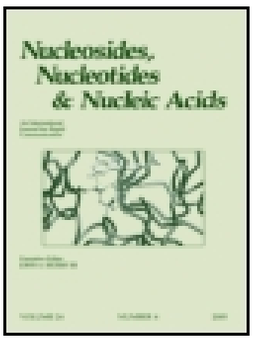

Nucleosides and Nucleotides

ISSN: 0732-8311 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/lncn19

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To cite this article: Oliver Jungmann & Wolfgang Pfleiderer (1997) Pteridine Nucleosides Analogs of 2'-Deoxyadenosine as Building Blocks for Oligonucleotide Synthesis, Nucleosides and Nucleotides, 16:5-6, 863-868, DOI: 10.1080/07328319708002964

To link to this article: http://dx.doi.org/10.1080/07328319708002964

Published online: 22 Aug 2006.

NUCLEOSIDES & NUCLEOTIDES, 16(5&6), 863-868 (1997)

PTERIDINE NUCLEOSIDES ANALOGS OF 2'-DEOXYADENOSINE AS BUILDING BLOCKS FOR OLIGONUCLEOTIDE SYNTHESIS

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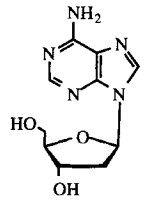
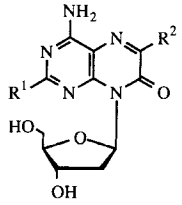
ABSTRACT. The synthesis, configuration and conformation of adenosine analogous pteridine nucleosides and their conversion into the monomeric building blocks for the automated application in an DNA synthesizer is described.

Introduction. Oligonucleotide probes containing fluorophores are increasingly used to study intra- and intermolecular interactions regarding energy-transfer phenomena, hybridisation and stacking effects in oligonucleotide and nucleic acid chemistry. Fluorophores are very sensitive and reflect changes in their immediate environment through measurable differences in fluorescence proporties.

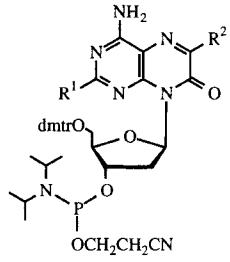
Pteridines are well known for their strong fluorescence and have been considered as an alternative possibility to label oligonucleotides, although these heterocyclic ring systems reveal some difficulties from a synthetic and chemical point of view.



2'-Deoxyadenosine



4-Amino-8-(2-deoxy-β-D-ribo-furanosyl)-7(8H)-pteridinones



Monomeric building blocks for oligonucleotide synthesis

Pteridine nucleosides can be regarded as structural analogs of the naturally ocurring pyrimidine and purine nucleosides, respectivley, and were therefore synthesized in our laboratories since 1970, expecting some biological activity. However, so far no antibacterial,

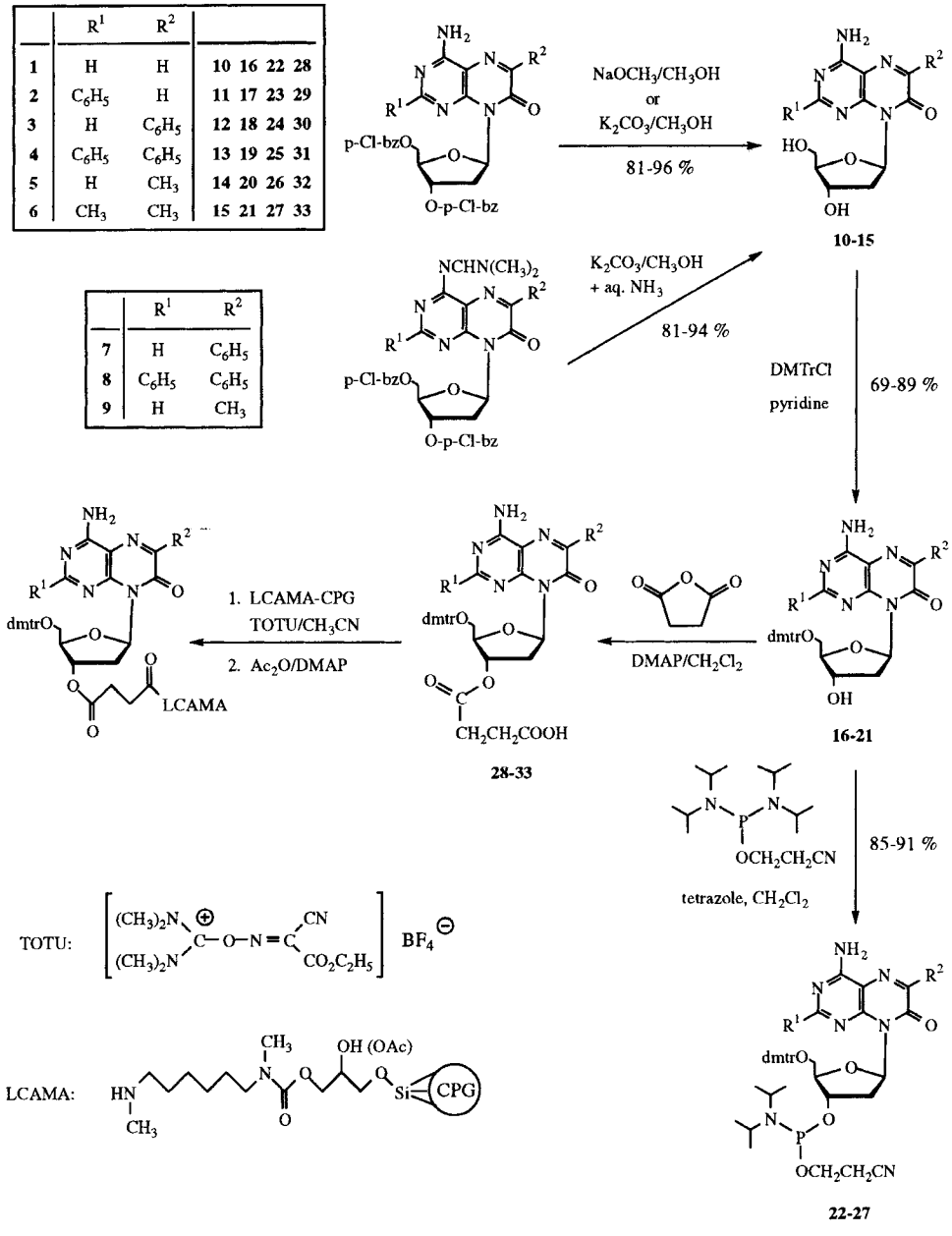
antiviral or antitumor activity was found. The physical proporties of these compounds are still of interest due to the fluorescence of the nucleobases.

The high quantum yields of 4-amino-7(8H)-pteridinone derivatives called our attention to the synthesis of the 7(8H)-pteridinone-N(8)-2'deoxy-β-D-ribonucleosides in a dimethoxytrityl, phosphoramidite form, which can be site-specifically inserted into oligonucleotides through a 3',5'-phosphodiester linkage using an automated approach on solid support material in a DNA synthesizer. The 4-amino pteridinones show a close relationship to 2'-deoxyadenosine and offer their application for the formation of modified oligonucleotides to a real time assay for the HIV-1 intergrase 3'-processing reaction[1].

Synthesis. Recently we published a new efficient method which allows the regio- and stereospecific synthesis of 7(8H)-pteridinone-N(8)-2'-deoxy-β-D-ribonucleosides[2]. In this way the 4-amino- as well as the 4-dimethylaminomethyleneamino-7(8H)-pteridinone derivatives react in form of their DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) salts in a clean S_N2-type mechanism with 3,5-di-O-(4-chlorobenzoyl)-1-chloro-2-deoxy-α-D-ribofuranose to the 4-amino- (**1-6**) and 4-dimethylaminomethyleneamino-8-(2-deoxy-β-D-ribofuranosyl)-7(8H)-pteridinones (**7-9**), respectively, by low formation of the corresponding α-anomers.

In accordance to G. Zemplen[3] the treatment of the compounds (**1-6**) with sodium methoxide or potassium carbonate in methanol resulted in the cleavage of the sugar protecting groups leading to the free 2,6-substituted pteridine nucleosides (**10-15**) in 81-96 % yield. Removal of the dimethylaminomethylene group afforded an additional treatment with aqueous ammonia. Reaction of the 3'-O- and 5'-O-unsubstituted building blocks (**10-15**) with dimethoxytrityl chloride in pyridine formed the appropriate 5'-O-tritylated nucleosides (**16-21**)[4]. The 5'-O-substitution was favoured due to the higher reactivity of the primary 5'-OH function, but still 8-19 % of the 3',5'-di-O-tritylated products were isolated after workup and flash chromatography. Finally, the reaction with β-cyanoethoxy-bis-(diisopropylamino)phosphane under 1H-tetrazole catalysis led to the 3'-O-(β-cyanoethyl)-diisopropylphosphoramidites (**22-27**) in high yields[5]. Purification of the phosphoramidite building blocks was achieved by flash chromatography on silica gel giving colourless and yellow foams, suitable for the synthesis of 3'-5' linked oligodeoxynucleotides in solution or on a solid support by a repetitve cycle.

A second series of building blocks for the solid phase synthesis, the 3'-O-hemisuccinates (**28-33**) of the six pteridine nucleosides were prepared by the almost quantitative reaction with succinic anhydride and 4-(dimethylamino)pyridine (DMAP)[6]. **28-33** were subsequently coupled to a special modified LCAMA-CPG [(long chain alkyl)methylamino contolled pore



Synthesis of the monomeric building blocks for oligonucleotide chemistry

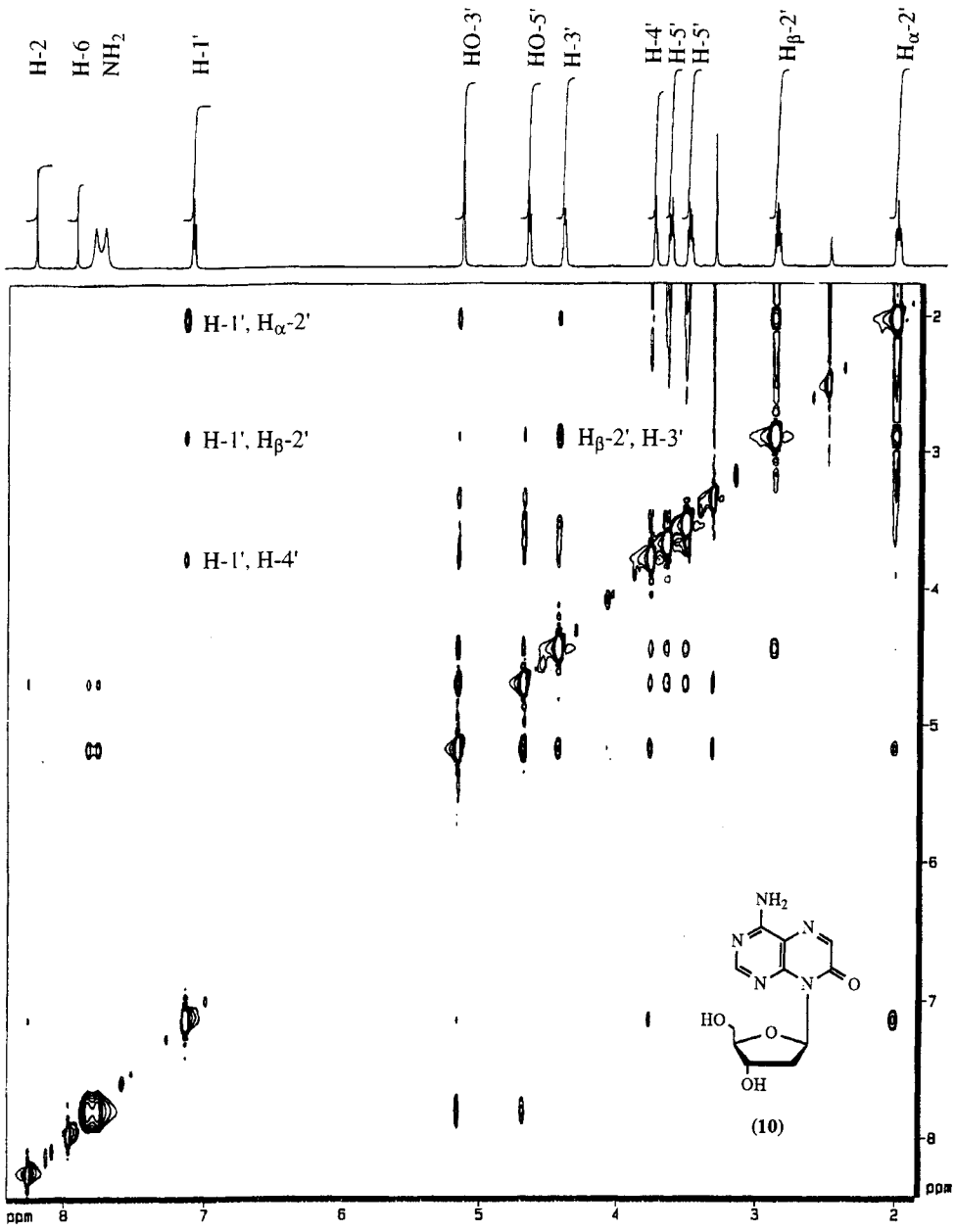
glass)] solid support material in the presence of the condensing reagent O-[cyano(ethoxycarbonyl) methylideneamino]-1,1,3,3-teramethyluronium tetrafluoroborate (TOTU) followed by a capping process with Ac_2O /DMAP in pyridine. Loadings between 29 and 39 μmol/g were reached with a 500 Å-CPG material.

Structure. Syntheses of nucleosides by glycosylation of heterocycles typically result in isomeric products and called therefore several chemical and biochemical methods[7] as well as CD[8] and NMR[9] measurements providing more or less suitable information about configurational and conformational parameters. For example, the structural assigment of regioisomers based on the chemical shifts of glycosidic and base protons in the ^{1}H-NMR spectra, and that of α/β-anomers on the signal pattern of the H-C(1').

For the β-configurated pteridine nucleosides (**10-15**) we found in analogy to former investigations[7c] a characteristic large chemical shift difference Δδ of the H_α-C(2') and the H_β-C(2') resonance in the ^{1}H-NMR spectra. The value of Δδ (250 MHz) in DMSO-d_6 and $CDCl_3$ ranges between 0.6 and 0.9 ppm for the β-anomers (**10-15**) whereas the same signals lie fairly close to one another in the α-anomers (Δδ = 0-0.1 ppm). That seems to be an indication of a more favoured and more restricted S-type puckered conformation in the β-anomeres (**10-15**) whereby the distance between H_β-C(2') and the nucleobase is shortened to a minimum. In the α-anomers a more flexible situation is assumed. However, we have to confess, that the solvent dependence of chemical shifts and coupling constants, based on the influence of steric and electronic effects to the conformational equilibria, makes such generalisations questionable.

In accordance to the more reliable results of F. Seela[10] and G. E. Wright[11], the two dimensional ROESY ^{1}H-NMR spectra show typical distance related cross relaxations for the β-configurated pteridine nucleosides (**10-15**). In general, the β-anomers display intensive crosspeaks between the protons H-C(1')/H_α-C(2'), H-C(1')/H-C(4') and H_β-C(2')/H-C(3'), which are located in the whole range of N- to S-typ conformations on the same side of the sugar moiety. Consequently, only weak crosspeaks between H-C(1') and H_β-C(2') are obtained because they are positioned on contrary faces.

We also learned from our two dimensional NMR investigations that the absence of ROE interactions between the base and the glycosidic protons points to a prefered *anti*-conformation of the pteridines with respect to the sugar. Therefore the β-glycosidic torsion angle χ, enclosed by C(1')-O(4') and N(8)-C(8a), ranges in analogy to the purine nucleosides[12] between 180 ± 90°. Only in the case of **10** and **11** weak crosspeaks of the pteridine subsituents H-C(2) and Ph-C(2), respectively, with HO-C(5') and H-C(3') are observed, implicating a higher *syn/anti* glycosidic bond ratio.



*600 MHz ROESY ^{1}H-NMR spectrum of 4-amino-8-(2-deoxy-β-D-ribofuranosyl)-7(8H)-pteridinone (**10**) in DMSO-d_6.*

However, the energy barrier between the *syn* and *anti* conformation is usually low, implying dynamic equilibria which might be more or less directed towards one side. Therefore a statement based on ROE intensities in analogy to the pyrimidine and purine nucleosides[13] is very difficult. Perhaps, the use of ROE intensities together with other structure constraints leads to a general approach to the conformational analysis.

Acknowlegment. We are thankful to M. Cavegen and Dr. A. Geyer, Universität Konstanz, for the determination and discsssion of the two dimensional ROESY ^{1}H-NMR spectra.

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