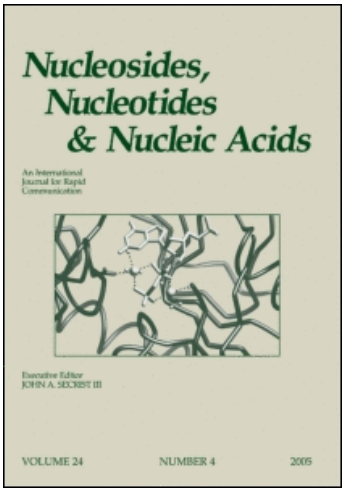

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SYNTHESIS OF 8-(2-DEOXY-β-D-RIBOFURANOSYL)-ISOXANTHOPTERINS NEW FLUORESCENT ANALOGS OF 2'-DEOXYGUANOSINE

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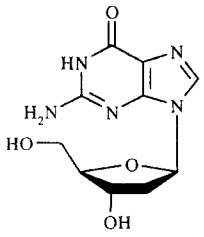
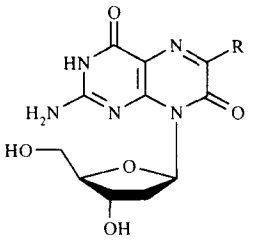
ABSTRACT. We have synthesized isoxanthopterin and 6-phenylisoxanthopterin nucleosides in form of their 5'-O-dimethoxytritylated 3'-phosphoramidites to be used as fluorescence markers directly in the synthesis of oligonucleotides by a machine-aided solid-support approach. The preparation of the monomers and some results of the oligonucleotide synthesis will be described.

INTRODUCTION. Pteridine-N^8-nucleosides have recently attracted much attention due to their structural relationship to the natural occurring purine-nucleosides in combination with interesting fluorescence properties. Especially the isoxanthopterin derivatives showing a guanine like pyrimidine ring substitution reveal high quantum yields which makes them favoured candidates as marker molecules for labelling experiments in molecular biology and biochemistry. Only lately the corresponding 2'-deoxynucleosides of 3-methylisoxanthopterin[1] and 3-methyl-2-N-(2-phenylethyl)-isoxanthopterin[2] were successfully synthesized and incorporated into oligonucleotides, the first one mentioned was used as marker in a real time assay for the HIV-I-integrase. If used in double stranded oligomers the different substitution in the pyrimidine part will interfere with base pair formation by steric hindrance and will, therefore, reduce the duplex stability analogously with the methyl derivatives.

So it should be interesting to prepare isoxanthopterin-nucleosides with an unmodified pyrimidine moiety for incorporation into oligonucleotides. Our aim was to synthesize 2-amino-8-(2-deoxy-β-D-ribofuranosyl)-4,7(3H,8H)-pteridinedione and 2-amino-6-phenyl-8-(2-deoxy-β-D-ribofuranosyl)-4,7(3H,8H)-pteridinedione as fluorescent 2'-deoxy-guanosine analogs in form of their protected phosphoramidites as building blocks for solid support DNA synthesis.

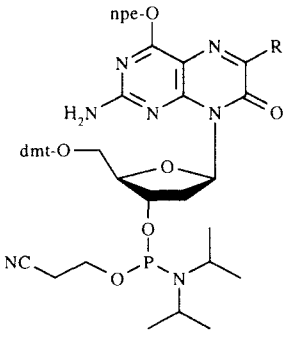


2'-Deoxyguanosine



R = H 8-(2-Deoxy-β-D-ribofuranosyl)-isoxanthopterin

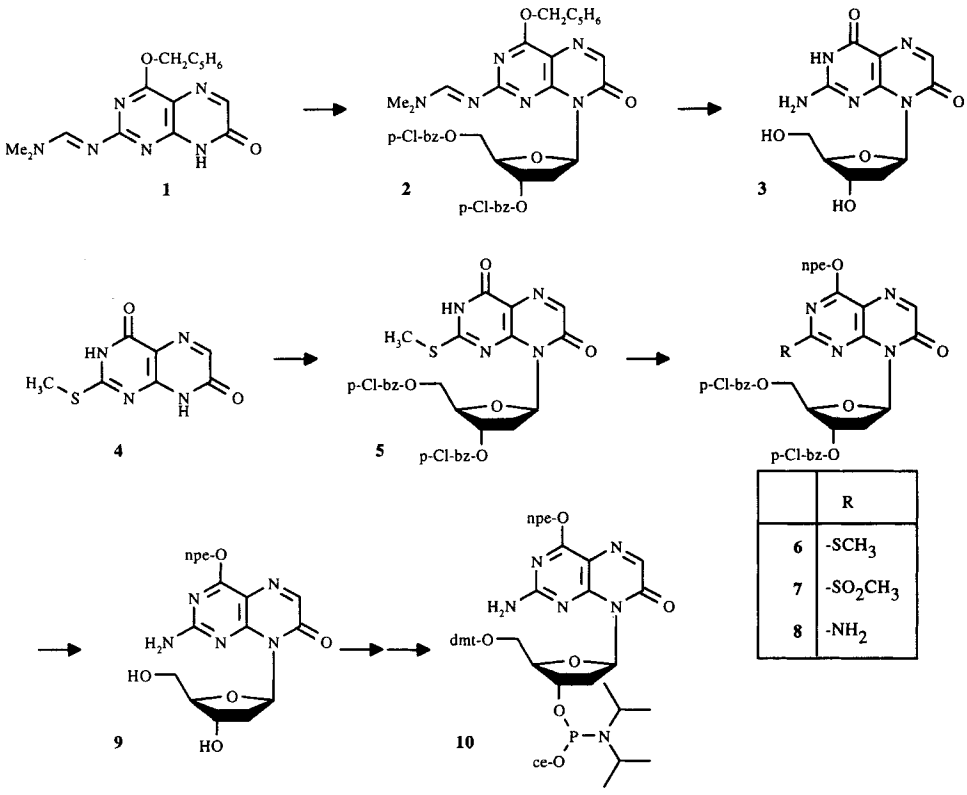
R = Ph 6-Phenyl-8-(2-deoxy-β-D-ribofuranosyl)-isoxanthopterin



building block for oligonucletide synthesis

SYNTHESIS OF THE MONOMERIC BUILDING BLOCKS. The isoxanthopterin itself is too insoluble in most organic solvents for direct glycosylation and the reaction using the persilylated heterocycle leads to a complex mixture of products due to the presence of two lactam and an exocylic amino function in combination with the problem of anomeric mixtures in the 2'-deoxy series. Those difficulties were overcome, after trying various protecting group strategies by the use of two different synthetic pathways both in the synthesis of the isoxanthopterin and the 6-phenylisoxanthopterin building block using 2-amino- and 2-methylthio-substituted [3] pteridines. The stable methylthio function can be easily displaced with ammonia under mild conditions after oxidation to its methylsulfonyl stage. Therefore this way offers a general approach to 2-substituted isoxanthopterin nucleosides when different amines are used. For the glycosylation we used the procedure of O.Jungmann[4] which allows a stereo- and regioselective formation of the desired N^8-β-nucleosides and gave better results than other methods in the pteridine series.

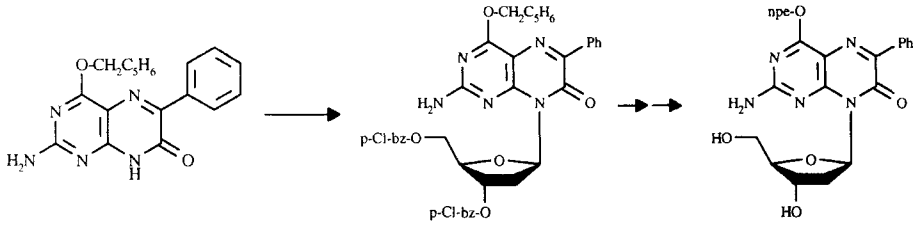
The first attempts to prepare an isoxanthopterin building block were made, starting with 2-amino-4-benzyloxy-7(8H)-pteridineone. To achieve a better solubility the exocyclic amino function was protected using N.N-dimethylformamide diethylacetal in DMF to give **1** in 96% yield. The glycosylation of **1** with the 3.5-di-O-p-chlorobenzoyl-2-deoxy-α-D-ribofuranosyl chloride worked best with DBU in acetonitrile where the N^8-β-isomer **2** separated as a colorless solid and could be isolated in 48% after recrystallisation to separate a small amount of the α-anomer. Hydrogenolytic deblocking of **2** by Pd/H_2 and subsequent treatment with conc. ammonia led to the isoxanthopterin-N^8-2'-deoxy-β-D-riboside **3** in 49%.



Because of difficulties in the tritylation of **3** we decided to choose the second pathway starting from the 2-methylthio-4,7(3H,8H)-pteridinedione (**4**). The glycosylation of **4** in presence of DBU afforded the corresponding N^8-β-glycoside **5** in 33-35% yield after chromatographical workup followed by introduction of the 2-(4-nitrophenyl)ethyl group in a Mitsunobu reaction leading to **6** in 78%. The oxidation of **6** was the crucial step in this synthetic pathway, due to a simulteanous oxidation at C(6) by common oxidizing agents leading to a leucopterin nucleoside[3,5]. After several attempts to achieve a thioselective oxidation the only efficient reagent found was the dimethyldioxirane (DMDO), which was prepared as an acetonic solution according to the procedures of Adam et al[6]. The synthesis of **7** was carried out in CH_2Cl_2 at room temperature. The progress was monitored by tlc showing both the intermediate 2-methylsulfinyl and the methylsulfonyl stage. Evaporation of the solvent afforded pure **7** in quantitative yield. The displacement with ammonia worked best in CH_2Cl_2 and treatment of **8** with NaCN/MeOH cleaved off the sugar blocking groups to form the O-4-protected isoxanthopterin nucleoside **9** in 65%. Dimethoxytritylation and phosphitylation led to the

protected isoxanthopterin building block **10** in only very low yields which were caused by cleaving of the npe function during the chromatographical workup. The nucleoside **9** can also be further deprotected by DBU to **3.**

For the preparation of the 6-phenylisoxanthopterin building block **21** we also used two pathways. The first one started with the 2-amino-4-benzyloxy-6-phenyl-7(8H)-pteridineone (**11**), which needed no further protection. The glycosylation afforded **12** in 31% yield, debenzylation and subsequent introduction of the npe protective group into the 4-O-position by a Mitsunobu reaction led to **13** in 96% and 84% yield, respectively.

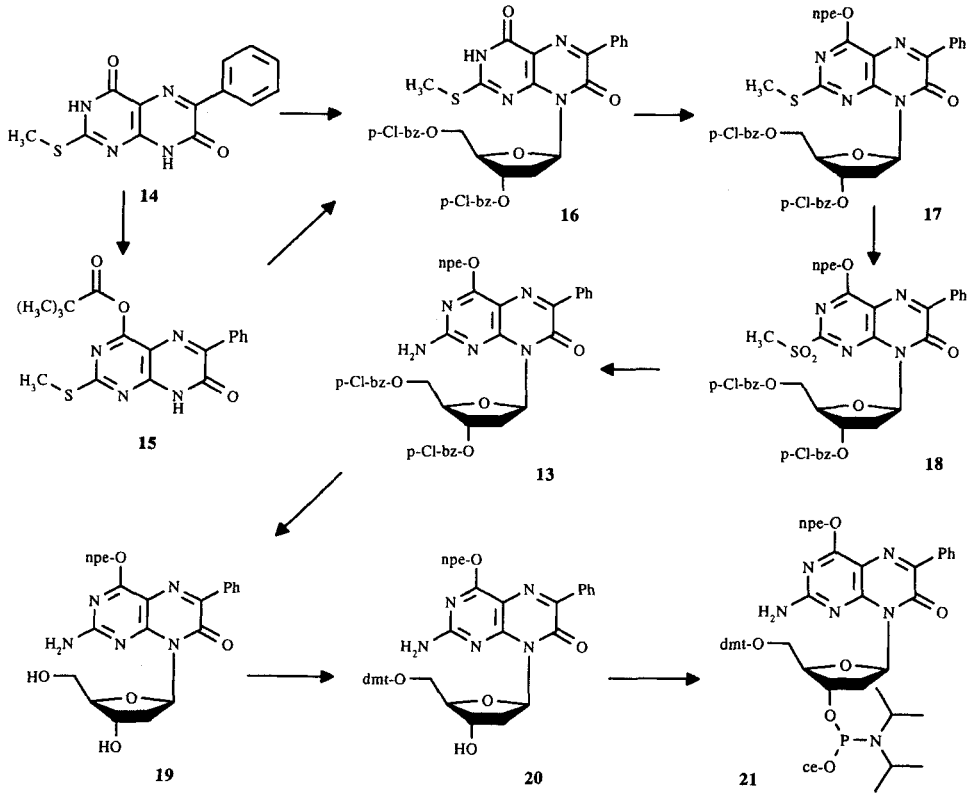


11 **12** **13**

The second approach to compound **13** started with the 2-methylthio-6-phenyl-4,7(3H,8H)-pteridinedione (**14**). Selective protection could be achieved by pivaloylchloride in pyridine, which prefers the 4-O- against the sterically hindered 7-O-position. The glycosylation of **15** with DBU in acetonitrile afforded the nucleoside **16** after depivaloylation in 33% yield over both steps.

Since the direct glycosylation of **14** yielded also 30% of **16** the direct interconversion was favoured. Introduction of the npe function led in 86% yield to **17**. The oxidation step was carried out with m-chloroperbenzoic acid and led to **18** in 85%. A 6-substituted pteridine system like **18** cannot be further oxidized at C(6), so it was not necessary to use dimethyldioxirane. The exchange of the methylsulfonyl function was done as mentioned above with gaseous ammonia in CH_2Cl_2. The product was obtained in 96% yield and was identical with **13** prepared by the other route. Deblocking of the sugar was again accomplished with NaCN/MeOH which leads to the 2-amino-4-O-[2-(4-nitrophenyl)ethyl]-6-phenyl-8-(β-D-2-deoxyribofuranosyl)-4,7(3H,8H)-pteridinedione (**19**), which was dimethoxytritylated and phosphitylated to **20** in 65% and **21** in 68% yield, respectively.

SYNTHESIS OF PTERIDINE MODIFIED OLIGONUCLEOTIDES. The monomeric building blocks were incorporated directly into oligonucleotides with an Applied Biosystems DNA synthesizer model 392 B, following standard procedures according to



the npe-/npeoc-strategy[7,8,9]. The marker phosphoramidite was placed in bottle position 5, dissolving the isoxanthopterin derivative in CH_2Cl_2 whereas the 6-phenylisoxanthopterin analog could be treated in the same way as the standard bases. Removal of the npe-protecting groups was done by treatment with 1 M DBU in acetonitrile followed by cleavage of the oligomer with conc. ammonia from the solid support.

The selected sequence was a 21-mer out of the U5 terminus of the HIV genome since the 3-methylisoxanthopterin had been used for the same target. The isoxanthopterin and the 6-phenylderivative were incorporated in different positions substituting one ore two guanosines. The synthesis of the oligomers worked well with good coupling yields for the pteridine phosphoramidites and only minor occurrence of failure sequences. The oligonucleotides were purified if necessary by HPLC to determine the corresponding melting temperatures on hybridisation with the complementary sequence. The following table lists the sequences of the newly synthesized oligonucleotides, the sites of the substitution of isoxanthopterin (I) and 6-phenylisoxanthopterin (P) for guanosine and the observed melting temperatures of the duplexes.

For the determination of the T_m values we used a Na^+-concetration of 0.15 M.

Sequence	T_m °C
5' d(GTG TGG AAA ATC TCT AGC AGT) 3'	59,2
5' d(GT**P** TGG AAA ATC TCT AGC AGT) 3'	58,7
5' d(GTG TG**P** AAA ATC TCT AGC AGT) 3'	59,0
5' d(GTG TGG AAA ATC TCT A**P**C AGT) 3'	57,6
5' d(GTG TGG AAA ATC TCT AGC A**P**T) 3'	59,1
5' d(GTG T**PP** AAA ATC TCT AGC AGT) 3'	57,9
5' d(GT**I** TGG AAA ATC TCT AGC AGT) 3'	57,6
5' d(GTG TGG AAA ATC TCT AGC A**I**T) 3'	57,6

CONCLUSION. The phosphoramidites of isoxanthopterin **10** and the 6-phenylisoxanthopterin **21** have been synthesized as suitable building blocks for machine aided oligonucleotide synthesis applying the npe-/npeoc-strategy, and were site specifically inserted into oligomers. The marked oligonucleotides showed intensive fluorescence and only a low disturbance of duplex formation which makes them very interesting for labelling experiments.

In the course of the pteridine nucleoside synthesis the two nucleoside building blocks **7** and **18** have been prepared in order to allow a simple entry to various 2-substituted isoxanthopterin nucleosides by nucleophilic displacement reaction of the methylsulfonyl function.

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