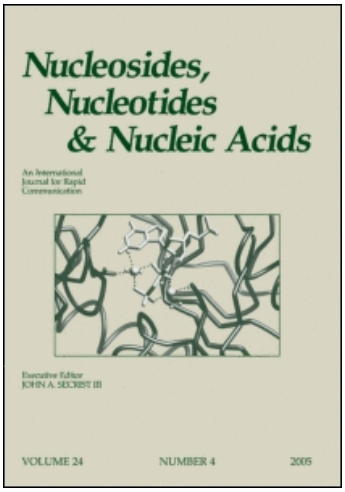

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Eritja, Ramon , Acedo, Montse , Aviñó, Anna and Fà, Carme(1995) 'Preparation of Oligonucleotides Containing Non-natural Base Analogues', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 821 — 824
To link to this Article: DOI: 10.1080/15257779508012480
URL: http://dx.doi.org/10.1080/15257779508012480

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 821-824 (1995)

PREPARATION OF OLIGONUCLEOTIDES CONTAINING NON-NATURAL BASE ANALOGUES.

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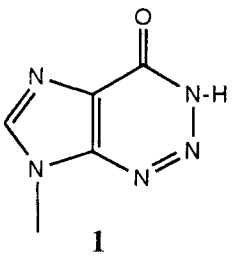
Abstract: The preparation of a protected derivative of 2-aza- 2'-deoxyinosine carrying a photolabile protecting group is described. The new derivative is useful to prepare oligonucleotides containing 2-azahypoxanthine. The synthesis of oligonucleotides containing 2-fluorohypoxanthine and O^4-alkylthymine is also described.

There is a growing interest on modified oligonucleotides containing non-natural base analogues. Specifically, we are interested in the preparation of oligonucleotides containing hypoxanthine derivatives (2-azahypoxanthine **1**, 2-fluorohypoxanthine **2**), in order to improve the base-pairing properties of hypoxanthine with natural bases. The preparation of oligonucleotides containing the mutagenic bases O-4-benzylthymines and O-4-butylthymines (**3**) will also be described.



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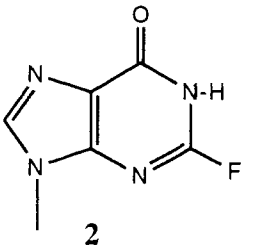

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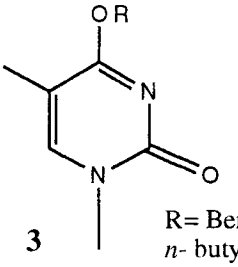

3

R= Benzyl, *n*- butyl

Previous attempts of synthesis of oligonucleotides containing 2-azahypoxanthine (**1**) failed due to an unexpected side-reaction observed on the N^1-(N,N-diphenylcarbamoyl) protected derivative of 2-azahypoxanthine[2]. The preparation of a new derivative of 2-azahypoxanthine is described. The new methodology uses the photolabile (2-nitrobenzyl) group for compound **1**. This protecting group has been previously described in oligonucleotide synthesis for the protection of the 2'-OH in ribonucleotides[3] and the 2'-

deoxyribose residue[4]. It has been shown to be stable during the conditions used in DNA synthesis including ammonia deprotection and can be removed by long-wavelength UV irradiation in conditions which are safe for oligomer integrity. Starting from AICA-riboside, the protected derivative of 2'-deoxy-2-azainosine was prepared in good yields following the scheme shown in FIGURE 1. The protected derivative of 2'-deoxy-2-azainosine was introduced into hexamers 5'CCCAAAI^{Az} 3', 5'GTCAAI^{Az} 3' and pentadecamer 5' TAGAGGI^{Az}TCCATTGC 3' (I^{Az} being 2-azahypoxanthine) sequences, using an automated DNA synthesizer. Treatment of oligonucleotide-supports with concentrated ammonia gave the desired oligonucleotides containing protected N^1-(2-nitrobenzyl)-2-azahypoxanthine. After HPLC purification, oligonucleotides were dissolved in water and irradiated for 6 hours with a 150 W black-light lamp (350 nm). In those conditions, the 2-nitrobenzyl group was removed yielding the desired oligonucleotide containing unprotected 2-azahypoxanthine that had a shorter retention time on HPLC. The presence of 2-azahypoxanthine was confirmed by enzyme digestion and HPLC analysis of nucleosides.

The selection of appropiate protecting groups for natural bases is the critical point for the synthesis of oligonucleotides containing 2-fluorohypoxanthine (**2**) and O^4-alkylthymine (**3**) residues. Due to the sensitivity of these bases to ammonia, the use of non-standard deprotection conditions is needed in order to avoid concentrated ammonia. Phosphoramidites containing *tert*-butylphenoxyacetyl (Expedite™) groups[5] were used for the synthesis of the following oligonucleotides containing O-4-benzylthymine : 5' TCCCAGTCACGACGT^{bz} 3' and 5' GCAATGGAT^{bz}CCTCTA 3'. O^4-Benzylthymine phosphoramidite was prepared essentially as described by Fernandez-Forner et al[6]. Deprotection was carried out by treatment of oligonucleotide-supports with a solution of benzyl alcohol, 1,8-diazabiciclo[5.4.0]undec-7-ene (DBU) and dioxane at 50°C for three days. In these conditions, alkoxide ions, generated by DBU, remove the protecting groups without harming the O^4-alkylthymine residue[7]. The low nucleophilicity of benzyloxide ions made the deprotection of the natural bases very slow. Only when natural bases were protected with the more labile *tert*-butylphenoxyacetyl protecting groups, oligonucleotides were efficiently deprotected by benzyl acohol / DBU solutions, giving clean products. It was also very important to use *tert*-butylphenoxyacetic anhydride as capping reagent instead of acetic anhydride because transacetylation occurs and the resulting acetyl groups are not removed with benzyl alcohol / DBU solutions. When the same sequences were synthesized but containing O^4-*n*-butylthymine and using a *n*-butanol / DBU solution for deprotection, some side products were observed. These side products are assigned to alkylation of bases by acrylonitrile generated from 2-cyanoethyl phosphate protecting

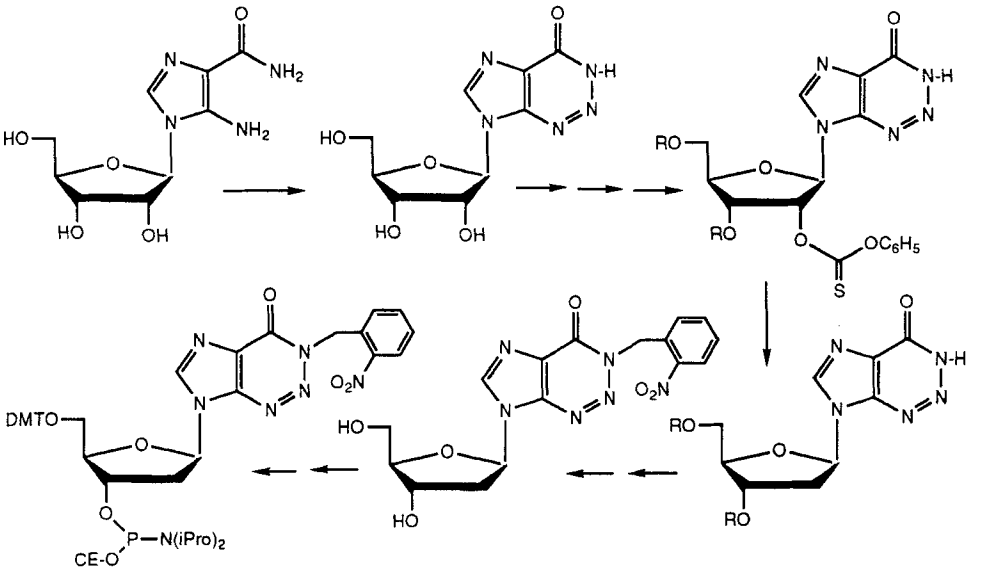

FIGURE 1 : Preparation of the phosphoramidite derivative of N^1-(*o*-nitrobenzyl)-2-aza-2'-deoxyinosine.

group. It has been observed that, when a non-nucleophillic base is present during the deprotection of 2-cyanoethyl group, alkylation of the bases may occur[8b].

Finally, 2-(*p*-nitrophenyl)ethyl (Npe and Npeoc)-protected phosphoramidites[8,9] were used for the preparation of oligonucleotides containing O^4-butylthymine (**3**) and 2-fluorohypoxanthine (**2**) residues. Based on previous work from Dr. Pfleiderer's group[9], a 2-(*o*-nitrophenyl)ethyl (Npe) linkage between oligonucleotide and support was designed[8b]. When Npe and Npeoc-protected phosphoramidites are used together with the Npe linkage, oligonucleotides can be prepared using DBU solutions in aprotic solvents avoiding the use of any nucleophile during deprotection[8]. Using this methodology we have prepared the following oligonucleotides : 5' CTACAT^{bu}CTTGA 3', 5'AAI^{F}CAC 3' and 5' TTACAI^{F}CTGGA 3' (T^{bu} : O^4-*n*-butylthymine **2**, I^F : 2-fluoro-2'-deoxyinosine **3**). 2-(*p*-Nitrophenyl)ethyl group was used instead of 2-cyanoethyl group for the protection of phosphate groups[8a]. The position 6 of 2-fluoro-2'-deoxyinosine was also protected with the Npe group. This group was used for protection of position 6 during the synthesis of 2-fluoro-2'-deoxyinosine from dG[10] and it was left during the preparation of the phosphoramidite and the assembly of the sequences. Deprotection was carried out by treatment of oligonucleotide-supports with a 0.5 M DBU solution in pyridine containing 5 mg of thymine for 15 hours at room temperature[8a]. The resulting solutions were

neutralized with acetic acid aqueous solution and concentrated to dryness. Purification of products was performed by HPLC (after desalting with a Sephadex G-10 column) using the standard DMT-on and DMT-off purification protocols. The presence of the desired base analogues was confirmed by phosphodiesterase and alkaline phosphatase digestion. Melting studies with these polymers are currently being done.

ACKNOWLEDGEMENTS

We are grateful to CICYT (PB 92-0043) for financial support. We thank Drs Manjit Dosanjh, Bea Singer, Fernando Albericio and Nanda Sinha for helpful suggestions and continued interest in this work.

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