DNA Binding Properties of Oligodeoxynucleotides Containing Pyrrolidino *C*-nucleosides

Supporting Information

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Synthesis of oligonucleotides

All oligomers were synthesized on the 1.3 μm scale on a *Pharmacia LKB Gene Assembler Special DNA synthesizer* using standard phosphoramidite chemistry. Deoxy-pseudouridine phosphoramidite was from Glen. The pyrrolidino phosphoramidites were prepared as described in Ref. 5. As starting units, commercially available natural nucleosides bound to CPG-solid support were chosen. The standard synthetic procedure was used. Coupling efficiencies were > 97 % as determined by trityl assay, both with tetrazole (0.4 Min CH_3CN) and *S*-benzylthiotetrazole (0.25 M in CH_3CN) as activator. The oligonucleotides were then cleaved from the solid support and deprotected in conc. aq. ammonia at rt. All strands were purified by ion exchange-HPLC (DEAE-HPLC) and obtained in yields of 17 to 42% (OD^{260nm}) after desalting on *Sep-Pak* cartridges. The purity of the oligomers was confirmed to be > 95 % by RP-HPLC, and their structural integrity, especially the cleavage of the Fmoc- and benzoyl-protecting groups, was proven by negative electrospray mass spectrometry analysis. The difference between the calculated and the measured mass was always lower than 0.05 %. Table 1 summarizes synthesis and analytical data of all modified oligonucleotides **1-6**.

UV-melting curves were carried out on a *Varian Cary 3E* UV/Vis spectrophotometer. Absorbances were monitored at 260 nm and the heating rate was set to 0.5°C/min. A heating-cooling-heating cycle in the temperature range 20-90°C or 0-90°C was applied. The absorbance melting curves were smoothed and the first derivative curves obtained using the *Varian WinUV* software. For temperatures < 20°C, nitrogen was passed through the spectrophotometer to avoid condensation on the cuvettes. To avoid evaporation of the solutions, 6-8 drops of dimethylpolysiloxane were added at the top of the samples in the cell.

Table 1.

TFO	**Yield**[a] *$OD_{260\ nm}$*	**ESI-MS**[b] *m/z found.* *m/z calc.*	**DEAE-HPLC**[c] *method* *t_R [min]*	**RP-HPLC**[d] *method* *t_R [min]*	**Deprotection**[e] *temp. [°C]* *time [h]*
1, X=dpψU	27.8	4409.4 4410.9	DEAE3 23.5	RP2 25.5	rt 18
4, X=dpψU	25.8	4395.6 4395.9	DEAE2 26.4	RP2 17.4	rt 18
5, X=dpψU	27.6	4395.8 4395.9	DEAE2 22.2	RP2 17.3	rt 18
6, X=dpψU	31.3	4350.0 4350.9	DEAE1 24.0	RP1 23.2	rt 40
2, X=dpψU	42.5	4410.6 4410.9	DEAE3 19.2	RP2 19.6	rt 18
3, X=dpψU	22.9	4410.6 4410.9	DEAE3 19.6	RP2 19.8	rt 18
1, X=dpψT	23.2	4424.6 4424.9	DEAE3 21.1	RP2 20.0	rt 18
4, X=dpψT	17.3	4423.5 4423.9	DEAE2 24.6	RP2 19.2	rt 18
5, X=dpψT	22.5	4423.6 4423.9	DEAE2 22.8	RP2 20.1	rt 18
2, X=dpψT	28.3	4424.5 4424.9	DEAE3 21.8	RP2 20.9	rt 18
3, X=dpψT	27.8	4424.6 4424.9	DEAE3 21.7	RP2 21.0	rt 18
1, X=dψU	23.5	4410.3 4411.9	DEAE4 22.4	RP1 27.3	rt 18
4, X=dψU	31.0	4396.5 4397.9	DEAE4 22.3	RP1 28.0	rt 18
5, X=dψU	21.4	4397.6 4397.9	DEAE3 23.6	RP2 22.1	rt 18
2, X=dψU	20.2	4411.9 4411.9	DEAE3 21.2	RP2 22.6	rt 18
3, X=dψU	22.0	4411.6 4411.9	DEAE3 21.7	RP2 22.4	rt 18

[a] after isolation by DEAE-HPLC

[b] the oligonucleotides were dissolved in a 1:1 mixture of MeCN/H_2O, containing 1 % NEt_3

[c] ET 125/4 Nucleogen DEAE 60-7, 125 x 4.0 mm (*Macherey-Nagel*); A = 20 mM KH_2PO_4 in H_2O/MeCN 4:1, pH 6.0; B = 1 M KCl, 20 mM KH_2PO_4 in H_2O/MeCN 4:1, pH 6.0; flow 1 ml/min; detection wavelength: 260 nm; rt
methods: DEAE1: 15-40 % B in 30 min; DEAE2: 22-45 % B in 30 min; DEAE3: 27-50 % B in 30 min; DEAE4: 32-55 % B in 30 min; DEAE5: 40-75 % B in 30 min

[d] Aquapore RP-300, 7 µm, 220 x 4.6 mm (*Brownlee*); A = 0.1 M triethylammonia acetate in H_2O, pH 7.0; B = 0.1 M triethylammonia acetate in H_2O/MeCN 1:4, pH 7.0; flow 1 ml/min; detection wavelength: 260 nm; rt
methods: RP1: 0-20 % B in 30 min; RP2: 5-25 % B in 30 min

[e] 25 % aq. NH_3 soln.

Determination of the pK_a of pyrrolidino *C*-nucleoside 2

To determine the pK_a of the free nucleoside, NMR absorptions of **2** in D_2O at different pH values were measured. For this purpose either DCl (20 % in D_2O) or NaOD (30 % in D_2O) were added to the NMR solvent. The chemical shift of H-C(5) was plotted against the pH (Figure 2).

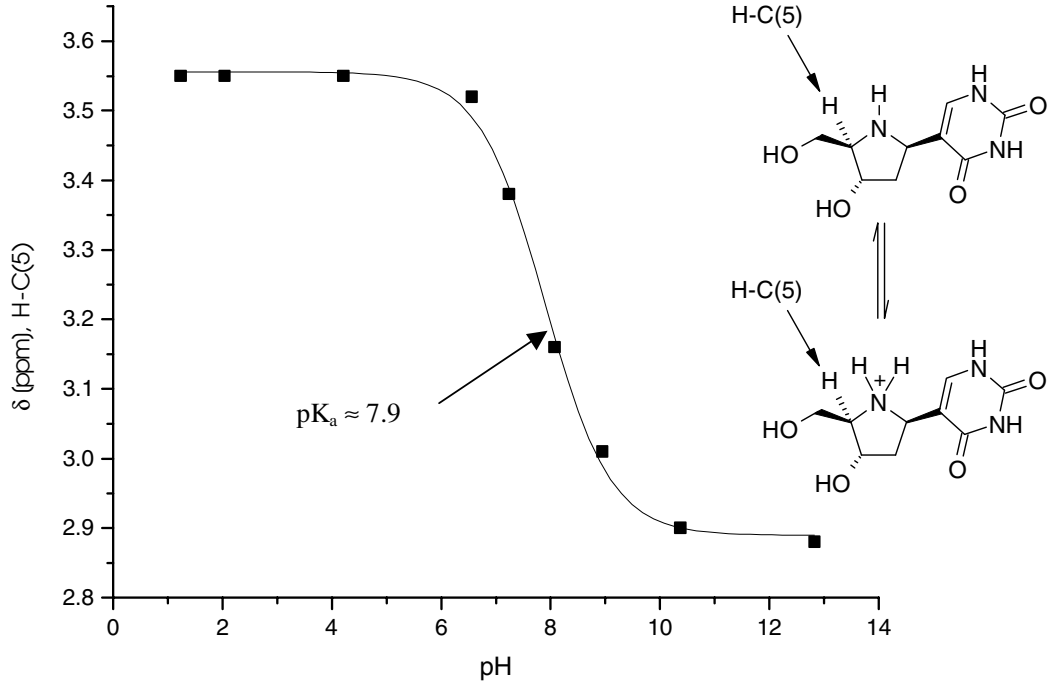

Figure 2. Determination of the pK_a of dpψU. Solvent: D_2O.