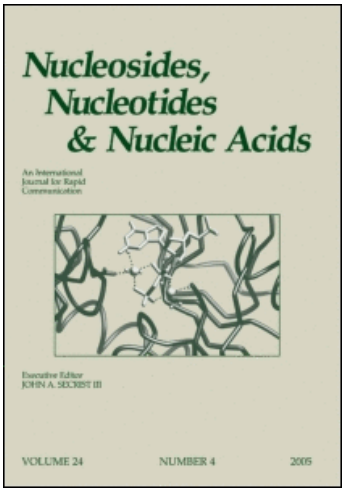

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PROTECTION OF 2,6-DIAMINOPURINE 2'-DEOXYRIBOSIDE

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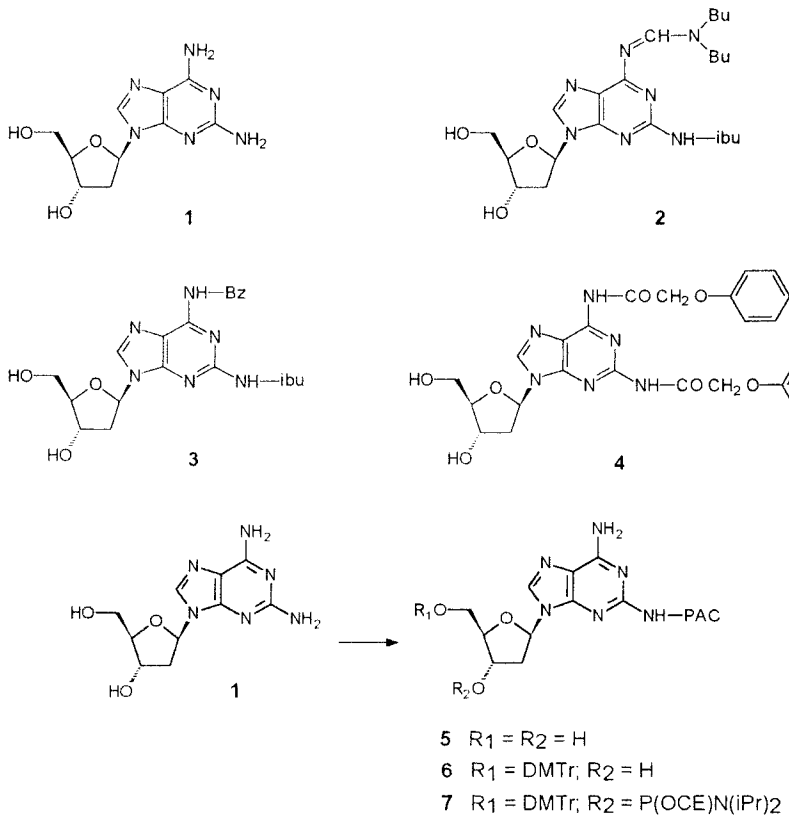
ABSTRACT: Protection of both amine moieties of 2,6-diaminopurine 2'-deoxyriboside as a dimethylformamidine group did not proove feasable, while protection with a phenoxyacetyl group afforded only a mono-protected analogue **5**. This analogue can be incorporated into oligonucleotides without difficulties. However, oligonucleotides with diaminopurine substituted for adenine did not yield more stable duplexes for the two sequences tested here.

A lot of research efforts in the past have been devoted to find a practical and straightforward method for efficient incorporation of 2,6-diaminopurine 2'-deoxyriboside (DAPdR, 2-amino-dA, **1**) into oligonucleotides. The presence of the 2,6-diaminopurine (DAP) substituting for adenine at a predetermined site can be of great help in structural studies of DNA delineating certain protein-DNA interactions[1]. On the other hand, replacement of adenine with DAP theoretically increases the basepair stability with thymidine due to the additional hydrogen bond possibility between the 2-aminogroup of DAP and O^2 of thymine. Due to the presence of two nucleophilic amino groups (although of different reactivity), incorporation of DAP into oligonucleotides always focused on a double protection strategy of the base to avoid unwanted side reactions.

In contrast to the drastic hydrolysis conditions required for deprotection of either the N^2-ibu-N^6-(di-n-butylformamidine)-2-amino-dA (**2**)[1] or the N^2-ibu-N^6-bz-2-amino-dA (**3**) protected analogue[2], deprotection of phenoxyacetyl (PAC) protected DAPdR (**4**) requires less harsh conditions, and takes place concomitantly with the standard protecting groups of the natural bases[3,4]. Our intention was to protect **1** (prepared according to Fathi et al.[5]) with two dimethylformamidine (dmf) groups, which would be easily deprotected after chain assembly. However, this goal was not accomplished: reaction with a large excess of the acetal (monitored by FAB MS) was very slow, and the desired product could not be isolated as discussed below.

A small-scale reaction of diaminopurine **1** with 12 eq. of *N,N*-dimethylformamide dimethyl acetal was run in anhydrous DMF under nitrogen while monitoring by FAB MS. The reaction was almost complete after 4 days at room temperature (RT) with MS



indicating > 90% conversion to the double protected derivative. After addition of conc. aq. NH_3 and stirring for 4 more days at RT, MS showed complete deprotection back to **1**. A preparative run (11 mmol) of **1** was stirred for two weeks with 8 eq. of the acetal. While after 90 min. mass spectrometric analysis indicated a 1:1 ratio was obtained of the mono-adduct and **1**, the starting product had disappeared completely after 18 h and the bis-adduct was formed over 90% after 5 days. However, evaporation and column purification on silica gel (CH_2Cl_2-MeOH 8:2) after two weeks of reaction (complete conversion) only afforded decomposed products. A smaller scale trial afforded the same disappointing result.

We therefore turned our attention to the procedure of Eritja et al.[3], making use of two phenoxyacetyl groups, since deprotection of these groups would be straightforward and would be accomplished within the same time frame as deprotection of the natural bases.

TABLE 1 Melting points (°C) for A_{13}·T_{13} homopolymers and for their counterparts with one diaminopurine deoxynucleoside incorporated in the middle of the A_{13}-strand as determined in a buffer containing 0.1 M NaCl, 20 mM KH_2PO_4 pH 7.5, EDTA 0.1 mM and at 4 μM of each strand, with Tm determined as the inflection point of the melting curve.

$d(T)_6Xd(T)_6$ $d(A)_6Yd(A)_6$				
Y\X	G	C	A	T
A	20.0	17.9	18.5	33.4
DAP	21.6	21.1	20.3	32.8

Making use of trichlorophenyl phenoxyacetate for introduction of the amide moieties, the product isolated did not display the expected NMR spectrum. Complete analysis and integration of all signals proved **5** to contain only one protecting group, situated at the 2-position, leaving the N^6-position unaltered. The latter finding clearly followed from analysis of the non-decoupled and a selectively decoupled ^{13}C spectrum (Varian Unity 500 MHz). In the non-decoupled ^{13}C-spectrum the C-5 carbon signal at 116.2 ppm showed a doublet of triplets ($^3J_{5,8H}$=11.2Hz; 2 x $^3J_{5,NH}$=4.75Hz) and by selective decoupling of the NH_2-signal, this multiplet collapsed into a doublet, which clearly showed that the amino group in the 6-position is unsubstituted and the phenoxyacetyl group is attached to the NH-group at the C-2 position. Selective decoupling of the NHC(O)-proton signal left the multiplet for the C-5 signal unchanged, and showed a sharpening of the C-2 signal at 156.1 ppm.

Tritylation and phosphitylation of the mono-protected DAPdR derivative **5** afforded low yields analogously to Eritja et al.[3], but incorporation into oligonucleotides went unhampered following a standard protocol for oligo assembly (ABI 392). However, melting studies of duplexes containing the modified base showed that DAP replacing for adenine did not yield more stable duplexes (Tables 1 and 2). The lack of increase in stability - as well in a mixed base environment, as within a A/T homo-polymer - is in contrast with the results of Gaffney et al.[2], where from the published graph an approximate rise of 3°C per incorporated residue can be deduced. This may indicate that the effect of substitution of DAP for A is highly dependent on the sequence selected. Gryaznov likewise reported either a very moderate or no increase in duplex stability with DNA as the target, but a

TABLE 2 Hybridisation stability within a mixed base content
Tm (°C) of the duplexes 5'-CACCG**X**CGGCGCC-3'
3'-GTGGC**Y**GCCGCGG-5'
as determined under the same conditions as for table 1.

Y / X	A	T	G	C	ΔTm (°C)
A	61.5	70.0	67.0	58.3	11.7
T	70.3	59.0	65.0	56.5	13.8
G	67.0	64.9	66.7	72.8	7.9
C	60.1	58.8	73.5	54.9	18.6
DAP	63.1	70.7	66.3	62.6	8.1

substantial increase when targeting RNA[4]. However, the affinity of the mixed 13-mer for its mRNA target, likewise is reduced when DAP is replacing adenine at the central position [personal communication of T. Saison-Behmoaras].

In conclusion, we state that mono-protection of DAPdR with a PAC group is sufficient to allow straightforward incorporation into oligonucleotides. However, for the sequences tested, substitution of adenine by diaminopurine did not lead to a substantial increase in duplex stability.

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