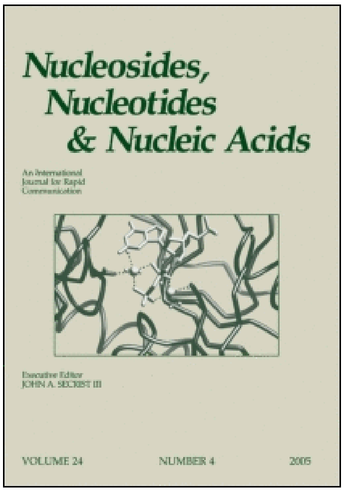

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Double Protection of the Heterocyclic Base of Xanthosine and 2'-Deoxyxanthosine

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DOUBLE PROTECTION OF THE HETEROCYCLIC BASE OF XANTHOSINE AND 2'-DEOXYXANTHOSINE

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Abstract - Reaction of O-protected xanthosines with p-nitrophenyl ethanol under Mitsunobu conditions yields the doubly alkylated O^2,O^6- and N^1-,O^2-derivatives. Deoxyxanthosine protected on both oxygens with a 2-(4-nitrophenyl)-ethyl group was synthesized starting from deoxyguanosine. Both protecting groups can be eliminated with DBU in pyridine.

INTRODUCTION

Screening of cloned DNA is often done with mixed-sequence probes. An ideal base analogue - one that would pair equally well with all four natural nucleosides - would reduce the vast number of synthetic sequences to be synthesized to just one. Deoxyxanthosine has been proposed as a possible candidate for this problem by us[1] and by others[2]. This paper describes the details for protection and deprotection of the base moiety, which enables one to incorporate deoxyxanthosine into oligonucleotides, or to use those protection techniques for synthesizing xanthosine analogues.

RESULTS AND DISCUSSION

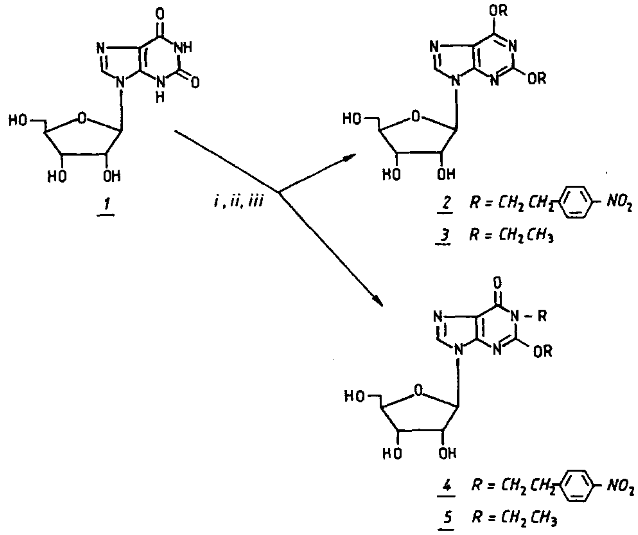
Protection of both lactam functions. In line with our research on inosine[3], the 2-(4-nitrophenyl)-ethyl (NPE) group was tested for protection of the xanthine base. This group was introduced in nucleic acid chemistry as a versatile protecting group by W. Pfleiderer.[4] The ease of introduction and elimination were tested first on the commercially available xanthosine. Mitsunobu reaction[5] on triacetyl xanthosine, followed by ester hydrolysis, gave two nucleoside products, which were separated on silica gel and isolated in 47 % and 18 % yield respectively.

Identification of both products was not straightforward. NMR measurements on a 90 MHz apparatus indicated both products to be doubly alkylated nucleosides. Of these 2 was probably the desired O^2,O^6-disubstituted xanthosine. The downfield position of both α-methylene groups (4.48-4.93 ppm) indicated alkylation on an oxygen atom (in comparison with the previously synthesized 1- and O^6-alkylated inosine derivatives). ^{13}C NMR confirmed this hypothesis with both signals of the α-methylene carbon around 67 ppm and the low field signals for C-2 and C-6 (160.2 and 160.7 ppm). Mass spectrometry gave no molecular ion peak, but a peak at m/e = 450 which corresponds to the (base + H) signal of the proposed structure, from which twice the NPE-group is cleaved as p-nitrostyrene (-149), giving the signals at m/e = 301 and 152.

The second product 4 gave the same MS signals and was therefore also doubly alkylated. NMR though showed one NPE-group to be on an oxygen atom and the other on a nitrogen: ^1H NMR gave the signals 3.26 ppm (CH_2-ar) and 4.47-4.80 ppm (CH_2-O) for alkylation on the O-atom, and 2.84 ppm (CH_2-ar) and 3.88-4.28 ppm (CH_2-N) for alkylation on a N-atom. ^{13}C NMR gave the signals 68.6 ppm (CH_2-O) and 41.8 ppm (CH_2-N) respectively. However, determination of the position of the alkyl groups was not possible.

Alkylation with ethyl alcohol instead of NPE-OH to get less complex products, gave an analogous mixture. Compound 3 proved to be the O^2,O^6-diethyl-xanthosine and product 5 was also an O- and N-alkylated xanthosine, the structure of which remained to be determined. The UV spectrum of 3 gave the same maximum at λ = 265 nm as for the O^2,O^6-dimethyl-xanthosine, synthesized previously by Schaeffer and Thomas in a different manner[6]. This confirmed the proposed structure.

Structure assignment of 4. Structure assignments of the products 2, 4 and 5 were done on a Bruker AM 300 WB spectrometer by 2-D-H,C-COLOC-NMR experiments. All scalar coupled proton spin systems were identified from $^1H,^1H$-COSY-NMR spectra and the non quarternary C-atoms were previously determined via $^1H,^{13}C$-COSY. Whereas scalar coupled proton spin systems are easily identified by two-dimensional homonuclear chemical-shift correlation ($^1H,^1H$-COSY) or ^1H-relayed-$^1H,^1H$-COSY experiments, it is much more difficult to assign spin systems which are separated by hetero or



i : benzoylchloride-pyridine ; ii : triphenylphosphine, DEAD, R-OH
iii : NH_3-MeOH

Fig. 1. Alkylation of xanthosine by means of a Mitsunobu reaction.

quarternary C-atoms. The assignment of these atoms could be solved by means of the Correlation spectroscopy via LOng range Couplings (COLOC).[7] With this pulse sequence it is possible to induce $^{1}H,^{13}C$-couplings ($^{3}J_{CH}$) in a high selectivity. Fig. 2 gives the consecutive numbering of the atoms for structure 4. Figure 3 gives the 300 MHz-$^{1}H,^{1}H$-COSY-NMR spectrum, Figure 4 describes the $^{1}H,^{13}C$-COSY-NMR data, and Figure 5 finally gives the $^{1}H,^{13}C$-COLOC-NMR data. According to COLOC-NMR structure 4 seems to be the right one. Important $^{3}J_{CH}$-couplings for all other putative structures are missing. On the other hand one can observe in the COLOC-NMR a correlation between H-10 and H-19 and a common carbon (C-2) atom (see Fig. 5). These correlations could also be ascribed to the putative structure 15, but in the infrared spectra of the different compounds one can observe a strong carbonyl absorption band for 4 and 5. This makes structure 15 (1-,O^{6}-bis-NPE-xanthosine) impossible. All other correlations are in agreement with the proposed structure 4. In addition to the experimental results we estimated the ^{13}C chemical shifts for the

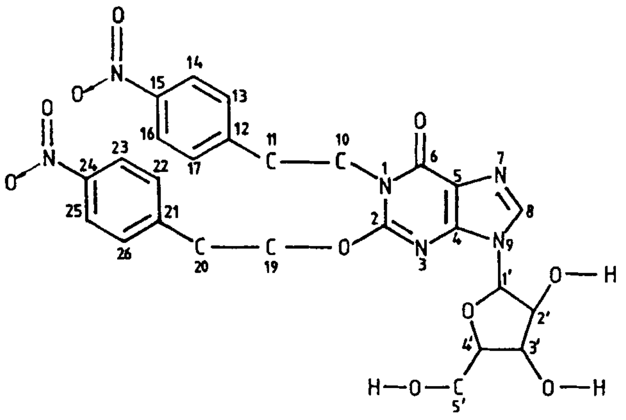


Fig. 2. Formula of structure 4 with consecutive numbering of the atoms excluding hydrogen.

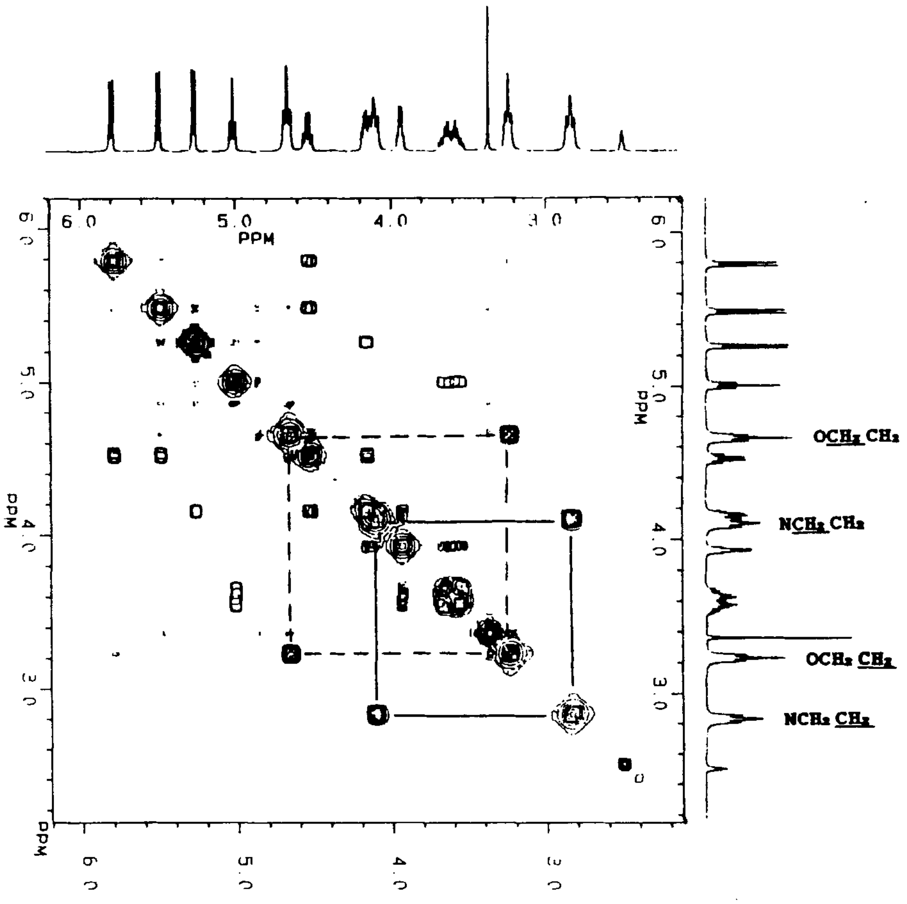


Fig. 3. Relevant part of the symmetrized 300 MHz-^{1}H,^{1}H-COSY-NMR spectrum of compound 4.

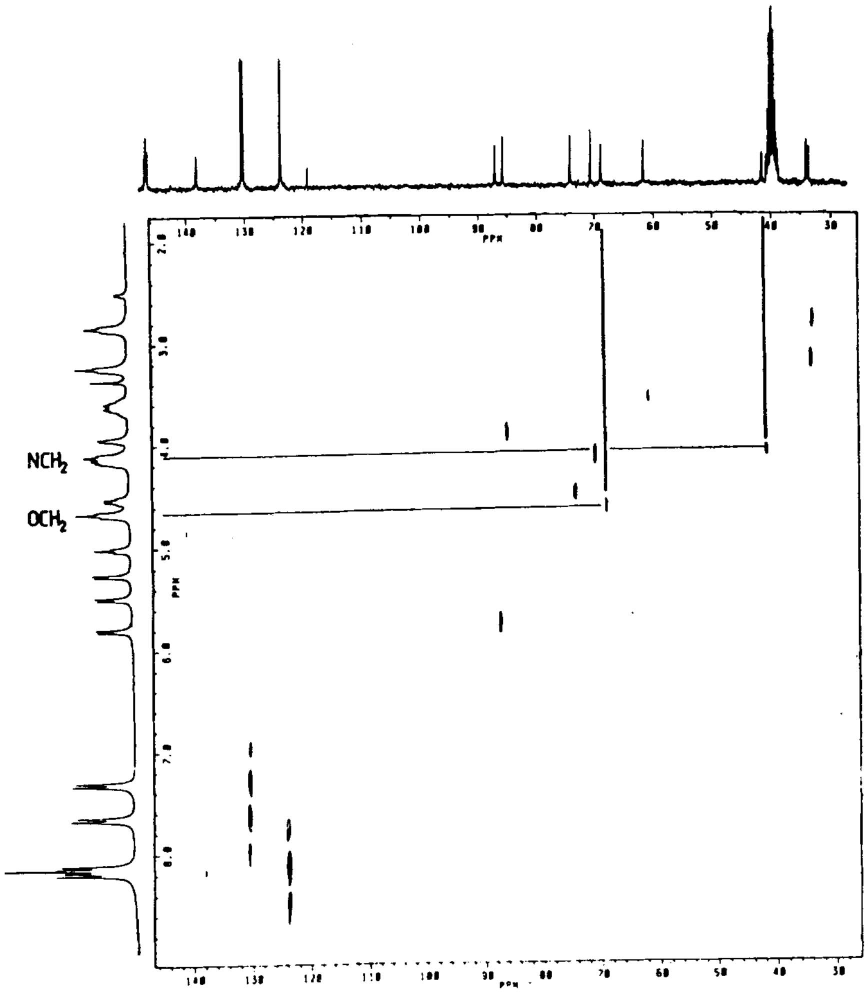


Fig. 4. 300 MHz-^{1}H,^{13}C-COSY-NMR spectrum of compound 4 for identification of the carbon signals.

putative structure of compound 4. They were computer generated and calculated with the help of known ^{13}C chemical shift data which are included in a ^{13}C-database[8,9]. Our estimated data for the structure of 4 are in total agreement with the experimental data.

Removal of the protecting groups. Elimination of both 2-(4-nitrophenyl)-ethyl groups of compound 2 was possible with 0.5 M DBU in pyridine. Quantitative studies were done after 5'-monomethoxytritylation of 2, in

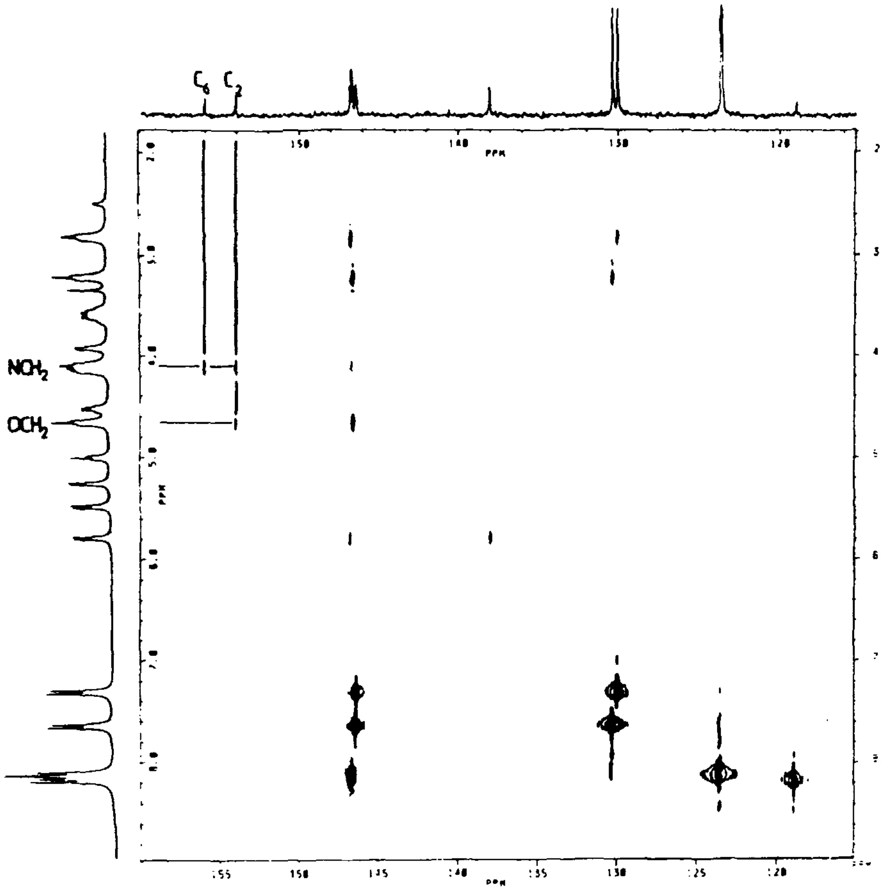
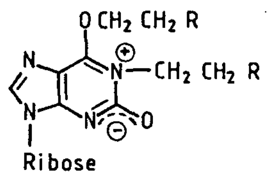


Fig. 5. Relevant part of the 300 MHz-^{1}H,^{13}C-COLOC-NMR spectrum of compound 4. One can observe the correlation between H-10 (N-$\underline{CH}_2$) and H-19 (O-$\underline{CH}_2$) and a common carbon atom (C-2).

order to be able to isolate the deprotected compounds. 0.5 M DBU in pyridine at room temperature (20 eq.) caused fast deprotection of one NPE-group, giving rise mostly to the O^2-monoalkylated xanthosine. This could be isolated in 42 %, out of the mixture with its O^6-isomer and the starting and end product. If the reaction proceeded for 15 h only the 5'-monomethoxytritylated xanthosine could be detected, which was isolated in 69 % yield. Side reactions as with the inosine derivatives[3] were not observed. Both NPE-groups were also eliminated within 15 h by treatment with a 1 M TMG-oximate solution at 70°C (cfr. fast deprotection method of oligonucleotides[10]).

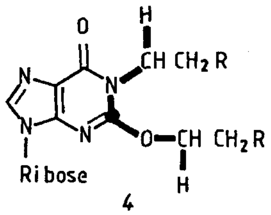
Table 1. 300 MHz-^{1}H,^{13}C-COLOC-NMR data

^{13}C[ppm]	Splitting	^{1}H[ppm]	Splitting	Correlation	$^{x}J_{HC}$
118.94	s	8.21	s	C-5/8-H	3
123.54	d	8.15	m	C-14,16/14,16-H	3;1
123.62	d	8.15	m	C-23,25/23,25-H	3;1
129.97	d	2.85	t	C-13,17/11-H	3
129.97	d	7.33	d	C-13,17/13,17-H	3;1
130.31	d	3.25	t	C-22,26/20-H	3
130.31	d	7.67	d	C-22,26/22,26-H	3;1
138.00	d	5.80	d	C-8/1'-H	3
146.33	s	7.33	d	C-15/13,17-H	3
146.41	s	7.33	d	C-24/22,26-H	3
146.58	s	3.25	t	C-21/20-H	2
146.58	s	4.68	t	C-21/19-H	3
146.58	s	8.15	d	C-21/23,25-H	3
146.65	s	2.85	t	C-12/11-H	2
146.65	s	4.13	t	C-12/10-H	3
146.65	s	8.15	d	C-12/14,16-H	3
146.77	s	5.80	d	C-4/1'-H	3
146.77	s	8.21	d	C-4/8-H	3
154.02	s	4.13	t	C-2/10-H	3
154.02	s	4.68	t	C-2/19-H	3
156.00	s	4.13	t	C-6/10-H	3



15

N^{1}, O^{6} - bis - NPE-X



4

N^{1}, O^{2} - bis - NPE-X

Fig. 6. Putative structures 4 and 15. The correlation between H-10 and H-19 and one common carbon atom is shown for structure 4 by thick lines.

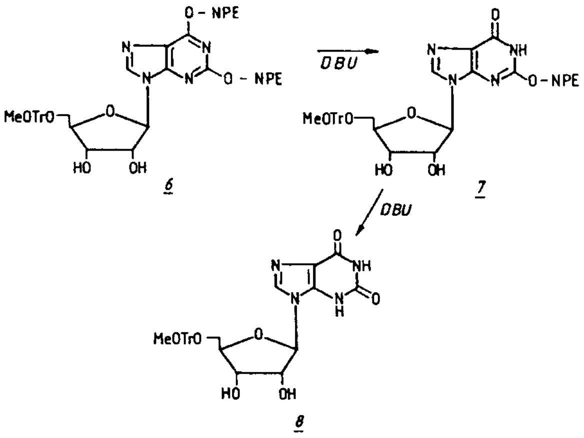


Fig. 7. Elimination of both protecting groups by the strong base DBU.

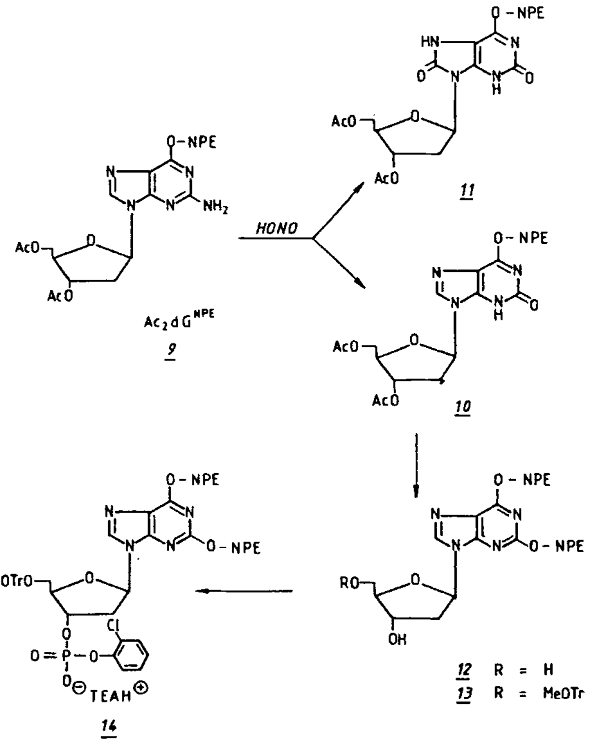


Fig. 8. Synthesis of the base-protected deoxyxanthosine monomer.

Deoxyxanthosine protection. Synthesis of the doubly protected deoxyxanthosine 12 was done starting from the O^6-protected deoxyguanosine 9. Deamination of 9 with nitrous acid (according to a deamination protocol established for the synthesis of uric acid derivatives[11]), keeping the temperature between 5 and 7°C, produced the diacetylated O^6-protected dX 10 in 59 % yield. The major side product was the uric acid derivative 11 which resulted from concomitant oxidation of 9 at C-8. The same strategy was used by Eritja et al.[2], although in a different manner, yielding 47 % of 10. The same product was also formed as a side product in the synthesis of the O^6-protected dI[3], and was identified by NMR and MS. The structure of 11 was resolved with MS giving the molecular ion at m/e = 517 and the signals at m/e = 368, 317 and 168, indicating the uric acid structure. As expected the H-8 proton was missing in the ^1H NMR spectrum, which gave a D_2O exchangeable proton at 10.15 ppm, confirming structure 11.

The second NPE-group was introduced under Mitsunobu reaction conditions and the mixture was deacetylated with MeOH saturated with ammonia, giving 12 in 81 % yield. UV, MS, ^1H and ^{13}C NMR analysis were analogous to 2. The doubly-protected 2'-deoxyxanthosine 12 was monomethoxytritylated and phosphorylated with o-chlorophenyl dichlorophosphate[12], and could be used as such for incorporation into oligonucleotides.

In contrast, Eritja et al.[2] protected only the O^6-carbonyl function, and reported that no side reactions occurred during oligonucleotide synthesis using such monomers when using the phosphoramidite method for coupling reactions. However, when 10 was treated under the conditions for phosphorylation or condensation following phosphotriester protocols, we observed the formation of side products. Hence double protection of the base moiety was found necessary for triester strategies. Synthesis of uric acid 5'-monophosphate likewise was accomplished after triple protection of the heterocyclic base[11].

Incorporation into oligonucleotides. To evaluate the incorporation into oligonucleotides, as for the O^6-alkylated dI monomer, a heptamer with 3 internal deoxyxanthosine residues was synthesized (TXTXXTT) and enzymatically degraded with nuclease P1 to its mononucleotide components.

After synthesis the solid support was divided in two. One fraction was treated with a 1 M TMG-oximate solution at 70°C for 24 h, to remove

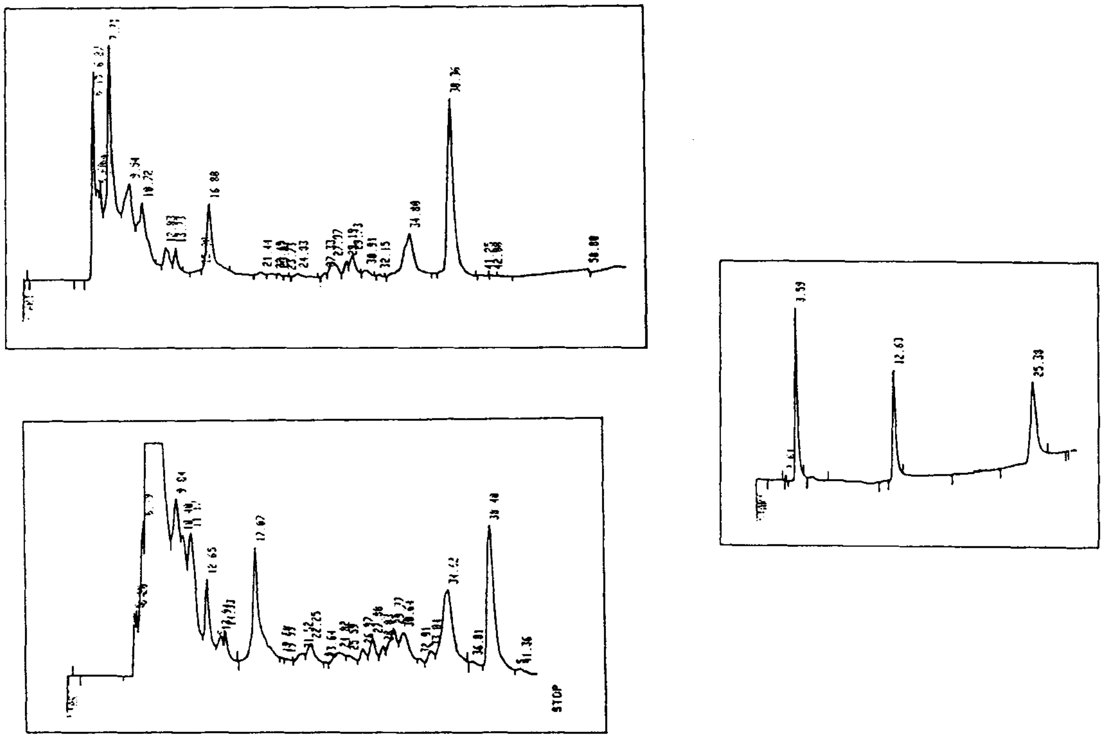


Fig. 9. A and B) HPLC purification on a Partisil 10 SAX column of the deprotected heptamer (TXTXXTT). Buffer A, 0.01 M KH$_2$PO$_4$, pH 6.3. Buffer B, 0.3 M KH$_2$PO$_4$, pH 6.3, both in formamide-water (6:4). Gradient, 0-70 % B in 45 min, 0.5 ml/min, ambient temperature.

A) Two step deprotection: TMG-oximate 0.5 M, for 15 h followed by 1 M DBU in pyridine for 24 h.

B) Fast deprotection: TMG-oximate 1 M, 70°C, 24 h.

C) Analysis of the isolated heptamer on a Partisil 10 SAX column after nuclease P1 digestion; Buffer A, 0.01 M KH$_2$PO$_4$, pH 4.0. Buffer B, 0.2 M KH$_2$PO$_4$, pH 4.0. Gradient, 0 % B for 5 min, 0-100 % B in 20 min.

Order of elution : thymidine, thymidine 5'-phosphate, deoxyxanthosine 5'-phosphate.

the base labile groups. The second part was submitted to a two step procedure involving a 0.5 M TMG-oximate treatment at 37°C for 15 h, followed by a treatment with a 1 M DBU solution in anhydrous pyridine for 24 h at room temperature. Detritylation of both fractions was done with 80 % acetic acid. HPLC purification on a Partisil 10 SAX column showed the two step procedure to be advantageous (Fig. 9). The peak at 34.6 minutes is probably due to incompletely deprotected nucleotidic material and the peak at 38 minutes was collected and desalted. The deprotected dX heptamer is eluted later on the anion exchange column in comparison with the dI heptamer[3], because of the negative charge on the heterocyclic base at pH = 6.3.

The purified heptamer was desalted on a reversed phase disposable cartridge, and enzymatically degraded to thymidine, thymidine 5'-phosphate and deoxyxanthosine 5'-phosphate with nuclease P1, as for dI. Subsequent analysis by HPLC showed no side products. Further treatment with bacterial alkaline phosphatase gave only two products dT and dX (identified by its UV spectrum).

Conclusion. The above results demonstrate that complete protection of the heterocyclic base of 2'-deoxyxanthosine is feasable with two 2-(4-nitrophenyl)-ethyl groups. Care has to be taken as alkylation of unprotected xanthosine yields 1, O^2-bis-alkylated-xanthosine as a side product. Deprotection is possible with DBU or tetramethylaguanidine, yielding almost quantitavely the deprotected compound. Incorporation into oligonucleotides is possible with a phosphotriester strategy as demonstrated above. This work was put to an end however as Eritja *et al.*[2] showed xanthosine not to be interesting as a base analogue for avoiding the synthesis of mixed probes. Yet, in view of the search for new antiviral and antitumoral compounds, the present methodology of protecting and deprotecting xanthosine can be considered when sugar modified analogues of xanthosine are needed.

EXPERIMENTAL

Materials and Methods

Solvents and reagents for oligonucleotide synthesis were purified as described previously, as well as synthesis of the functionalised support and the protected monomer nucleotides[10]. Other chemicals and methods were as described in the previous paper[3]. M.p.s. were measured using a Büchi-Tottoli apparatus and are uncorrected. I.R. spectra were recorded on a Perkin Elmer Model 580B spectrophotometer. UV measurements were done with a Beckman UV 5230 spectrophotometer. NMR measurements were obtained on a Jeol FX900 spectrometer with shifts in δ units. Me_4Si was used as internal reference for ^1H NMR and the solvent resonance as reference for ^{13}C measurements (DMSO at 39.6 and $CDCl_3$ at 76.9 p.p.m. from Me_4Si). The carbon with the nitro substituent of the 2-(4-nitrophenyl)-ethyl group is designated as C-para (C_p); C-ortho = C_o; C-meta = C_m. Mass spectra were recorded on a single-focusing AEI MS-12 mass spectrometer (Kratos Ltd., Manchester, U.K.) operated at an accelerating voltage of 8 kV, trap current 100 µA, ionisation energy 70 eV and ion source temperature as described. Relative intensity is included between brackets. NMR analysis of <u>4</u> and <u>5</u> were done on a Bruker AM 300 WB spectrophotometer (computer: Aspect 3000) operating at 75.5 MHz for ^{13}C-NMR and at 300 MHz for ^{1}H-NMR spectra. One-dimensional and two-dimensional homo- (H,H) and heteronuclear (H,C) COSY-NMR spectra were recorded using standard pulse sequences. For recording the heteronuclear 2-D-H,C-COLOC-NMR the following pulse sequence was used: $\Delta_1-(\pi/2\ ^{1}H)-t_1/2-(\pi\ ^{1}H,\ \pi\ ^{13}C)-(\Delta_2-t_1/2)-(\pi/2\ ^{1}H,\ \pi/2\ ^{13}C)-\Delta_3-AQ,\ ^{1}H$ BB. Number of experiments : 104 to 128 scans each; 4K datapoints; acquisition time: 0.67s; relaxation delay: Δ_1 = 2,3s, Δ_2 = 28ms, Δ_3 = 28ms; total measuring time: 15 hours; spectral width in F2: 45ppm, in F1: 7.2ppm, squared sine bell was applied for F2, shift=2 and an unshifted sine bell window in the F1 direction; before Fourier transformation the spectrum was zerofilled to 8K in the F2 direction and to 512 datapoints in F1; concentration: 0.25M solution in DMSO-d_6; measuring at room temperature with TMS as internal reference.

Thin layer chromatography was done on Merck Kieselgel $60F_{254}$ aluminium sheets and plates were developed in one of the following systems: A: $CHCl_3$-MeOH (9:1); B: $CHCl_3$-MeOH (95:5); C: $CHCl_3$-MeOH (8:2); D: $CHCl_3$-acetone (8:2). Elemental analyses were done at the University of Konstanz.

Mitsunobu reaction on xanthosine.

3.2 g xanthosine dihydrate <u>1</u> (10 mmol) was acylated in 100 ml dry DMF-pyridine (8:2) with 10 ml acetic anhydride for 2 h at room temperature, giving the triacetyl derivative. The mixture was evaporated *in vacuo* and was alkylated under Mitsunobu conditions with 6.55 g (25 mmol) of triphenylphosphine, 4 ml (25 mmol) of diethyl azodicarboxylate (DEAD) and 4.175 g (25 mmol) NPE-OH in 150 ml dry dioxane. After 15 min, two new nucleoside products were detected on TLC (solvent $CHCl_3$-acetone) along with the starting product. The ratio of these products didn't change anymore the next hour and 10 mmol extra triphenylphosphine and DEAD were added, colouring the reaction mixture deep red. After 15 min the starting product had disappeared, water was added and after a few minutes the mixture was evaporated in vacuo. The residue was treated overnight with 400 ml MeOH saturated with ammonia. Evaporation and column chromatography on 150 g silica gel with a MeOH gradient in $CHCl_3$ (3-15 %), gave after crystallisation from acetone-diethylether 2.72 g (4.67 mmol, 47 %) of O^2,O^6-NPE_2-X 2.

O^2,O^6-di-[2-(4-nitrophenyl)-ethyl]-xanthosine 2. M.p.: 140-142°C (recrystallised from ethyl alcohol-water). UV: (MeOH) λ_{max}=268 nm (ε=29750), shoulder at 246 nm; TLC: Rf=0.25 (solvent A), Rf=0.54 (solvent C);
MS: m/e = (no molecular ion detectable, neither after silylation of the sugar hydroxyl groups) 450 (base + H, 1 %), 301 (450 - 149, 5 %), 152 (301-149, 30 %), 149 (100 %), 136 (13 %), 119 (20 %), 103 (50 %), 91 (25 %), 77 (65 %) (NPE-fragments), 133 (sugar, 5%).
NMR: ^1H: (DMSO-d_6/D_2O) δ=3.13-3.37 (m, 4 H, 2 x CH_2-ar), 3.65 (m, 2 H, 2 H-5'), 4.00 (m, 1 H, H-4'), 4.22 (m, 1 H, H-3'), 4.48-4.93 (m, 5 H, H-2' + 2 x CH_2-O), 5.91 (d, 1 H, H-1', J=5.7 Hz), 7.61 (d, 4 H, ortho-H), 8.16 + 8.17 (2 x d, 4 H, meta-H), 8.39 (s, 1 H, H-8) ppm.
^{13}C: (DMSO-d_6) δ =34.4 (2 x CH_2-ar), 61.3 (C-5'), 66.4 + 67.0 (2 x CH_2-O), 70.2 (C-3'), 73.5 (C-2'), 85.5 (C-4'), 87.4 (C-1'), 116.8 (C-5), 123.3 (4 C_m), 130.1 (4 C_o), 140.9 (C-8), 146.2 + 146.4 + 146.8 (2 C_x + 2 C_p), 153.4 (C-4), 160.2 + 160.7 (C-2 + C-6) ppm.
Anal. Calcd. for $C_{26}H_{26}N_6O_{10}.H_2O$: C, 52.00; H, 4.70; N, 14.00.
Found : C, 51.86, H, 4.71; N, 13.89.

Further elution of the column afforded 1.04 g (1.8 mmol, 18 %) of 1-,O^2-NPE_2-X, 4, crystallised from ethyl alcohol-water.

1-,O^2-di-[2-(4-nitrophenyl)-ethyl]-xanthosine, 4. M.p.: 195-196°C (ethyl alcohol-water); UV: (MeOH) λ_{max}=266 nm (ε=21050), shoulder at 250 nm; IR: 1665 cm^{-1} (strong C:O absorption band); TLC: Rf=0.10 (solvent A), Rf=0.39 (solvent C);
MS: m/e = (no molecular ion detectable, neither after silylation of the sugar hydroxyl groups) 450 (base + H, 10 %), 433 (450 - OH, 2 %), 301 (450 - 149, 7 %), 300 (base - 149, 40 %), 152 (301 - 149, 90 %), 149 (100 %), 136 (42 %), 119 (35 %), 103 (65 %), 91 (40 %), 77 (90 %) (NPE-fragments), 133 (sugar, 12 %)
NMR: ^1H: (DMSO-d_6) δ = 2.85 (t, 2 H, N-CH_2-$\underline{CH}_2$-ar), 3.25 (t, 2 H, O-CH_2-$\underline{CH}_2$-ar), 3.63 (m, 2 H, 2 H-5'), 3.93 (m, 1 H, H-4'), 4.13 (t, 2 H, N-$\underline{CH}_2$), 4.17 (m, 1 H, H-3'), 4.54 (q, 1 H, H-2'), 4.68 (t, 2 H, O-$\underline{CH}_2$), 5.03 (t, 1 H, 5'-OH), 5.29 (d, 1 H, 3'-OH), 5.50 (d, 1 H, 2'-OH), 5.80 (d, 1 H, H-1', J= 6Hz), 7.33 (d, 2 H, ortho-H N-NPE, J= 9Hz), 7.67 (d, 2 H, ortho-H O-NPE, J= 9Hz), 8.15 (m, 4 H, meta-H), 8.21 (s, 1 H, H-8) ppm.
^{13}C : (DMSO-d_6) δ= 33.47 (N-CH_2-$\underline{CH}_2$-ar), 33.84 (O-CH_2-$\underline{CH}_2$-ar), 41.36 (CH_2-N), 61.47 (C-5'), 68.69 (CH_2O), 70.48 (C-3'), 73.95 (C-2'), 85.61 (C-4'), 87.00 (C-1'), 118.94 (C-5), 123.54 + 123.62 (4 C_m), 129.97 +

130.31 (4 C_o), 138.00 (C-8), 146.33 (C_p, N-aryl), 146.41 (C_p, O-aryl), 146.58 (C_x, O-aryl), 146.65 (C_x, N-aryl), 146.77 (C-4), 154.02 (C-2), 156.00 (C-6) ppm.

Anal. Calcd. for $C_{26}H_{26}N_6O_{10}.H_2O$: C, 52,00; H, 4.70; N, 14.00.
Found: C, 51.96; H, 4.47; N, 13.84.

Diethylxanthosine derivatives

As for the synthesis of 2 and 4, 2.4 g (7.5 mmol) of xanthosine 1 was acetylated with acetic anhydride in 75 ml DMF-pyridine (8:2), and was next alkylated with 5.25 g (20 mmol) of triphenylphosphine, 3.2 ml (25 mmol) of DEAD and 2 ml (+/- 30 mmol) dry ethyl alcohol in 100 ml anhydrous dioxane. Workup as for the previous reaction afforded after crystallisation from MeOH-diethylether 706 mg (2.07 mmol, 27 %) of O^2,O^6-diethylxanthosine 3 and 420 mg (1.25 mmol, 17 %) of 1-,O^2-diethylxanthosine 5.

O^2,O^6-diethylxanthosine 3. M.p.:173-174°C (methanol-diethylether) UV: (MeOH) λ_{max}=265 nm (ε=12075) and 236 nm (ε=10400)), shoulder at 243 nm; min. at 251 nm (ε=7600 (3.88)); TLC: Rf=0.22 (solvent A);
MS: m/e = 340 (M^+, 35 %), 310 (M - 30, 6 %), 251 (M - 89, 44 %), 237 (base + 30, 100 %), 209 (base + 2 H, 55 %), 208 (base + H, 70 %), 180 (208 - C_2H_4, 25 %), 152 (208 - 2 x C_2H_4, 35 %), 133 (sugar, 2 %), analysis at 160°C;
NMR: ^1H: (DMSO-d_6/D_2O) δ=1.37 + 1.41 (2 x t, 6 H, 2 CH_3), 3.68 (m, 2 H, 2 H-5'), 4.00 (m, 1 H, H-4'), 4.21 (m, 1 H, H-3'), 4.39 (t) + 4.56 (t) + 4.64 (m) (5 H, 2 CH_2-O + H-2'), 5.90 (d, 1 H, H-1', J= 5.7Hz), 8.37 (s, 1 H, H-8) ppm.
^{13}C: (DMSO-d_6) δ = 14.6 (2 x CH_3), 61.7 (C-5'), 63.1 + 63.4 (2 x CH_2-O), 70.6 (C-3'), 73.6 (C-2'), 85.5 (C-4'), 87.9 (C-1'), 117.1 (C-5), 141.1 (C-8), 153.6 (C-4), 160.9 + 161.3 (C-2 + C-6) ppm.
Anal. Calcd. for $C_{14}H_{20}N_4O_6$: C, 49,45; H, 5.93; N, 16.48.
Found: C, 48.85; H, 5.99; N, 16.29.

1-,O^2-diethylxanthosine 5. M.p.:181°C (methanol-diethylether); UV: (MeOH) λ_{max}=241 nm (ε=12575), shoulders at 247 and 260 nm;
IR: 1685 cm^{-1} (strong C:O absorption band); TLC: Rf=0.07 (solvent A);
MS: m/e = 340 (M^+, 21 %), 251 (M - 89, 4 %), 236 (base + CHO, 50 %), 208 (base + H, 100 %), 193 (208 - CH_3, 18 %), 180 (208 - C_2H_4, 70 %), 165 (180 - CH_3, 21 %), 152 (208 - 2 x C_2H_4, 55 %), 109 (152 - HNCO, 50 %), analysis at 160°C;
NMR: ^1H: (DMSO-d_6/D_2O) δ=1.18 (t, 3 H, $\underline{CH_3}$-CH_2-N, J= 7.2 Hz), 1.41 (t, 3 H, $\underline{CH_3}$-CH_2-O, J= 7.2 hz), 3.64 (m, 2 H, 2 H-5'), 3.85-4.27 (m, 4 H, H-3' + H-4' + CH_3-$\underline{CH_2}$-N), 4.50 (m, 3 H, CH_3-$\underline{CH_2}$-O + H-2'), 5.80 (d, 1 H, H-1', J= 5.7 hz), 8.15 (s, 1H, H-8) ppm.
^{13}C: (DMSO-d_6) δ=13.7 + 14.3 (2 x CH_3), 36.4 (CH_3-$\underline{CH_2}$-N), 61.7 (C-5'), 65.5 (CH_3-$\underline{CH_2}$-O), 70.6 (C-3'), 73.8 (C-2'), 85.7 (C-4'), 87.5 (C-1'), 119.2 (C-5), 138.3 (C-8), 147.0 (C-4), 154.5 + 156.4 (C-2 + C-6) ppm.

Anal. Calcd. for $C_{14}H_{20}N_4O_6$: C, 49.45; H, 5.93; N, 16.48.

Found: C, 49.30; H, 6.04; N, 16.48.

5'-Monomethoxytrityl-O^2,O^6-di-[2-(4-nitrophenyl)-ethyl]-xanthosine 6

The doubly protected xanthosine 2 (2.413 g, 4.14 mmol) was coevaporated twice with dry pyridine, and was tritylated overnight at room temperature with 1.2 eq. monomethoxytritylchloride. The reaction was quenched by addition of MeOH, and the mixture was purified on 70 g silica gel (elution with $CHCl_3$-MeOH (99:1)). Fractions containing the desired product were pooled, concentrated and precipitated from 500 ml hexane-diethylether (9:1) to yield 3.37 g of the title compound 6 (3.95 mmol, 95 %).

UV: ($CHCl_3$) λ_{max}=240 and 270 nm; TLC: Rf=0.42 (solvent B);
NMR: ^1H: ($CDCl_3/D_2O$) δ = 3.03-3.54 (m, 6 H, 2 x CH_2-ar + 2 H-5'), 3.73 (s, 3 H, CH_3-O), 4.23-4.86 (m, 7 H, H-4' + H-3' + H-2' + 2 x CH_2-O), 5.96 (d, 1 H, H-1', J= 5.5 Hz), 6.74 (d, 2 H, trityl), 7.10-7.54 (m, 16 H, trityl + 2 x ortho-H NPE), 7.95 (s, 1 H, H-8), 8.11 + 8.12 (2 x d, 4 H, meta-H NPE) ppm.
^{13}C: ($CDCl_3$) δ = 34.9 (2 x CH_2-ar), 55.0 (CH_3-O), 63.4 (C-5'), 66.8 + 67.2 (2 x CH_2-O), 71.4 (C-3'), 75.0 (C-2'), 84.6 (C-4'), 86.7 (ϕ_3-C), 89.0 (C-1'), 116.8 (C-5), 123.5 (4 C_m), 129.6 (4 C_o), 139.3 (C-8), 145.4 + 145.6 + 146.7 (2 C_x + 2 C_p), 152.8 (C-4), 160.4 + 161.1 (C-2 + C-6), + trityl signals (113.0 + 126.9 + 127.7 + 128.1 + 130.1 + 134.8 + 143.7 + 158.5) ppm.

Elimination of the 2-(4-nitrophenyl)-ethyl groups from 6

A: 5'-monomethoxytrityl-O^2-[2-(4-nitrophenyl)-ethyl]-xanthosine 7

860 mg (1 mmol) 6 was coevaporated twice with dry pyridine and was taken up in 10 ml 0.5 M DBU in pyridine. The reaction was monitored by TLC (solvent A), and the complete deprotected product could be detected after 45 min. After 65 min the reaction was quenched by addition of 5 ml of a 1 M acetic solution acid in pyridine. TLC revealed that approximately 20 % of the starting product was still present, 10 % and 50 % of the mono alkylated xanthosine derivatives (Rf=0.17 and 0.27), and 20 % of the complete dealkylated product (Rf=0).

The mixture was evaporated and the residue was taken up in 50 ml $CHCl_3$ and washed with 2 x 100 ml water. The water phases were reextracted twice with 50 ml $CHCl_3$. The most polar product (MeOTr-X, 8) was removed mostly with the water layer. Silica gel column chromatography (60 g) and elution with $CHCl_3$-MeOH (98:2), followed with (96:4) afforded 297 mg (0.42 mmol, 42 %) of 7 after precipitation in 100 ml pentane-diethylether (3:2).

UV: (MeOH): λ_{max}=235 nm (ε=25200), 262 nm (ε=19400); TLC: Rf=0.17 (solvent A);
NMR: ^{1}: ($DMSO/D_2O$) δ= 3.00-3.40 (m, CH_2-ar + 2 H-5'), 3.73 (s, CH_3-O), 4.11 (m, H-4'), 4.43 (m, H-3' + CH_2-O), 4.72 (m, H-2'), 5.90 (d, H-1', J= 5.5 Hz), 6.80 (d, trityl), 7.10-7.45 (m, trityl), 7.52 (d, ortho-H NPE), 8.06 (s, H-8), 8.14 (d, meta-H NPE) ppm.
^{13}C: (DMSO) δ = 33.9 (CH_2-ar), 55.9 (CH_3-O), 64.1 (C-5'), 67.5 (CH_2-O), 70.4 (C-3'), 73.2 (C-2'), 83.3 (C-4'), 86.0 (ϕ_3-C), 88.1 (C-1'), 120.2 (C-5), 123.1 (2 C_m), 130.0 (2 C_o), 137.9 (C-8), 145.4 + 145.6 (C_x + C_p), 148.2 (C-4), 155.1 + 157.0 (C-2 + C-6) ppm, + trityl signals.

B: 5'-monomethoxytrityl-xanthosine 8

1710 mg (2 mmol) of 6 was coevaporated twice with dry pyridine and taken up in 80 ml anhydrous pyridine containing 0.5 M DBU. The reaction was stirred for 15 h at room temperature. TLC showed complete deprotection of the base moiety, with only one nucleosidic product formed. 40 ml of a 1 M acetic acid solution in pyridine was added under stirring. The mixture was evaporated *in vacuo* and the residu was taken up in 100 ml of a 5 % $NaHCO_3$ solution and extracted with 3 x 100 ml EtOAc. Only a small fraction of the product was extracted by the organic phase, which was evaporated. The residue was taken up in $CHCl_3$-diethylether which slowly caused precipitation. To the water phase was added brine, causing a voluminous precipitate. Washing and drying of the precipitates yielded a total of 802 mg (1.38 mmol, 69 %) of the title product 8 as its sodium salt.

UV: (MeOH): λ_{max}=235 nm (ε=17670), 274 nm (ε=8850), shoulder at 246 nm, (H_2O, 50 mM phosphate buffer): pH 4: λ_{max}=232 nm (ε= 19230), 262 nm (ε=9060); pH = 7, 9, 12: unchanged, λ_{max}=235 nm (ε=16650), 276 nm (ε=8930);
TLC: Rf=0.28 ($CHCl_3$-MeOH (7.3));
NMR: ^{1}H: ($DMSO/D_2O$) δ = 3.12-3.40 (m, 2 H-5'), 3.73 (s, CH_3-O), 3.95-4.20 (m, H-4' + H-3'), 4.24-4.45 (m, H-2'), 5.78 (d, H-1', J= 5.5 hz), 6.86 (d, trityl), 7.10-7.50 (m, trityl), 7.53 (s, H-8), 9.49 (s, N^1-H?) ppm.
^{13}C: (DMSO) δ = 55.2 (CH_3-O), 64.1 (C-5'), 70.4 (C-3'), 74.2 (C-2'), 82.6 (C-4'), 86.0 (ϕ_3-C), 86.9 (C-1'), 114.7 (C-5), 132.7 (C-8), 154.1 (C-4), 159.3 + 160.0 (C-2 + C-6), + trityl signals (113.3 + 127.0 + 128.0 + 128.1 + 130.1 + 135.1 + 144.3 + 158.3) ppm.

3',5'-di-O-acetyl-O^6-(2-[4-nitrophenyl]-ethyl)-2'-deoxyxanthosine (Ac_2dX^{NPE}) 10

5 g (10 mmol) of the O^6-protected 2'-deoxyguanosine (Ac_2dG^{NPE}) (9) was dissolved in 100 ml acetone, and the solution was cooled in an ice bath. A solution of 2.8 g (40 mmol) of $NaNO_2$ in 20 ml water was added under stirring at 0°C. Over a period of 10 min 3.6 ml (60 mmol) of $HClO_4$ 70 % in 10 ml water were added, keeping the temperature below 7°C. The mixture was stirred for 90 min maintaining the temperature between 5°C and 7°C. TLC in $CHCl_3$-MeOH (95:5) revealed no more starting product to be present, and the mixture was neutralised with a 5 % $NaHCO_3$ solution. Acetone was evaporated and the remaining water phase was extracted 3 x with EtOAc. The organic phases were washed with a 5 % $NaHCO_3$ solution, and with brine. TLC (solvent B) showed two important products with Rf=0.27 (the desired 10) and Rf=0.36 (side product 11), and several minor impurities. Chromatography on 120 g silica gel with an acetone gradient in $CHCl_3$ (0-30 %) yielded, after crystallisation from $CHCl_3$-diethylether, 2.957 g (5.9 mmol, 59 %) of 10 and 580 mg (1.12 mmol, 11 %) of 11 from benzene-$CHCl_3$.

9-β-D-(3',5'-di-O-acetyl-2'-deoxyribofuranose)-O^6-(2-[4-nitrophenyl]-ethyl)-uric acid 11. M.p.: 142-144°C (benzene-$CHCl_3$);
UV: (MeOH) λ_{max}=279 nm (ε=23025), 238 nm (ε=10700); TLC: Rf=0.13 (solvent D), Rf=0.36 (solvent B);
MS: m/e = 517 (M^+, 0.3 %), 368 (M - 149, 0.3 %), 317 (base + H, 6 %), 201 (sugar, 3 %), 168 (317 - 149, 10 %), 149 (NO_2-C_6H_4-C_2H_3, 27 %), 140 (201 - HOAc - H, 18 %), 119 (149 - NO, 10 %), 103 (C_6H_4 - C_2H_3, 15 %), 98 (140 - CH_2CO, 30 %), 81 (sugar - 2 HOAc, 85 %), 43 (acetyl, 100 %), analysis at 200°C;
NMR: ^{1}H: ($CDCl_3$) δ=2.05 + 2.08 (2 s, 6 H, 2 CH_3), 2.10-2.70 (m, 1 H, H-2'), 3.06-3.56 (m) + 3.19 (t) (3 H, H-2" + CH_2-ar, J= 6.5 Hz), 4.15-4.75 (m, 5 H, 2 H-5' + H-4' + CH_2-O), 5.59 (m, 1 H, H-3'), 6.36 (t, 1 H, H-1', J= 6.7 Hz), 7.48 (s, 2 H, ortho-H NPE, J= 8.8 Hz), 8.13 (d, 2 H, meta-H NPE, J=8.8 Hz), 10.15 (s, 1 H, N-H, exchanged with D_2O) ppm;
^{13}C: ($CDCl_3$) δ =20.5 + 20.6 (2 CH_3), 33.7 (C-2'), 34.8 (CH_2-ar), 63.9 (C-5'), 66.6 (CH_2-O), 74.6 (C-3'), 81.9 (C-4' + C-1'), 100.3 (C-5), 123.4 (2 C_m), 129.9 (2 C_o), 145.0 + 146.8 (C_x + C_p), 150.0 + 153.2 + 153.5 + 158.7 (arom.), 170.3 + 171.4 (2 C:O) ppm.

Anal. Calcd. for $C_{22}H_{23}N_5O_{10}$: C, 51.11; H, 4.48; N, 13.55.
Found: C, 50.73; H, 4.47; N, 13.22.

O^2,O^6-di-(2-[4-nitrophenyl]-ethyl)-2'-deoxyxanthosine, 12

2.005 g (4 mmol) of Ac_2dX^{NPE} (10) were coevaporated twice with dry dioxane and dissolved in 60 ml dry dioxane. To this solution were added under stirring 1.6 g of triphenylphosphine, 1 g of NPE-OH and 1 ml of DEAD (each 6 mmol). The mixture warmed up and coloured violet on addition of the DEAD, and became brown-yellow after a few minutes. TLC (solvent D) showed the formation of a new nucleosidic product with Rf= 0.30. The mixture was evaporated in vacuo after quenching the reaction with water, and the residue was treated overnight with 150 ml NH_3-MeOH at room temperature. The next morning a fraction of the title product was crystallised from the reaction mixture. The filtrate was evaporated and chromatographed on 70 g silica gel with a MeOH gradient in $CHCl_3$. The fractions with Rf=0.34 were crystallised from $CHCl_3$-diethylether, yielding a total of 1.832 g (3.24 mmol, 81 %) of 12.

M.p.: 124-125°C ($CHCl_3$-diethylether); UV: (MeOH): λ_{max}=268 nm (=32750), shoulder at 247 nm; TLC: Rf=0.34 (solvent A);
MS: m/e = (no molecular ion detectable, neither after silylation of the sugar hydroxyl groups), 450 (base + H, 0.2 %), 301 (450 - 149, 1 %), 149 (100 %), 119 (15 %), 103 (50 %), 91 (20 %) + 77 (75 %) (NPE-fragments), 117 (sugar, 5 %);
NMR: ^1H: (DMSO-d_6/D_2O) δ=2.15-2.86 (m, 2 H, 2 H-2'), 3.13-3.37 (m, 4 H, 2 x CH_2-ar), 3.58 (m, 2 H, 2 H-5'), 3.87 (m, 1 H, H-4'), 4.40 (m, 1 H, H-3'), 4.61 + 4.77 (2 x t, 4 H, 2 x CH_2-O), 6.31 (t, 1 H, H-1', J= 6.8 Hz), 7.61 (d, 4 H, ortho-H NPE), 8.16 + 8.17 (2 d, 4 H, meta-H NPE), 8.35 (s, 1 H, H-8) ppm.
^{13}C: (DMSO-d_6) δ =34.5 (2 x CH_2-ar), 39.6 (C-2'), 61.8 (C-5'), 66.6 + 67.2 (2 x CH_2-O), 70.9 (C-3'), 83.8 (C-4'), 88.0 (C-1'), 117.0 (C-5), 123.5 (2 C_m), 130.3 (2 C_o), 140.9 (C-8), 146.4 + 146.6 + 147.0 (2 C_x + 2 C_p), 153.3 (C-4), 160.4 + 160.8 (C-2 + C-6) ppm;
Anal. Calcd for $C_{26}H_{26}N_6O_9$: C, 55.12; H, 4.64; N, 14.84.
Found : C, 54.98; H, 4.75; N, 14.87.

5'-Monomethoxytrityl-O^2,O^6-di-(2-[4-nitrophenyl]-ethyl)-2'-deoxyxanthosine 13

566 mg (1 mmol) of 12 was coevaporated twice with dry pyridine, and dissolved in 15 ml of anhydrous pyridine. Reaction with 370 mg (1.2 mmol) of monomethoxytritylchloride for 3 hours, and workup as for 6, yielded after precipitation in 150 ml hexane 604 mg of the title compound 13 (720 µmol, 72 %).

UV: ($CHCl_3$) λ_{max}=240 and 270 nm; TLC: Rf=0.46 (solvent B);
NMR: ^1H: ($CDCl_3$/D_2O) δ=2.20-2.90 (m, 2 H, H-2'), 3.05-3.55 (m, 6 H, 2 x CH_2-ar + 2 H-5'), 3.75 (s, 3 H, CH_3-O), 4.20-4.83 (m, 6 H, H-4' + H-3' + 2 x CH_2-O), 6.33 (t, H-1', J= 6.6 Hz), 6.74 (d, 2 H, trityl), 7.12-7.58 (m, 16H, trityl + 2 x ortho-H NPE), 7.97 s (1 H, H-8), 8.11 + 8.12 (2 x d. 4 H, meta-H NPE) ppm.

^{13}C: ($CDCl_3$) δ=34.9 (2 x CH_2-ar), 39.3 (C-2'), 55.0 (CH_3-O), 63.5 (C-5'), 66.6 + 67.1 (2 x CH_2-O), 71.2 (C-3'), 84.4 (C-4'), 86.6 (Φ_3-C), 88.7 (C-1'), 116.6 (C-5), 123.5 (4 C_m), 129.7 (4 C_o), 139.4 (C-8), 145.4 + 145.6 + 146.7 (2 C_x + 2 C_p), 152.8 (C-4), 160.4 + 161.1 (C-2 + C-6), + trityl signals (113.0 + 126.9 + 127.7 + 128.1 + 130.1 + 134.8 + 143.7 + 158.5) ppm.

5'-Monomethoxytrityl-3'-(o-chlorophenylphosphate)-O^2,O^6-di-(2-[4-nitrophenyl]-ethyl)-2'-deoxyxyanthosine triethylammonium salt, 14

Phosphorylation of 545 mg (650 μmol) of 13 was carried out as for the protected deoxyinosine monomer.[3] Chromatography on 40 g silica gel and elution with $CHCl_3$-MeOH-TEA (95:4:1) afforded after precipitation from 150 ml hexane-diethylether (8:2) 712 mg (630 μmol, 97 %) of the title product 14.

UV: ($CHCl_3$) λ_{max}=240 and 270 nm; TLC: $CHCl_3$-MeOH-TEA (90:10:2) Rf=0.25; NMR: ^{13}C: ($CDCl_3$) δ=8.3 (3 x CH_3), 34.9 (2 x CH_2-ar), 39.2 br (C-2'), 45.6 (3 x CH_3-CH_2-N^+), 55.0 (CH_3-O), 62.6 + 64.1 (C-5'), 66.5 + 67.2 (2 x CH_2-O), 76.9 + 78.0 (C-3'), 81.5 (C-1'), 83.9 + 85.2 (C-4'), 86.7 (Φ_3-C), 113.0 - 161.0 (aromatic signals) ppm.

Deprotection of the heptamer and enzymatic degradation

Deprotection of the heptamer was basicly done the same way as for the dI heptamer[3]. Times and concentrations are mentioned in the discussion section.

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REFERENCES

1. A. Van Aerschot, B. Peters and H. Vanderhaeghe, VIIth International Round Table, Konstanz, Sept. 29-Oct. 3, 1986.
2. R. Eritja, D. Horowitz, P. Walker, J.P. Ziehler-Martin, M. Boosalis, M. Goodman, K. Itakura and B. Kaplan, Nucleic Acids Res. 14, 8135 (1986).

3. A. Van Aerschot, P. Herdewijn, G. Janssen and H. Vanderhaeghe, Nucleosides & Nucleotides. In press.
4. F. Himmelsbach, B. Schulz, T. Trichtinger, R. Charubala and W. Pfleiderer, Tetrahedron 40, 59 (1984).
5. O. Mitsunobu, Synthesis 1 (1981).
6. H.J. Schaeffer and H.J. Thomas, J. Am. Chem. Soc. 80, 3788 (1958).
7. H. Kessler, C. Griesinger, J. Zarbock and H.R. Loosli, J. Magn. Res. 57, 331 (1984).
8. W. Bremser, Z. Anal. Chem. 286, 1 (1977).
9. W. Bremser, H. Wagner and B. Franke, Org. Magn. Res. 15, 178 (1981).
10. H. Rink, M. Liersch, P. Sieber and F. Meyer, Nucleic Acids Res. 12, 6369 (1984).
11. B. Schulz and W. Pfleiderer, Nucleic Acids Res. Symp. Ser. 16, 157 (1985).
12. M. Gait, H. Matthes, M. Singh, B. Sproat and R. Titmas, Chemical and Enzymatic Synthesis of Gene Fragments, H. Gassen and A. Lang, ed., p. 1 (1982), Verlag Chemie, Weinheim.
13. A. Van Aerschot, P. Herdewijn and H. Vanderhaeghe, Nucleosides and Nucleotides, in press.

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