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FLEXIBLE AGLYCONE RESIDUES IN DUPLEX DNA

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Abstract: Nucleotide analogues with a primary amide function attached by a polymethylene chain to C_1 of 2-deoxy-D-ribofuranose were synthesized and incorporated into short double helices. The thermodynamic stability of these duplexes was studied. A higher melting temperature was observed when a hydrophobic hydrocarbon chain was attached to C_1, instead of the more hydrophilic amide group.

Unusual base pairs are present in natural DNA : some nucleotides are methylated, defects transiently appear, like apurinic / apyrimidinic sites, and mismatches. The structure of relaxed B DNA is little affected by these local alterations, as demonstrated by NMR[1,2,3,19,20] or X-ray crystallography[4,5]. Chemical DNA synthesis allows to further vary the nature of aglycone residues : 5-bromouracil and 2,6-diaminopurine give more stable base pairs than respectively thymine and adenine[6]. Hypoxanthine[7] and N^4-methoxycytosine[8] feature a relative lack of specificity of hydrogen bonding. They are ambiguous nucleosides. The introduction in a single hybridization probe of an ambiguous nucleoside at sites of codon degeneracy is an alternative to the synthesis of mixed-sequence oligonucleotides.

We wished to evaluate compound **4** as an ambiguous nucleoside. The conformational mobility of the polymethylene chain should indeed allow the terminal amide function to hydrogen bond indifferently with A, C, T or G. The flexible aglycone chain of **4** contains four carbons, in order to mimic the N_1-C_6-C_5-C_4 and the N_9-C_4-C_5-C_6 connections of, respectively, pyrimidines and purines. This paper reports the synthesis of short double helices incorporating **4** and the evaluation of their thermodynamic stability.

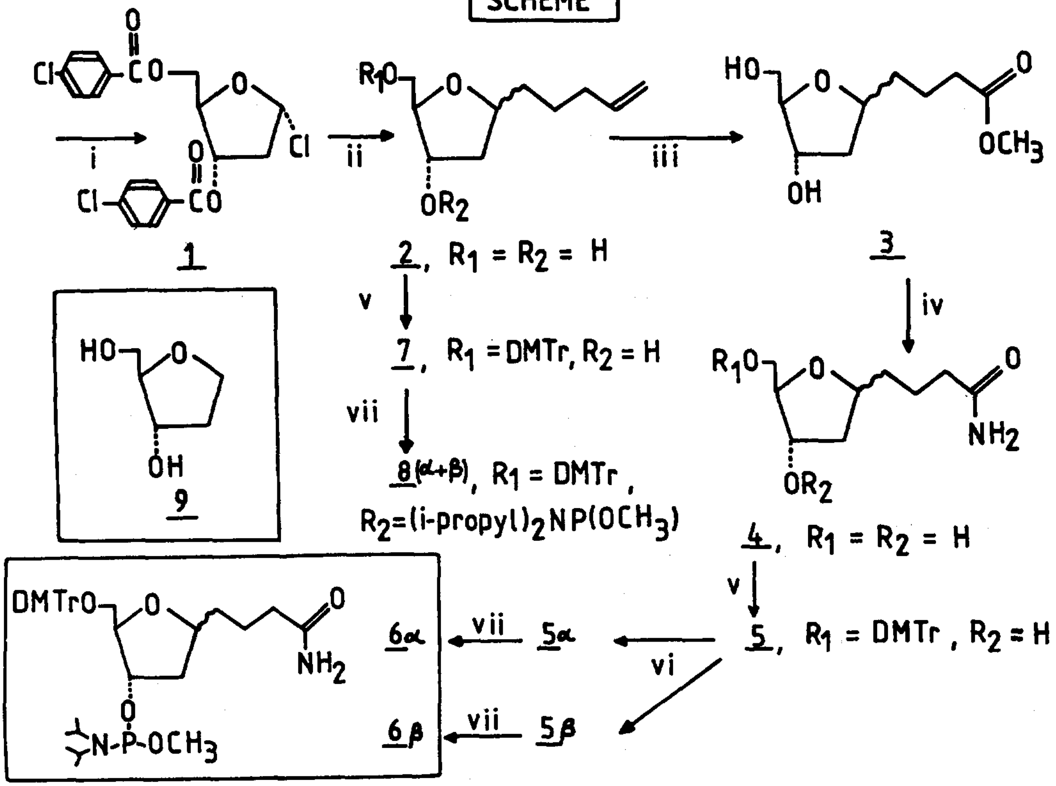
The synthesis of the building blocks required for oligonucleotide synthesis **6α**, **6β**, and the synthesis of **8**(α+β), also introduced in oligomers for comparison, are described in the scheme. Solid α-1-chloro-3,5-di-O-(p-chlorobenzoyl)-2-deoxy-D-ribofuranose **1** (3.9 g, 10 mmoles) was added in one fraction to an excess of 5-pentenylmagnesium bromide (obtained by the iodine catalyzed reaction of magnesium (3.04 g, 125 mmoles) with 5-pentenyl bromide (14 ml, 120 mmoles) magnetically stirred in dry ether (110 ml). After 3 h at RT, the mixture was poured in a slurry of cold (0°c) water-ether mixture (200 ml, 1:1, v/v). The pH was adjusted to neutral by acetic acid. The ethereal phase was discarded, and the aqueous phase was extracted with butanol (100 ml). Evaporation of the butanol, followed by coevaporation with toluene gave **2** as a mixture of diastereoisomers (1.69 g, 90%, oil, α / β = 2:1)[9]. Compound **2** (0.126 g) was dissolved in dry HCl / methanol (50 ml, 1M). The mixture was cooled to -78°c. Dry oxygen was bubbled (10 min), then ozone (till a stable blue color appeared, approx. 20 min), then nitrogen (30 min). The mixture was allowed to warm to about 0°c, when an exothermic reaction took place[10] (an ice bath was used). The residue after evaporation was chromatographed on silica to give **3** (eluent CH_2Cl_2, R_f= 0.22, visualized by using a $KMnO_4$ (0.5 g)/NaOH 1N (100 ml) spray). Yield : 0.105 g (72%, oil). IR (film) : 1730 cm^{-1}, $COOCH_3$. Treatment of **3** with conc. ammonia overnight gave **4** (IR (film) : 1690 cm^{-1}, $CONH_2$), that was dimethoxytritylated as

TABLE

Tmax (°c) of duplexes formed by sequences a-d paired with sequences e-j.
a-d = GTTTTCCCXGTCACGAC, e-j = GTCGTGACYGGGAAAAC
(nd : not determined)

	a, X=A	b, X=G	c, X=C	d, X=T
e, Y=C	48.3	nd	47.1	47.8
f, Y=T	55.4	52.3	47.2	49.5
g, Y=4β	46.4	48.0	43.5	45.2
h, Y=4α	46.3	47.8	44.7	46.3
i, Y= 2(α+β)	49.5	nd	47.9	48.5
j, Y=9	nd	nd	44.8	45.6

SCHEME



i, see ref. 23 ; ii, $CH_2=CH-(CH_2)_3-MgBr$, 12 eqv. ; iii, O_3 in HCl/CH_3OH (1M) ;
iv, conc. NH_3 ; v, DMTrCl, pyridine ; vi, prep. TLC ;
vii, $(\succ N)_2POCH_3$, $(\succ N)_2NH_2^+$  , CH_2Cl_2 .

usual[11] (**3** to **5**, 49% yield). At this stage, both diastereoisomers were separated by prep. TLC, eluting five times successively on the same plate (silica, eluent : CH_2Cl_2/CH_3OH 95:5, v/v + 1% $N(C_2H_5)_3$, **5α** front, **5ß** queue)[12]. The 500 MHz 1D and 2D COSY ^1H NMR spectra of **5α** and **5ß**, as well as those of related C-nucleosides of known stereochemistry were discussed by us elsewhere[13]. Both anomers were phosphitylated (65% yield) according to a known procedure [14] (81 MHz ^{31}P NMR, $CDCl_3$, delta$_{ppm}$, (H_3PO_4), **6α** 145.76 and 146.02; **6ß** 145.50). The anomeric mixture **8**(α+β) (α / β, 2:1) was obtained along the same lines (**2** to **8**, 35% yield. 81 MHz ^{31}P NMR, $CDCl_3$, 145.34 and 145.89).

Oligonucleotides **a-j** were assembled on an OMNIFIT manual DNA synthesizer, using the standard phosphoramidite strategy[11], except that no capping step was used for **g** and **h**, in order to avoid the acylation of the amide function[15]. The oligonucleotides were purified to homogeneity by anion exchange and reversed phase chromatography. Their sequences were checked by the solid-phase supported Maxam-Gilbert method (HYBOND, Amersham). An equimolar mixture of complementary sequences (0.003 mM in ssDNA) was dissolved in sodium cacodylate buffer (0.1 M, pH 7). After heating at 60°c for 5 min, cooling and degasing of the samples, the A_{260} / temperature curves (melting sigmoïds) were recorded. We used T_{max}, the temperature corresponding to the maximum of the first derivative of the melting sigmoïds, as a measure of the duplex stability. The results are reported in the table[16].

For the sake of clarity, the T_{max} may be classified into three categories:

- T_{max} > 50°c : duplex **af** (where all the bases are correctly paired) and **bf**, featuring a stable GT wobble base pair.
- 48°c < T_{max} < 50°c : some mismatched duplexes (**ae**, **df**) and, curiously, **ai** and **di**.
- T_{max} < 48°c : all the other duplexes, including **cj** and **dj** , where there is no aglycone residue at the Y position.

In terms of duplex stability and lack of hydrogen bonding selectivity, **4α** and **4ß** seem not better than **9**. Schneider and Benner recently described duplexes with flexible sugar backbone (glycerol)[17]. They also observed a large destabilization of their double helices. Preorganization, as Cram pointed out in the field of host-guest chemistry[18], is also a central determinant of binding power in DNA.

However, bulk effects like hydrophobicity could perhaps be used to create ambiguous and not destabilizing nucleotide analogues. An intriguing observation of our work is that duplexes **ai**, **ci** and **di** are in fact as stable as those featuring pyrimidine-pyrimidine mismatches, where stacking forces exist[19,20,21]. If the (admitedly weak) stabilization of duplexes incorporating **2**(α+β) instead of **9** or **4** is indeed due to an hydrophobic effect[22], it could be enhanced by lengthening the polymethylene chain. Further work along this line is currently in progress.

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9 500 MHz ^{1}H NMR of the mixture **2α**+**2ß** (DMSO, delta$_{TMS}$, ppm). The ^{1}H 2D COSY spectrum of the mixture was fully compatible with the attribution.

2α, 5.79 (m, $C\underline{H}=CH_2$), 5.04-4.92 (m, $CH=C\underline{H}_2$), 4.02 (m, $C\underline{H}$-OH), 3.87 and 3.57 (2m, $C\underline{H}$-O-$C\underline{H}$), 3.32 (m, $C\underline{H}_2$-OH), 2.17 (ddd, 1H, ring $C\underline{H}_2$(H endo), J_{gem}=13.6 Hz, $J_{vic1}=J_{vic2}$=7Hz), 2.02 (m, $C\underline{H}_2$-$CH=CH_2$), 1.27-1.57 (m, 5H, ring $C\underline{H}_2$(H exo) and $(C\underline{H}_2)_2$).

2ß, 5.79 (m, $C\underline{H}=CH_2$), 5.04-4.92 (m, $CH=C\underline{H}_2$), 4.02 (m, $C\underline{H}$-OH), 3.94 and 3.57 (2m, $C\underline{H}$-OC$\underline{H}$), 3.25 (m, $C\underline{H}_2$-OH), 2.02 (m, $C\underline{H}_2$-$CH=CH_2$), 1.74 (ddd, 1H, ring $C\underline{H}_2$(H exo), J_{gem}=13.6Hz, $J_{vic1}=J_{vic2}$<2Hz), 1.27-1.57 (m, 5H, ring $C\underline{H}_2$(H endo) and $(C\underline{H}_2)_2$).

125 MHz ^{13}C NMR of the mixture **2α**+**2ß** (DMSO, delta$_{TMS}$, ppm):

2α, 138.90 ($\underline{C}H=CH_2$), 114.93 ($CH=\underline{C}H_2$), 85.44 and 77.14 ($\underline{C}$H-O$\underline{C}$H), 71.72 ($\underline{C}$H-OH), 61.99 ($\underline{C}H_2$-OH), 40.00 (ring $\underline{C}H_2$), 35.72, 33.40 and 25.80 ($(\underline{C}H_2)_3$).

2ß, 138.90 ($\underline{C}H=CH_2$), 114.93 ($CH=\underline{C}H_2$), 87.29 and 77.48 ($\underline{C}$H-O-$\underline{C}$H), 72.28 ($\underline{C}$H-OH), 62.69 ($\underline{C}H_2$-OH), 40.00 (ring $\underline{C}H_2$), 34.95, 33.40 and 25.80 ($(\underline{C}H_2)_3$).

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5α, Calcd. for $C_{30}H_{35}O_6N.0.10(CH_2Cl_2)$: C, 70.32; H, 6.90. Found : C, 70.41; H, 7.01.

5ß, Calcd. for $C_{30}H_{35}O_6N.0.19(CH_2Cl_2)$: C, 69.50; H, 6.83. Found : C, 69.22; H, 7.04.

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