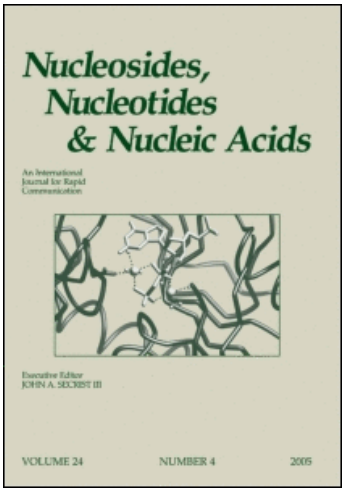

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Pyrazolo[3,4-d)Pyrimidine 2'-Deoxyribo and 2',3'-Dideoxyribo-Furanosides: Synthesis and Application to Oligonucleotide Chemistry

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PYRAZOLO[3,4-d]PYRIMIDINE 2′-DEOXYRIBO- AND 2′,3′-DIDEOXYRIBO-FURANOSIDES: SYNTHESIS AND APPLICATION TO OLIGONUCLEOTIDE CHEMISTRY

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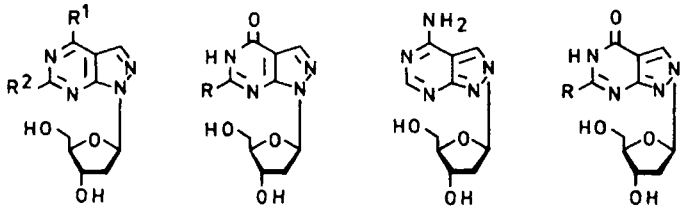
Abstract. - The synthesis of pyrazolo[3,4-d]pyrimidine 2′-deoxyribonucleosides with various substituents at C-4 and C-6 (1 - 4) is described employing either liquid-liquid or solid-liquid phase-transfer glycosylation. From 1a ($z^8c^7A_d$) and 2b ($z^8c^7G_d$) the phosphoramidites 12a,b and 15a,b were synthesized. They were used in automated solid-phase synthesis resulting in the oligonucleotides 16 - 25. Deoxygenation (3′-OH) of 1a and 2b yielded pyrazolo[3,4-d]-pyrimidine 2′,3′-dideoxynucleosides isosteric to ddA, ddG, and ddI.

Pyrazolo[3,4-d]pyrimidines as well as their ribofuranosides exhibit extraordinary therapeutic activities against severe diseases. Due to their closely related structure to purines incorporation of corresponding 2′-deoxyribofuranosides into DNA-fragments is of interest. Moreover, pyrazolo[3,4-d]pyrimidine 2′,3′-dideoxyribofuranosides may show anti-HIV activity which would make them useful in the chemotherapy of AIDS. Recently, we have synthesized a series of 2′-deoxy- [1,2] and 2′,3′-dideoxynucleosides [3,4] isosteric to the parent purine nucleosides. In addition the N-2 isomers were obtained.



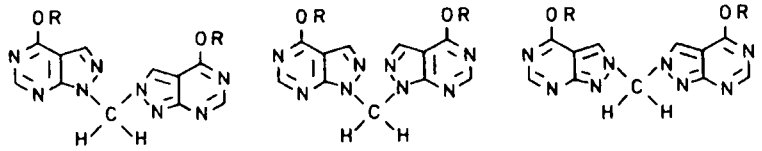
1a: $R^1 = NH_2$; $R^2 = H$
b: $R^1 = NH_2$; $R^2 = NH_2$
c: $R^1 = H$; $R^2 = NH_2$
d: $R^1 = H$; $R^2 = H$

2a : R = H
b : R = NH_2

3

4a : R = H
b : R = NH_2

Our first syntheses employed liquid-liquid phase-transfer glycosylation (50% aq. KOH-CH_2Cl_2, 10 mol-% of Bu_4NHSO_4). If CH_2Br_2 replaced CH_2Cl_2 in the presence of 70 mol-% of catalyst side reactions took place. They became main reactions if the halogenose 9 was omitted and the reaction time was increased to one hour. Compounds 5a-7a were isolated in a ratio of 16:8:1 and were converted into the bis-allopurinols 5b-7b.

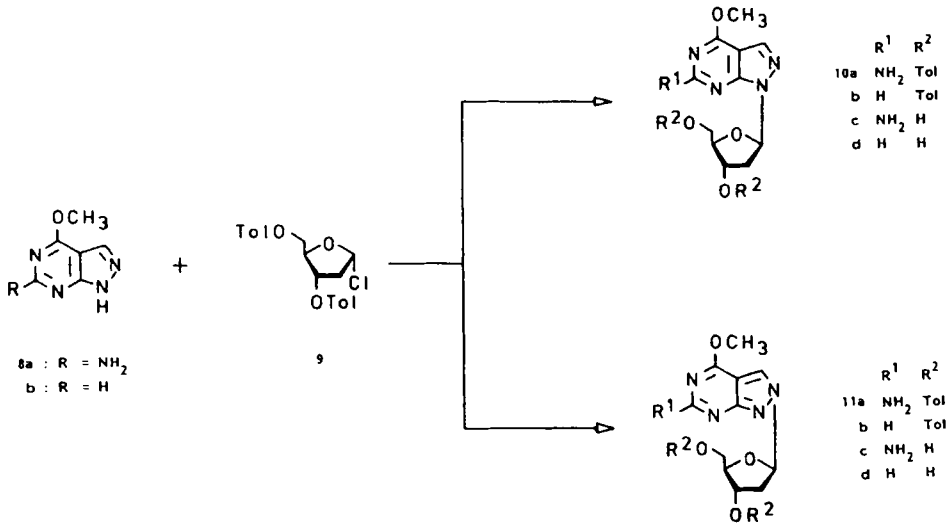


5a : R = CH_3
b : R = H

6a : R = CH_3
b : R = H

7a : R = CH_3
b : R = H

Glycosylation of the nucleobases 8a,b with the halogenose 9 was now carried out under solid-liquid phase-transfer conditions (solid KOH, TDA-1, MeCN) which increased the reaction yield.



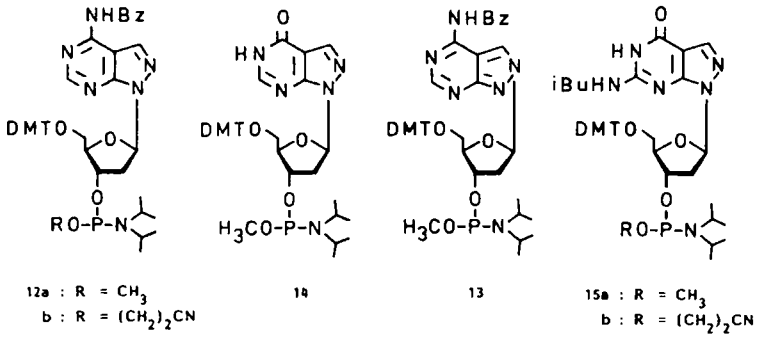
8a : R = NH_2
b : R = H

9

	R^1	R^2
10a	NH_2	Tol
b	H	Tol
c	NH_2	H
d	H	H

	R^1	R^2
11a	NH_2	Tol
b	H	Tol
c	NH_2	H
d	H	H

The crystalline N-1 isomers 10a and 10b were isolated as major reaction products apart from minor amounts of the N-2 isomers 11a and 11b. After Zemplen deprotection the 4-methoxy group of compounds 10c,d as well as 11c,d was nucleophilically displaced yielding the pyrazolo[3,4-d]pyrimidine nucleosides 1-4.

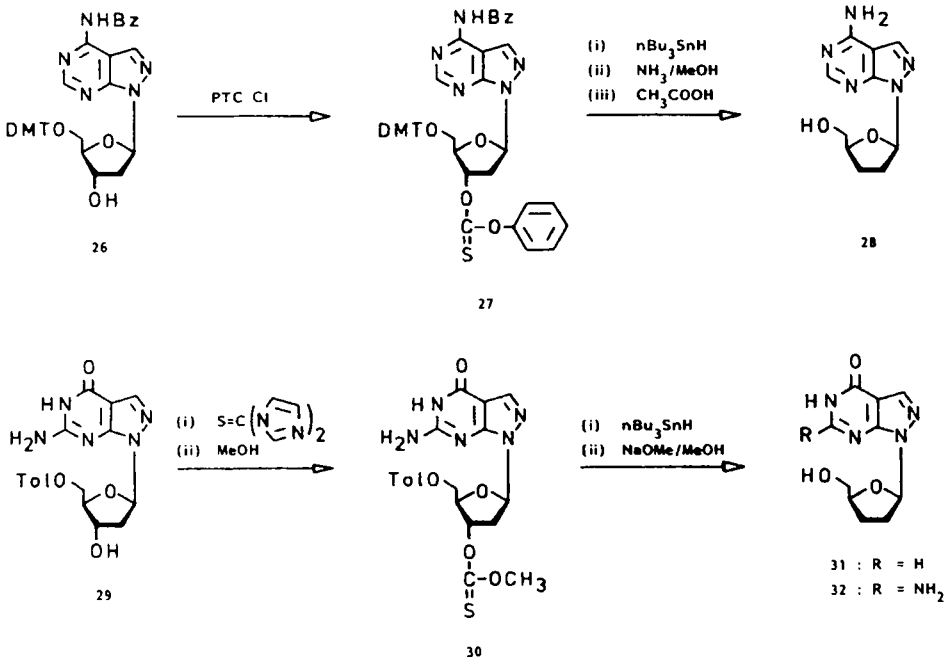


Next, appropriately protected phosphoramidites (12-15) were synthesized and employed together with those of regular nucleosides in solid-phase oligonucleotide synthesis using an automated DNA-synthesizer. The following oligomers have been obtained [4,5]:

(16) $d(Cz^8c^7G)_3$
(17) $d(z^8c^7GC)_3$
(18) $d(z^8c^7AT)_6$
(19) $d(z^8c^7A_{N-2}T)_6$
(20) $d(CTGGz^8c^7ATCCz^8c^7AG)$
(21) $d(CTGGATCCz^8c^7AG)$
(22) $d(CTGGz^8c^7ATCCAG)$
(23) $d(Gz^8c^7GAATTCC)$
(24) $d(z^8c^7Gz^8c^7GAATTCC)$
(25) $d(z^8c^7GGAATTCC)$

From the alternating oligonucleotides 16-19 it became apparent that incorporation of N-1 isomers such as 1a and 2b instead of dA or dG increased the T_m-values compared to the non-modified oligomers. Surprisingly also the oligomer 17 containing the N-2 isomer 3 formed a stable duplex with even a higher melting temperature (T_m increase ca. 15 °C). Finally, the palindromic oligonucleotides 20-25 containing compound 1a or 2b have been used as regioselective probes for the endodeoxyribonucleases Eco RI and Sau 3A. As these enzymes do not hydrolyze the modified DNA-fragments purine nitrogen-7 is required for enzymatic hydrolysis. Even a nitrogen nearby the original proton-acceptor site - N-2 of pyrazolo-[3,4-d]pyrimidines - was not accepted by the enzym.

In order to reduce cytotoxicity of already known purine 2′,3′-dideoxyribonucleosides we have converted the 2′-deoxycompounds 1a and 2b into the new 2′,3′-dideoxynucleosides 28 and 32. The route of protection and deoxygenation is shown in the next scheme.



26 27 28 29 30

While the DMT-residue was employed for OH-5′-protection of compound 1a, compound 2b required an acyl group for protection. Furthermore, compound 1 was deoxygenated via the phenoxythiocarbonylated 27; a methyl-thiocarbonate residue had to be used in case of 2b. Adenosine deaminase converted compound 28 into allopurinol 2′,3′-dideoxyribofuranoside 31, but with a lower rate than ddA into ddI. The pyrazolo[3,4-d]pyrimidine 2′,3′-dideoxyribofuranosides show slightly increased stability at their N-glycosylic bonds compared to the parent purine nucleosides. Antiviral evaluation of the 2′,3′-dideoxynucleosides 28, 31, and 32 is in progress.

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