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Oligonucleosides with a nucleobase-including backbone; synthesis and self-association of novel dinucleotide analogues

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The synthesis and self-association of protected oxymethylene-bridged UA analogues are described.

We are studying oligonucleotide analogues with a nucleobase-including backbone (Fig. 1, **A**), to determine whether the structural differentiation between nucleobase and backbone in DNA, RNA, and their analogues (Fig. 1, **B**), is a prerequisite for the formation of stable homo- and/or heteroduplexes.[1]†

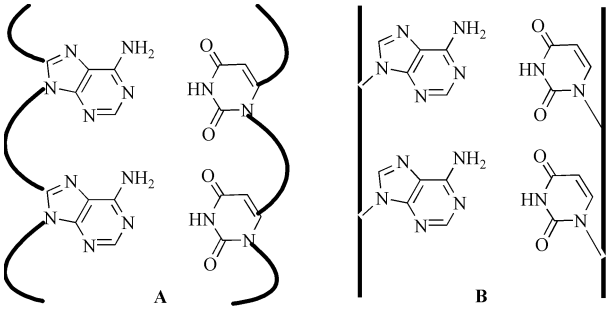


Fig. 1 Schematic representation of (**A**) oligonucleotide analogues with a nucleobase including backbone, and (**B**) oligonucleotides and analogues with a structural differentiation of nucleobase and backbone.

Tetrameric analogues of type **A**, derived from ethynediyl-linked adenosine and uridine, showed no evidence for homo-pairing, and a similar uridine hexamer did not hetero-pair with a complementary RNA strand.[2] Modeling suggested that an *anti* conformation of these analogues is a prerequisite for pairing, whilst NMR analysis of an adenosine dimer showed that a *syn* conformation is preferred.[3] Modeling studies also suggested that oxymethylene-bridged oligomers (Fig. 2), should pair in the *syn* conformation (Watson–Crick type hydrogen bonding), so far only known to occur in *Z*-DNA.[4] We have hence prepared the corresponding self-complementary UA dimer.

2′,3′-*O*-Isopropylideneuridine (**1**) was protected as the TIPS ether **2** (Scheme 1). Deprotonation with LDA,[5] followed by formylation with DMF and reduction with $NaBH_4$[6] gave C(6)-hydroxymethylated **3**. The C(8)-hydroxymethylated adenosine **7** was prepared *via* a similar route from protected adenosine **5**. Treatment of **3** with mesyl chloride gave the chloromethylated **4** (64% from **1**). 4,4′-Dimethoxytritylation of **7** yielded **8**, which was then desilylated to **9** (59% from **5**). The ether **10** (Scheme 2) was prepared by alkylation of alcohol **9** with the chloride **4**; *N*-debenzoylation gave the amine **11**. Detritylation or desilylation of **11** led to the monoalcohols **12** and **13**, respectively, which were further deprotected to give diol **14**. Acid hydrolysis[2] of **14** yielded the oxymethylene dimer **15**.‡

According to the chemical shift for H–C(2′),[7] the protected uridine **2** prefers an *anti* conformation (δH–C(2′) = 4.70 ppm), whilst the C(6)-substituted derivatives **3**, **4** and **10**–**13** prefer a *syn* conformation (δ = 5.19–5.40 ppm). Likewise, the protected adenosine **6** prefers the *anti* conformation (δH–C(2′) = 5.32 ppm), whilst the C(8)-substituted derivatives **7–13** prefer a *syn* conformation (δ = 5.70–6.03 ppm).

The ^{1}H-NMR spectra of **11–13** in $CDCl_3$ are characterised by a concentration dependent downfield shift of the uridine H–N(3), evidencing an intermolecular hydrogen bond. Association constants K_a (Table 1),[8] and the thermodynamic parameters $\Delta H°$ and $\Delta S°$ in $CDCl_3$ were calculated for **11–13** from the δ(H–N(3)) concentration and temperature dependence (Fig. 3).§ The δ(H–N(3)) of diol **14** in $CDCl_3$ was almost concentration independent (12.96–12.65 ppm from 33–1 mM), whilst the fully deprotected dimer **15** was insufficiently soluble to evidence association. Other solvent systems are under investigation. The data for **11** and **12** highlight the contribution of the lipophilic dimethoxytrityl group, but even the K_a value obtained for the

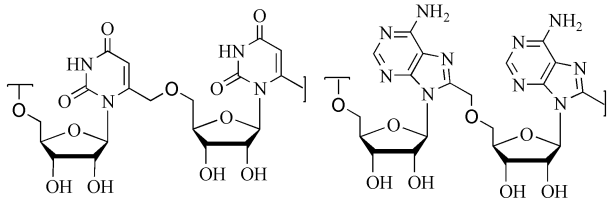
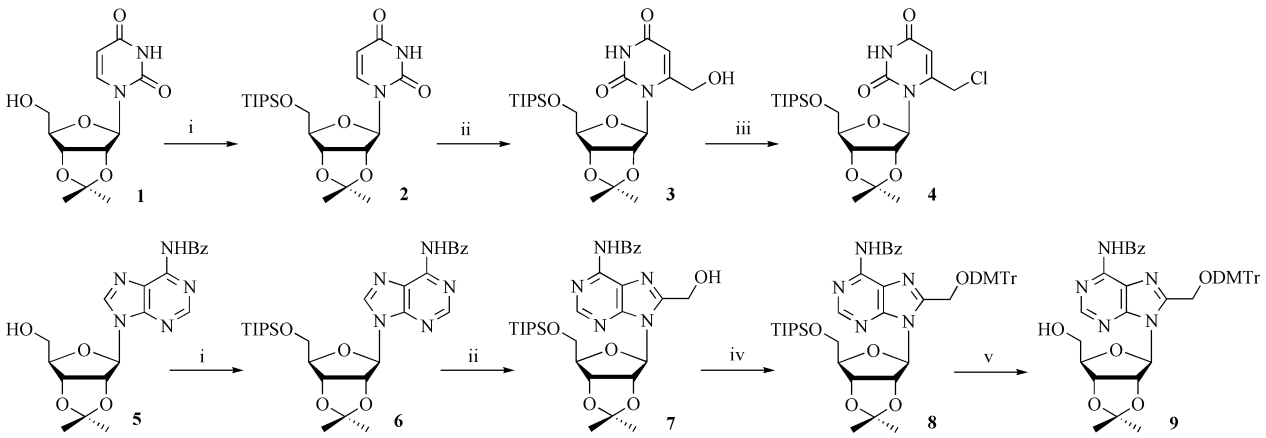


Fig. 2 Oxymethylene bridged oligonucleotide analogues.



Scheme 1 *Reagents and conditions*: i. TIPSCl, imidazole, CH_2Cl_2, rt, 98% (**2**), 96% (**6**); ii. a) LDA (5 eq.), THF, −78 °C; b) DMF, −78 °C to rt; c) AcOH, EtOH, $NaBH_4$, rt, 70% (**3**), 74% (**7**); iii. MsCl, C_5H_5N, 0 °C to rt, 94%; iv. DMTrCl, $EtN(iPr)_2$, CH_2Cl_2, 50 °C, 94%; v. TBAF, THF, rt, 89%.

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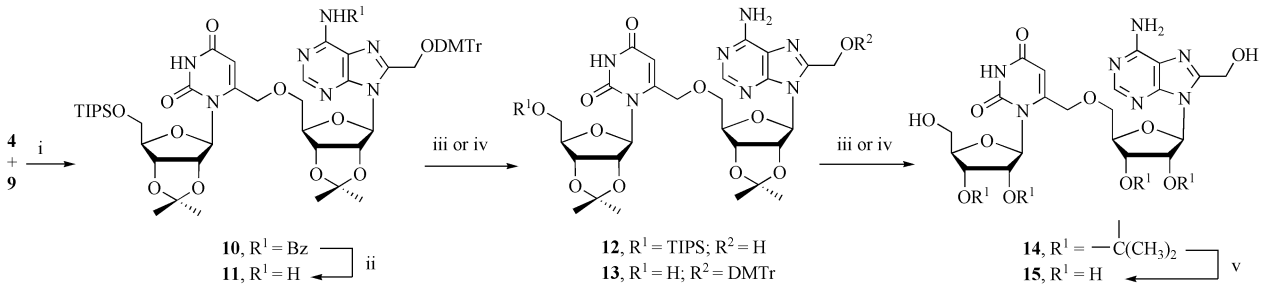

Scheme 2 *Reagents and conditions*: i. NaH, DMF:THF (2:1), 0 °C, 60%; ii. NH_4OH, MeOH, 95%; iii. HCO_2H, $MeNO_2$, rt, 84% (**12**), 82% (**14**); iv. TBAF, THF, rt, 91% (**13**), 85% (**14**); v. HCO_2H, H_2O (8:2), rt, 75%.

Table 1 Association constants and thermodynamic parameters for the dimers **11–13** in $CDCl_3$

	K_a (M^{-1})[a]	$-\Delta H°$ (kcal mol^{-1})	$-\Delta S°$ (e.u.)[b]
11	966	15.8	40.4
12	277	21.8	63.7
13	3222	24.4	64.8

[a] Determined at 22 °C, uncertainty in K_a estimated at 15%. [b] e.u. = entropy units (1 e.u. = 1 cal per (mol.K)).

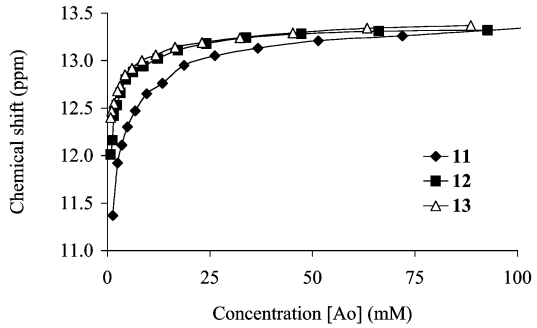

Fig. 3 Concentration dependence of δ(H–N(3)) for dimers **11**, **12** and **13** in $CDCl_3$ at 295 K.

detritylated **12** compares favourably with that determined for 3′,5′-di-*O*-acetyl-2′-deoxyuridine with a 2′-deoxyadenosine derivative (70 M^{-1}).[9] The high K_a value for **13**, and the inability to dissociate **14** appreciably in $CDCl_3$ correlate with a downfield shift of H–O(5′) as compared to **1** ($\Delta\delta \approx 1.0$ ppm), and are rationalised by the formation of a C(5′)O–H···O=C(2) intramolecular hydrogen bond.

Watson–Crick type base pairing is suggested by a cross-peak between the hydrogen bonded imino H–N(3) and the adenine H–C(2) in a 2D-NOESY experiment on associated dimer **11**.[9]

These results support the contention that a structural differentiation of nucleobases and backbone is not required for pairing. We are now investigating the details of base pairing, stacking, and hydrogen bonding.

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Notes and references

† For the sake of simplicity, we have designated these analogues as 'oligonucleotide analogues with a nucleobase-including backbone', while, strictly speaking, these systems do not possess a 'backbone'.

‡ All new compounds showed satisfactory NMR, IR, and MS data. **11**: I = adenosyl unit; II = uridyl unit. ^{1}H-NMR (500 MHz, $CDCl_3$): 13.09 (br s, H–N(3/II)); 8.37 (s, H–C(2/I)); 8.37–7.49 (m, 2 arom. H); 7.47–7.38 (m, 4 arom. H); 7.32–7.21 (m, 3 arom. H); 6.90 (br s, 2 H–N(6/I)); 6.86–6.83 (m, 4 arom. H); 6.22 (d, J = 1.3, H–C(1′/I)); 5.88 (dd, J = 1.3, 6.3, H–C(2′/I)); 5.75 (d, J = 1.0, H–C(1′/II)); 5.36 (s, H–C(5/II)); 5.34 (dd, J = 6.3, 3.8, H–C(3′/I)); 5.27 (dd, J = 1.0, 6.3, H–C(2′/II)); 4.87 (dd, J = 6.3, 4.5, H–C(3′/II)); 4.55, 4.41 (AB, J = 11.8, 2 H–C(10/I)); 4.44, 4.03 (AB, J = 13.3, 2 H–C(7/II)); 4.31 (ddd, J = 3.8, 5.3, 4.9, H–C(4′/I)); 4.15 (ddd, J = 4.4, 5.4, 7.1, H–C(4′/II)); 3.85 (dd, J = 10.5, 5.4, H–C(5′a/II)); 3.83 (dd, J = 10.5, 7.1, H–C(5′b/II)); 3.79 (s, MeO); 3.65 (dd, J = 10.5, 5.3, H–C(5′a/I)); 3.63 (dd, J = 10.5, 4.9, H–C(5′b/I)); 1.55, 1.55, 1.42, 1.41 (4s, Me_2C); 1.01–0.96 (m, $(Me_2CH)_3$–Si). ^{13}C-NMR (75 MHz, $CDCl_3$): 164.07 (s, C(2/II)); 158.50 (s, C(8/I)); 155.61 (s, C(6/I)); 151.91 (d, C(2/I)); 150.74 (s, C(6/II)); 150.24 (s,C(4/II)); 150.10 (s, C(4/I)); 148.81 (s, arom. C); 144.00 (s, arom. C); 135.08 (s, arom. C); 130.02 (d, arom. C); 128.10 (d, arom. CH); 127.81 (d, arom. CH); 126.90 (d, arom. CH); 118.45 (s, C(5/I));113.59 (s, *C*$(Me)_2$/I); 113.26 (s, *C*(Me)2/II); 113.17 (d, arom. CH);103.71 (d, C(5/II)); 91.39 (d, C(1′/II)); 90.00 (d, C(1′/I)); 89.65 (d, C(4′/II)); 87.50 (s, *C*Ar_3);86.74 (d, C(4′/I)); 84.37 (d, C(2′/II)); 83.57 (d, C(2′/I)); 82.26 (d, C(3′/II)); 81.67 (d, C(3′/I)); 69.80 (t, C(5′/I)); 68.08 (t, C(7/II)); 64.50 (t, C(5′/II)); 59.27 (t, C(10/I); 55.23 (q, 2 × MeO); 27.36, 27.36, 25.76, 25.76 (4q, *Me*$_2$C); 17.95 (q, *Me*$_2$CH)$_3$Si); 11.99 (d, Me_2*C*H)$_3$Si). HR-MALDI-MS: 303 (100%, $[DMTr]^+$); 1114.492 (23%, $[M + Na]^+$; calc. 1114.4936). IR ($CHCl_3$): 3488w, 3185w, 2993m, 2943m, 2866m, 2840w, 1712s, 1636m, 1608m, 1509s, 1446m, 1383m, 1157m, 1068s, 1036m, 882m, 831m.

§ NMR was performed at 295 K on a Varian Gemini300 spectrometer (300 MHz) in $CDCl_3$ passed through aluminium oxide immediately prior to use. Experiments started at the highest concentration, with stepwise replacement of 0.2 ml of the 0.7 ml solution with 0.2 ml pure $CDCl_3$. The data were analysed graphically and by nonlinear least-squares fitting.[8]

Thermodynamic parameters were determined by van't Hoff analysis. The uridyl δH–N(3) was monitored between 50 and −30 °C at a fixed concentration (between 20–80% of saturation). Linear fits of data collected below 0 °C were poor.

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