Synthesis and pairing properties of oligoribonucleotide analogues containing a metal-binding site attached to \( \beta \)-D-allofuranosyl cytosine

Xiaolin Wu and Stefan Pitsch*

Organisch-Chemisches Laboratorium der Eidgenössischen Technischen Hochschule, Universitätstrasse 16, CH-8092 Zürich, Switzerland

Received July 22, 1998; Revised and Accepted August 18, 1998

ABSTRACT

A method for the facile preparation of oligoribonucleotide analogues containing \( \beta \)-D-allofuranosyl nucleosides with additional functional groups tethered to the 6′-O positions is presented. It is based on the synthesis of two protected nucleosides carrying a 6′-O-bromopentyl and a 6′-O-methyaminopentyl substituent. By a simple two-step procedure, these key intermediates were transformed into two phosphoramidites carrying a 1-aza-18-crown-6 and a triethylenglycol group, respectively, each capable of complexing metal ions. By automated synthesis, these functionalized nucleoside analogues were efficiently incorporated into short oligoribonucleotides. Under physiological conditions (150 mM NaCl, 2 mM MgCl\(_2\), pH 7.4), incorporation of a single allofuranosyl cytosine substituted with a triethyleneglycol moiety led to a significant enthalpic stabilization of an A-type RNA duplex. This observation is in agreement with a metal ion-mediated stabilizing interaction between the two pairing strands.

INTRODUCTION

Modified and functionalized oligonucleotides play an important role as molecular tools and potential antisense drugs (1). Furthermore, there exists an as yet unexploited potential for catalysis of chemical reactions with tailored ribozymes containing additional side chains (2). So far, oligoribonucleotides have been functionalized at the nucleobases, at the 5′- or 3′-termini, at the phosphodiester linkages or at the 2′-O position (for recent examples see 3–6).

During our ongoing investigations of the properties of hexofuranosyl oligonucleotides we developed a method for the synthesis of oligoribonucleotides containing 6′-O-substituted \( \beta \)-D-allofuranosyl and \( \beta \)-L-talofuranosyl nucleosides, which can be regarded as C(5′)-substituted ribonucleosides. Preliminary melting curve studies revealed that single incorporations of \( \beta \)-allofuranosyl nucleosides in an A-type RNA duplex did not significantly change the pairing properties (relative to those of the parent duplex), whereas incorporation of \( \beta \)-allofuranosyl nucleosides resulted in substantial weakening of the duplex (7; X.Wu, unpublished results).

Here we present a synthesis of the two \( \beta \)-D-allofuranosyl cytosine building blocks 9 and 13, containing all protecting groups required for automated synthesis and a 6′-O-bromopentyl (electrophilic) or a 6′-O-methyaminopentyl (nucleophilic) substituent, respectively. The 2′-O positions were protected with the Pr\(_4\)SiOCH\(_2\) (TOM) group, which we recently introduced for the chemical synthesis of oligoribonucleotides under standard DNA coupling conditions (8; S.Pitsch, X.Wu, P.A.Weiss, S.Vonhoff and L.Jenny, in preparation).

The two reactive building blocks 9 and 13 potentially serve as starting materials for a variety of functionalized oligoribonucleotide analogues, allowing the straightforward introduction of different side chains at a very late stage of monomer synthesis. As first examples, we functionalized them with two metal chelating moieties and transformed them into phosphoramidites 15 and 17. Preliminary molecular model studies indicated the possibility of an interaction between a tethered metal complex (covalently bound to the 6′-O position of an alfofuransyl nucleoside) and the phosphodiester backbone of an unmodified partner strand across the major groove. When an appropriate alignment is realized, such an interaction can potentially stabilize the duplex electrostatically or catalyse a specific strand scission reaction by providing a correctly positioned Lewis acid. Triethyleneglycol and 1-aza-18-crown-6 were chosen as ligands for their known ability to form complexes with the biologically abundant metal ions Na\(^+\), K\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) (9,10).

RESULTS

Synthesis of monomers

For synthesis of the key intermediates 9 and 13 we first prepared the appropriately pre-functionalized sugar building blocks 3 and 4 which allowed an efficient, stepwise introduction of the nucleobase, the dimethoxytrityl group and the 2′-O protecting group (Scheme 1).

Selective alkylation of the primary hydroxy group in diol 1 (11) with 1,5-dibromopentane gave bromide 2 in good yields. This reaction was accomplished by first forming the cyclic dibutyl tin-derivative, followed by alkylation in the presence of tetrabutylammonium iodide and caesium fluoride according to Nagashima and Ohno (12). The common precursor 2 could be elaborated by a series of reactions into sugar building blocks 3 and 4 without intermediate purification. The bromopentyl sugar 3 was obtained by

*To whom correspondence should be addressed. Tel: +41 1 632 4481; Fax: +41 1 632 1136; Email: pitsch@xeragon.com
silylation of 2 with trisopropylsilyl triflate, cleavage of the ketal group with 50% trifluoroacetic acid and dibenzoylation with benzoyl chloride. The N-allyloxy carbonyl-protected methy lamino-pentyl sugar 4 was obtained by silylation of 2 with trisopropylsilyl triflate, substitution of bromide (and cleavage of the 3-O-benzoyl group) with methy lamine, selective N-acylation with allyl chloroformate, cleavage of the ketal group and perbenzoylation. Nucleosidation of 3 and 4 was achieved under Vorbrüggen conditions (13) with \textit{in situ} trimethylsilylated N4-benzoylcytosine using trimethylsilyl triflate (with 3 and 4) or SnCl4 (with 3) as Lewis acid. Without isolation of the nucleosidation products the Pr3Si groups were removed with a mixture of aqueous HF and HCl in MeCN and nucleosides 5 and 6 were isolated in good yields. From these the dimethoxytritylated diols 7 and 8 were obtained in excellent yields by treatment with dimethoxytrityl chloride in the presence of collidine and AgNO3 according to Hakimelahi et al. (14), directly followed by O-debenzoylation.

Regiosel ective introduction of the Pr3SiOCH2Cl (TOM) group at the 2′-O positions of diols 7 and 8 was carried out under conditions developed in our laboratory (11; S.Pitsch, X.Wu, P.A.Weiss, S.Vonhoff and L.Jenny, in preparation) and gave compounds 9 and 11 in satisfactory yields. The 3′-O-alkylated

**Scheme 1.** Reagents and conditions. (a) (i) Bu2SnO, toluene, reflux, (ii) Br(CH2)5Br, CsF, Bu4NI, DMF, room temperature. (b) For 3: (i) Pr3Si–OTf, Et(Pri)2N, CH2Cl2, room temperature, (ii) CF3COOH, H2O, room temperature, (iii) BzCl, DMAP, py, CH2Cl2, room temperature; for 4: (i) Pr3Si–OTf, Et(Pri)2N, CH2Cl2, room temperature, (ii) MeNH2, EtOH, room temperature, (iii) AllOC(O)Cl, Et(Pri)2N, CH2Cl2, room temperature, (iv) CF3COOH, H2O, room temperature, (v) BzCl, DMAP, py, CH2Cl2, room temperature. (c) (i) Bis(trimethylsilyl)acetamide, N4–benzoylcytosine, MeCN, 70°C, then Me3Si–OTf, (ii) HF, HCl, MeCN, room temperature; (d) (i) DMT–Cl, AgNO3, sym–collidine, CH2Cl2, room temperature, (ii) NaOH, THF/MeOH/H2O, 4°C. (e) Bu2SnCl2, Pr3Si–OCH2Cl, Et(Pri)2N, (CH2Cl)2, 70°C. (f) Pd(PPh3)4, Et2NH, PPh3, CH2Cl2, room temperature.

**Scheme 2.** Reagents and conditions. (a) 1-aza-18-crown-6, Bu4NI, Et(Pri)2N, EtOH, 75°C. (b) (2-cyanoethyl)(N,N-diisopropylamino)-chlorophosphite, Et(Pri)2N, CH2Cl2, room temperature. (c) Me(OCH2CH2)3Cl, Bu4NI, Et(Pri)2N, toluene, 95°C.
regioisomers 10 and 12 were isolated as minor products. Both pairs of regioisomers were unambiguously identified by their 1H NMR spectra according to Pitsch (11). From 11 the free methyl aminopentyl nucleoside 13 was obtained according to Hayakawa et al. (15).

**Functionalization**

Reaction of the bromopentyl-substituted nucleoside 9 and 1-aza-6-crown-18 led to the corresponding crown ether-substituted nucleoside. During this reaction partial loss of the benzoyl base-protecting group was observed. Therefore, it was completely removed with ammonia, protected again with Ac₂O and isolated as the N₄-acetylcytosine derivative 14 in fair yield. Under standard conditions, it was finally transformed into phosphoramidite 15 (Scheme 2).

Reaction of the methyl aminopentyl nucleoside 13 and diethylene glycol monomethyl monochloroethyl diether CH₃(OCH₂CH₂)₃Cl led to the corresponding triethylene glycol-substituted nucleoside 16, which was finally transformed into phosphoramidite 17 (Scheme 2).

**Synthesis of oligonucleotides**

For our initial hybridization studies we designed a non-self-complementary tetradecamer RNA sequence in which we incorporated phosphoramidites 15 and 17 at two different positions, one near the 3'- and one near the 5'-end (Table 1 and Fig. 2). The syntheses were carried out on a 1.5 µmol scale using the conditions in Table 1. Phosphoramidites 15 and 17 were efficiently incorporated (coupling yield >98%) using twice the coupling time required for standard, TOM-protected phosphoramidites (Fig. 1). The removal of base and phosphate protecting groups and cleavage from the solid support was carried out with 10 M MeNH₂ in EtOH/H₂O 1:1 at 25°C for 2 h. After evaporation, complete removal of all TOM protecting groups was achieved with 1 M Bu₄NF·3H₂O in THF at 25°C for 12 h. After work-up and desalting on Sephadex G-10, the sequences were purified by reversed phase HPLC and characterized by MALDI-TOF mass spectrometry according to Pieles et al. (16) (Table 1).

**Pairing properties**

Figure 2 illustrates the position of the metal-binding sites within duplexes formed by the functionalized tetradecamers C–F and the corresponding complementary sequence A. When the modified nucleosides are near the 3'-end of the sequence (Fig. 2a), they were located in the center of the duplex and the tethered functional groups could reach over the major groove to interact with the backbone of the other strand (duplexes CA and EA, which are internally functionalized). When the modified nucleosides are near the 5'-end of the sequence (Fig. 2b), the functional groups were located outside the duplex and not able to reach the other strand (duplexes DA and FA, which are externally functionalized).

We expected that an eventual positive interaction between the two pairing strands would lead to stabilization of the internally functionalized duplexes, but not of the externally functionalized ones. Therefore, the latter were prepared and investigated as...
were determined in 150 mM NaCl + 2 mM MgCl₂, varying the NaCl + MgCl₂. The results in Table 2 were obtained from pH 7.4 in the presence of 150 mM NaCl, 150 mM KCl and 150 mM were therefore performed under physiological conditions.

The thermodynamic stability of each duplex was determined at pH 7.4 in the presence of 150 mM NaCl, 150 mM KCl or 150 mM NaCl + 2 mM MgCl₂. In the second set, transition temperatures were determined at pH 7.4, varying the concentrations of NaCl, KCl and NaCl + MgCl₂. In the first set, the transition temperatures of all duplexes were determined in 10 mM Tris–HCl (pH 7.4) and 150 mM NaCl, 150 mM KCl or 150 mM NaCl + 2 mM MgCl₂. The thermodynamic data for the crown ether-containing RNA duplexes in KCl and NaCl solution the difference in ΔG° values is about −3 kcal/mol in favour of the internally functionalized duplex B·A, whereas in NaCl both sequences displayed about the same ΔG° value. In the presence of 2 mM MgCl₂, however, the duplex C·A displayed a stronger pairing than the parent duplex B·A and than the internally functionalized duplex C·A (relative to the parent duplex B·A). The more negative ΔG° value of duplex formation for the functionalized duplex C·A (relative to the parent duplex B·A) is the consequence of a more favourable ΔH° term, which below 50°C compensates for the less favourable ΔS° term (Fig. 3).

The ΔG° values of duplex formation at 37°C obtained from pairing of the triethylene glycol-substituted oligoribonucleotides C·A, C·E, and F·A indicate a stronger association of sequence E compared with sequence F in all three environments investigated. In KCl and NaCl solution the difference in ΔG° values was about −3 kcal/mol in favour of the internally functionalized duplex C·A. In the presence of 2 mM MgCl₂, however, a very large energy difference of −8 kcal/mol (35% of total at 37°C), again in favour of sequence E, was observed. The externally functionalized duplex C·A uniformly displayed a weaker pairing than the unmodified duplex B·A, whereas the internally functionalized duplex E·A showed an equal pairing in KCl (−0.3 kcal/mol), a slightly stronger pairing in NaCl (−2.3 kcal/mol) and a much stronger pairing in NaCl + MgCl₂ (−6.5 kcal/mol) than the unmodified duplex B·A. Again, the strong stabilization of the functionalized duplex C·A (relative to the unfunctionalized duplex B·A) is a consequence of a more favourable ΔH° term which below 65°C compensates for the less favourable ΔS° term (Fig. 3).

**DISCUSSION**

These thermodynamic data for duplex stability indicate that in the presence of Mg²⁺ ions pairing is slightly stabilized by a 1-aza-18-crown-6 group and strongly stabilized by a triethylene glycol group tethered to the 6'-O position of an allofuranosyl

**Table 2.** Data obtained from measurements in 10 mM Tris–HCl (pH 7.4) and 150 mM NaCl, 150 mM KCl or 150 mM NaCl + 2 mM MgCl₂

<table>
<thead>
<tr>
<th>Duplex</th>
<th>Conditions</th>
<th>Tm (1+1 mM) (°C)</th>
<th>ΔH° (kcal/mol)</th>
<th>ΔTS° (kcal/mol)</th>
<th>ΔG° (kcal/mol)</th>
<th>ΔG° (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B·A</td>
<td>NaCl</td>
<td>67.5</td>
<td>−123.1</td>
<td>−103.3</td>
<td>−19.8</td>
<td>−19.8</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>64.2</td>
<td>−140.9</td>
<td>−119.8</td>
<td>−21.1</td>
<td>−21.1</td>
</tr>
<tr>
<td></td>
<td>NaCl + MgCl₂</td>
<td>70.8</td>
<td>−162.1</td>
<td>−136.8</td>
<td>−25.3</td>
<td>−25.3</td>
</tr>
<tr>
<td>C·A</td>
<td>NaCl</td>
<td>63.8</td>
<td>−131.9</td>
<td>−112.0</td>
<td>−19.9</td>
<td>−19.9</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>61.3</td>
<td>−133.3</td>
<td>−113.5</td>
<td>−19.8</td>
<td>+1.3</td>
</tr>
<tr>
<td></td>
<td>NaCl + MgCl₂</td>
<td>67.9</td>
<td>−193.1</td>
<td>−166.2</td>
<td>−26.9</td>
<td>−1.6</td>
</tr>
<tr>
<td>D·A</td>
<td>NaCl</td>
<td>64.2</td>
<td>−146.6</td>
<td>−125.4</td>
<td>−21.2</td>
<td>−1.4</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>61.3</td>
<td>−138.7</td>
<td>−118.3</td>
<td>−20.4</td>
<td>+0.7</td>
</tr>
<tr>
<td></td>
<td>NaCl + MgCl₂</td>
<td>67.1</td>
<td>−151.8</td>
<td>−128.9</td>
<td>−22.9</td>
<td>+2.4</td>
</tr>
<tr>
<td>E·A</td>
<td>NaCl</td>
<td>63.4</td>
<td>−163.7</td>
<td>−141.6</td>
<td>−22.1</td>
<td>−2.3</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>61.5</td>
<td>−163.2</td>
<td>−141.8</td>
<td>−21.4</td>
<td>−0.3</td>
</tr>
<tr>
<td></td>
<td>NaCl + MgCl₂</td>
<td>68.8</td>
<td>−240.1</td>
<td>−208.3</td>
<td>−31.8</td>
<td>−6.5</td>
</tr>
<tr>
<td>F·A</td>
<td>NaCl</td>
<td>63.0</td>
<td>−127.1</td>
<td>−107.8</td>
<td>−19.3</td>
<td>+0.5</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>61.2</td>
<td>−120.1</td>
<td>−101.8</td>
<td>−18.3</td>
<td>+2.8</td>
</tr>
<tr>
<td></td>
<td>NaCl + MgCl₂</td>
<td>68.2</td>
<td>−156.2</td>
<td>−132.6</td>
<td>−23.6</td>
<td>+1.7</td>
</tr>
</tbody>
</table>

The thermodynamic data were extracted from concentration-dependent transition curves according to Marky and Breslauer (17). The ΔG° values are relative to the change in free energy of the parent duplex B·A.
A comparison of the temperature dependance of ΔG° values among the parent duplex B·A, the crown ether-substituted duplexes C·A and D·A (a) and the triethyleneglycol-substituted duplexes E·A and F·A (b). Data were obtained from concentration-dependant transition curves measured in 150 mM NaCl + 2 mM MgCl₂, 10 mM Tris–HCl (pH 7.4).

In the absence of Mg⁡²⁺ ions only the triethyleneglycol-substituted duplex is slightly stabilized.

Structurally, these observations indicate a specific interaction of the Mg⁡²⁺-complexed ligand of the modified nucleosides with the negatively charged backbone of the other strand. The open chain ligand present in oligonucleotide E forms a relatively weak complex with the Mg⁡²⁺ ion, still offering additional coordination sites. It was concluded that the strong duplex stabilization observed results from formation of a complex between the ethyleneglycol moiety, a Mg⁡²⁺ ion and a phosphodiester group of the partner strand A. Thereby, one hydrated Mg⁡²⁺ ion within the major groove is replaced by a chelated Mg⁡²⁺ ion (Fig. 4). The cyclic ligand present in oligonucleotide C forms a very strong complex with the Mg⁡²⁺ ion and no additional coordination to the phosphodiester backbone is possible. The weak duplex stabilization observed is concluded to be the result of an electrostatic interaction between the positively charged Mg⁡²⁺ complex and the negatively charged phosphodiester backbone of the partner strand.

The additional non-covalent intramolecular interactions were reflected in the enthalpic stabilization of duplexes C·A and E·A. On the other hand, the conformational changes within the tethered group and/or the backbone required for such an interaction led to an entropic destabilization, which compensated largely, but at low temperature not entirely, for the enthalpic stabilization. In all cases where no stabilization of the duplex could be observed no significant differences in enthalpy and entropy terms were measured, indicating that the additional functional group was pointing into the solution.

We were unable to detect any structural changes upon introduction of the functionalized allofuranosyl cytidines into RNA strands by CD spectroscopy. All duplexes essentially had the same CD spectrum, typical for an A-type RNA duplex.

CONCLUSION AND OUTLOOK

The strategy presented here allows a straightforward preparation of a variety of functionalized oligonucleotides and revealed the 5'-position of nucleosides as a new and promising site for modification, labelling and conjugate formation. The metal-binding derivatives which have been prepared as first examples constitute a new principle for stabilizing oligonucleotide duplexes. We now are preparing reactive allofuranosyl and 2'-deoxyallofuranosyl nucleosides with the other three nucleobases and with different tethers. We will also determine whether 5'-triphosphates derived from those can be incorporated enzymatically into DNA or RNA. Employing nucleotide analogues related to those presented in this paper, we are trying to find oligoribo- and oligodeoxyribonucleotide analogues eventually capable of stabilizing RNA and/or DNA structure, catalysing specific strand scission reactions and enhancing cellular uptake.

MATERIALS AND METHODS

General

Work-up implies distribution of the reaction mixture between CH₂Cl₂ and saturated aqueous NaHCO₃ solution, drying of the organic layer with MgSO₄, filtration and evaporation of the filtrate. TLC: unless otherwise mentioned, precoated silica gel plates from Macherey & Nagel (exceptionally pre-coated Al₂O₃ plates from Merck), stained by dipping into a solution of 10 ml anisaldehyde, 10 ml concentrated H₂SO₄, 2 ml AcOH in 180 ml EOH and subsequent heating with a heat gun. Column chromatography (CC): unless otherwise mentioned, silica gel 60 (230–400 mesh) from Fluka (exceptionally Al₂O₃, activity III, from ICN Adsorbentien).
Optical rotation (α) Jasco-DIP-370, all measurements in CHCl₃ (1 g/100 mL). UV spectra: Uvikon 931, λmax in nm, ε (dm³/mol/cm) indicated in parentheses, all measurements in MeOH. NMR: Varian-Gemini 300 (1H, 300 MHz; 31P, 121 MHz), chemical shift δ in p.p.m. (MeSi as internal standard), δp in p.p.m. (85% H₃PO₄ as external standard), all measurements in CDCl₃, coupling constants J in Hz. MS: VG-ZAB2-SEQ, all samples measured in FAB⁺ mode, 3-nitrobenzyl alcohol as matrix, relative intensity in % as indicated in parentheses.

Oligonucleotide synthesis

The oligoribonucleotides were assembled on CPG supports (1.5 μmol scale) on a Pharmacia Gene Assembler using the methods in Table 1. The TOM-protected phosphoramidites and solid supports were purchased from Xeragon AG (Switzerland). Average coupling yields were >99% (detritylation assay). Deprotection was carried out as described in the text. The crude product was purified by reversed phase HPLC and finally desalted according to Pitsch (11).

Thermal denaturation studies

Absorbance versus temperature profiles were recorded in fused quartz cuvettes at 260 nm on a Cary Bio-1 spectrophotometer equipped with a Peltier temperature control device. The samples were prepared under sterile conditions from stock solutions of the oligonucleotide, 1 M Tris–HCl buffer (pH 7.4) and 5 M NaCl were prepared under sterile conditions from stock solutions of the samples measured in FAB⁺ mode, 3-nitrobenzyl alcohol as matrix and subsequently degassed. A layer of silicon oil was placed on the surface of the solution. The studies were carried out at 0.5, 1, 2, 4 and 8 μM concentrations of both strands. Prior to the measurements, each sample was briefly heated to 80°C. The curves were obtained with both a cooling and heating ramp of 0.5°C/min.

The transition temperatures were obtained after differentiation of the melting curves and analysed according to Marky and Breslauer (17).

3-O-Benzoyl-6-O-(5-bromopentyl)-1,2-O-isopropyliden-α-β-allofuranose (2)

In a Dean-Stark apparatus, a solution of 3-O-benzoyl-1,2-O-isopropyliden-α-β-allofuranose (1) (11) (9.72 g, 30 mmol) and Bu₂SnO (11.2 g, 33 mmol) in toluene (75 ml) was refluxed for 1 h. The toluene was evaporated, the residue diluted with DMF (75 ml) and treated with CsF (6.84 g, 45 mmol), 1,5-dibromopentane (163.2 g, 120 mmol) and Bu₃NF (16.62 g, 45 mmol). The suspension was kept at room temperature for 1 h. Work-up and CC (hexane/EtOAc 9:1–7:3) gave 3αβ/β (4.7 g, 54%, αβ/β 1:2 by NMR) as a colourless oil. TLC (hexane/EtOAc 4:1) 0.66; δq 0.66–1.15 (21 H, m, Pr₃Si), 1.33–1.53 (m, 2 CH₂), 1.65–1.82 (m, CH₂), 3.21–3.24 (m, H-6), 3.34–3.46 (3 H, m, H-6, H-2), 5.30–5.62 (m, CH₂), 4.30 [m, H–C(5)], 4.47 [dd, J = 4.4, 5.6, H-4(α)], 4.97 [m, H-3(β)], 5.60 [dd, J = 4.8, 5.6, H-2(β)], 5.90 [dd, J = 2.2, 5.3, H-3(β)], 6.03 [dd, J = 5.3, 5.5, H-3(α)], 6.09 [dd, J = 1.9, 6.5, H-2(α)], 6.61 [dd, J = 2.2, 6-H(α)], 6.87 [dd, J = 4.4, 6-H(β)], 7.17–7.61 (m, 9 ArH), 7.76–8.10 (m, 6 ArH); m/z 801 (6, MH⁺).

N⁴-Benzoyl-1′-6′-O-(5-bromomethyl)-2′,3′-di-O-benzoyl-β-β-allofuranosylcytosine (5)

A suspension of 3αβ/β (5.4 g, 6.75 mmol), N⁴-benzoylcytosine (18) (1.6 g, 7.4 mmol) and bis(trimethylsilyl)acetamide (4.2 ml, 16.9 mmol) in CH₂CN (27 ml) was stirred at 70°C for 1 h, treated with SnCl₄ (3.2 ml, 27 mmol) and stirred at 70°C for 20 min. After work-up, the residue was dissolved in CH₂CN (200 ml), treated with HCl (conc.) (2 ml) and HF (40% in H₂O) (4 ml) and stirred at room temperature for 8 h. Work-up and CC (hexane/ EtOAc 8:2–4:6) gave 5 (3.6 g, 71%) as a white foam. TLC (hexane/EtOAc 2:8) 0.55; δq 0.57–97.2; λmax 261 (21 000), 229 (28 900); δq 1.44–1.64 (4 H, m, 2 CH₂), 1.75–1.90 (m, CH₂), 3.37 (t, J = 6.8, CH₂), 3.48–3.53 (m, 2 H-6’), 3.65–3.67 (3 H, m, OCH₂, OH-5’), 4.30 (m, H-5’), 4.45–4.49 (m, H–C(4’)), 5.88 (dd, J = 5.6, 6.6, H-2’), 5.99 (dd, J = 2.5, 5.6, H-3’), 6.58 (d, J = 6.8, H-1’), 7.26–8.03 (m, 15 ArH, H-5’), 8.35 (d, J = 7.8, H-6), 8.77 (s, NH); m/z 736 (11, MH⁺).

N⁴-Benzoyl-1′-6′-O-(5-bromomethyl)-5′-O-(4,4′-dimethoxytrityl)-β-β-allofuranosylcytosine (7)

A suspension of 5 (2.7 g, 3.6 mmol), AgNO₃ (612 mg, 3.6 mmol) and syn-collidine (1.2 ml, 9 mmol) in CH₂Cl₂ (12 ml) was treated with 4,4′-dimethoxytrityl chloride (1.84 g, 5.4 mmol) for 1 h at room temperature. After filtration and evaporation, the residue was dissolved in an ice-cold solution of THF/MEOH/H₂O 5:4:1 (150 ml), treated with 10 N aqueous NaOH (3 ml) at 4°C for 15 min, then neutralized with AcOH (1.9 ml) and concentrated to 40 ml. Work-up and CC [CH₂Cl₂/CH₂Cl₂/MeOH 97.3 (+2% NEt₃)] gave 7 (2.46 g, 84%) as a colourless foam. TLC (MeOH/CH₂Cl₂ 8:2:9) 0.50; δq 0.57 [28.4; δmax 262 (14 700), 238 (22 300); δq 1.44–1.63 (m, 2 CH₂), 1.80–1.92 (m, CH₂), 3.19–3.27 (m, OCH₂, H-6’), 3.42 (t, J = 6.5, CH₂), 3.40–3.45 (m, H-6’), 3.55–3.64 (m, H-5’, OH), 3.80 (s, 2 OMe), 4.21–4.27 (m, H-2’, H-4’), 4.44 (s, OH), 4.67 (dd, J = 4.4, 5.6, H-5’, 5.86 (d, J = 4.4, H-1’), 7.22–7.63 (m, 12 ArH, H-5’), 7.88–7.89 (m, 2 ArH), 7.92 (d, J = 7.5, H-6), 8.20 (s, NH); m/z 829 (18, MH⁺), 303 (100).

N⁴-Benzoyl-1′-6′-O-(5-bromomethyl)-5′-O-(4,4′-dimethoxytrityl)-2′-O-[(triisopropylsilyloxy)methyl]-β-β-allofuranosylcytosine (9)

A solution of 7 (2.46 g, 3.0 mmol) and Pr₂NEt (2.1 ml, 12 mmol) in CICH₂CH₂Cl (10 ml) was treated with Bu₃SnCl₂ (1.1 g,
mixtures of diastereoisomers). TLC (Al₂O₃, MeOH/CH₂Cl₂ 4:96) 0.52; λ<sub>max</sub> 239 (23 600); δ<sub>H</sub> 1.00–1.07 (21 H, m, Pr₃Si), 1.13–1.21 (m, 4 Me), 1.32–1.36 (m, 3 CH₂), 2.21 (s, MeCO), 2.42–2.44 (m, CH₂), 2.54 and 2.61 (21, J = 6.5, CH₂), 2.73 (t, J = 5.8, CH₂), 2.97–3.08 (m, CH₂, H-6'), 3.39–3.67 (25 H, m, 37) (2 s, O Me), 4.27 and 4.32 (2d, J = 5.0, 5.3, H-2'), 4.40 (m, H-4'), 4.64–4.77 (m, H-3'), 4.96–5.06 (m, OCH₂O), 6.05 (0.5 H, d, J = 3.7, H-1'), 6.06 (0.5 H, d, J = 4.7, H-1'), 6.81–6.84 (m, 4 ArH), 7.23–7.75 (m, 10 ArH, H-5), 7.61–7.66 (m, H-6), 9.18 (s, NH); δ<sub>OH</sub> 150.2, 149.8; ml<sub>c</sub> 1336 (100, M⁺).

6-O-[5-(N-Allyloxycarbonyl-methylamino)-pentyl]-1,2,3-tri-O-benzoyl-5-O-[[tripropylsilyloxy]-methyl]-β-D-allofuranosyl-cytosine (4)[α/β]

At room temperature, a solution of 2 (9.78 g, 21 mmol) in CH₂Cl₂ (70 ml) was treated with Pr₃Nı (10.6 ml, 63 mmol) and Pr₃SiOCH₂Cl (3.74 ml, 27.3 mmol) for 1 h. Work-up gave a crude product, which was treated with MeNH₂ in EtOH (8 M, 70 ml) for 1 h at room temperature. After evaporation and work-up, the residue was dissolved in CH₂Cl₂ (70 ml) and treated with Pr₃Nı (7.2 ml, 44 mmol) and allyl chlorofomate (2.2 ml, 21 mmol) at room temperature for 0.5 h. Work-up gave a yellow oil. The crude product obtained by treatment of the resulting oil with CF₃COOH (50 ml) and H₂O (50 ml) for 1 h at room temperature was dissolved in pyridine (15 ml) and CH₂Cl₂ (30 ml) and treated with benzoyl chloride (5.6 ml, 48 mmol) and dimethylaminopyridine (293 mg, 2.4 mmol) for 12 h. Work-up and CC (hexane/EtOAc 9:1–7:3) gave 4 (2 g, 43%, α/β 1.2 by 'H NMR). TLC (hexane/EtOAc 7:3) 0.42; δ<sub>H</sub> 1.05–1.15 (7 H, m, Pr₃Si), 1.21 (m, CH₂), 1.44 (m, 2 CH₂), 2.83 (s, CH₃), 3.09–3.11 (m, 3 CH₂), 3.31–3.41 (2 H, 2 H-6), 3.59 (d, J = 5.9, CH₂), 4.26 (m, H-5(α)), 4.31 (m, H-5(β)), 4.55 (m, CH₃), 4.66 (dd, J = 4.3, 5.1, H-4(α)), 4.78 (dd, J = 1.8, 1.9, H-4(β)), 5.23 (m, CH₃), 5.60 (dd, J = 4.3, 6.5, H-2(β)), 5.90 (dd, J = 2.5, 5.3, H-2(α)), 5.92–5.94 (m, CH₃), 6.02 (dd, J = 2.2, 5.3, H-3(α)), 6.08 (dd, J = 1.9, 6.5, H-3(β)), 6.61 (d, J = 2.1, 1.1(α)), 6.87 (d, J = 4.4, H-1(β)), 7.17–7.61 (m, 9 ArH), 7.75–8.12 (m, 6 ArH); ml<sub>c</sub> 833 (2, M⁺), 710 (100).

N<sup>4</sup>-Benzoyl-1-[6-O-(5-bromopentyl)-5′-O-(4,4′-dimethoxy-trityl)-3′-O-[[tripropylsilyloxy]-methyl]-β-D-allofuranosyl]-cytosine (15)

A solution of 14 (192 mg, 0.17 mmol) in CH₂Cl₂ (0.75 ml) was treated consecutively with Pr₃Nı (73 µl, 0.43 mmol) and (2-cyanoethyll)N₃-dissopropyloxyporphosphate (48 mg, 0.20 mmol). After stirring for 1 h at room temperature, the mixture was subjected to CC (Alox, hexane/EtOAc 6:4:3:7) and 15 (210 mg, 92%) was obtained as a pale yellow foam (1:1 mixture of diastereoisomers). TLC (Al₂O₃, MeOH/CH₂Cl₂ 4:96) 0.52; λ<sub>max</sub> 239 (23 600); δ<sub>H</sub> 1.00–1.07 (21 H, m, Pr₃Si), 1.13–1.21 (m, 4 Me), 1.32–1.36 (m, 3 CH₂), 2.21 (s, MeCO), 2.42–2.44 (m, CH₂), 2.54 and 2.61 (21, J = 6.5, CH₂), 2.73 (t, J = 5.8, CH₂), 2.97–3.08 (m, CH₂, H-6'), 3.39–3.67 (25 H, m, 37) (2 s, O Me), 4.27 and 4.32 (2d, J = 5.0, 5.3, H-2'), 4.40 (m, H-4'), 4.64–4.77 (m, H-3'), 4.96–5.06 (m, OCH₂O), 6.05 (0.5 H, d, J = 3.7, H-1'), 6.06 (0.5 H, d, J = 4.7, H-1'), 6.81–6.84 (m, 4 ArH), 7.23–7.75 (m, 10 ArH, H-5), 7.61–7.66 (m, H-6), 9.18 (s, NH); δ<sub>OH</sub> 150.2, 149.8; ml<sub>c</sub> 1336 (100, M⁺).
MeOH/H2O 5:4:1 (120 ml), 10 N aqueous NaOH (2.4 ml), AcOH (1.5 ml). Work-up and CC [CH2Cl2 to CH2Cl2/MeOH 98:2 (+2% NEt3)] gave 8 (2.1 g, 91% as a white foam. TLC (MeOH/CH2Cl2 8:2) 9.05% d, 1.4% (m, H-3′)
3.80 (s, 2 OMe), 4.10–4.14 (m, H-2′)
1050 (4, MH+), 303 (100).

5.23 (2d, J 7.2, H-2, H-6″), 8.79–7.89 (m, 2 ArH, H-5), 7.91 (d, J 7.2, H-6); m/z 965 (100, M+).

N4-Benzoyl-1-{6-O-(N-methyl-5-[2-[2-methoxy-ethoxy]-ethoxy]ethylenamino)-pentyl}-5′-O-(4,4′-dimethoxytrityl)-2′-O-\{[(trisopropyl)silyloxy]methyl\}-β-o-allofuranosylcytosine (16) A solution of 13 (244 mg, 0.253 mmol), Bu4NI (187 mg, 0.506 mmol), Pr2NEt (0.17 ml, 1.0 mmol) in 0.8 ml toluene was treated with MeOCH2CH2Cl (18) (110 mg, 0.61 mmol) and stirred for 8 h at 95°C. Work-up and CC [CH2Cl2 to MeOH/CH2Cl2 3:97 (+2% Et3N)] gave 16 (120 mg, 44%) as a yellow foam. TLC (Al2O3, MeOH/CH2Cl2 2:97) 0.41; λmax 259 (19700), 234 (28700); δ1H 1.05–1.09 (21 H, m, Pr3Si), 1.26–1.29 (m, 2 CH2), 2.25 (s, NMe), 2.33–2.38 (m, NCH2), 2.55–2.59 (m, 2 CH2), 3.14–3.21 (m, OCH2H-6′), 3.36 (s, OMe), 3.50–3.66 (m, OH-3′, 2 CH2H-6′, H-5′), 3.80 (2, OMe), 4.14–4.21 (m, H-2′, H-4′), 4.62–4.66 (m, H-3′), 5.13 and 5.23 (2 d, J 4.7, OCH2O), 6.01 (d, J 2.8, H-1′), 6.84–6.86 (m, 4 ArH), 7.25–7.63 (m, 12 ArH, H-5), 7.82–7.85 (m, 2 ArH), 7.88 (d, J 7.2, H-6); m/z 1113 (3, MH+), 303 (100).

N4-Benzoyl-1-{6-O-(N-methyl-5-[2-[2-methoxy-ethoxy]-ethoxy]ethylamino)-pentyl}-5′-O-(4,4′-dimethoxytrityl)-2′-O-\{[(trisopropyl)silyloxy]methyl\}-β-o-allofuranosylcytosine (17) As described for 15, with 16 (120 mg, 0.11 mmol), CH2Cl2 (0.3 ml), Pr2NEt (47 μl, 0.28 mmol) and (2-cyanoethyl)(N,N-diisopropyl-phosphoramidite) (17) 0.28 mmol) and (2-cyanoethyl)(N,N-diisopropylamino)chlorophosphite (31 mg, 0.12 mmol). CC (Al2O3, hexane/AcOEt 6:4–2:8) gave 17 (130 mg, 90%) as a pale yellow foam (1:1 mixture of diastereoisomers). TLC (Al2O3, MeOH/CH2Cl2 4:96) 0.52; λmax 261 (18700), 239 (27700); δ1H 1.02–1.08 (21 H, m, Pr3Si), 1.16–1.25 (m, 4 Me), 1.26–1.28 (m, 2 CH2), 1.34–1.39 (m, 2 CH2), 2.24 (s, NMe), 2.31–2.34 (m, NCH2), 2.54–2.63 (m, 2 CH2), 2.92–3.08 (m, OCH2H-6′), 3.36 and 3.37 (2s, CH3), 3.43–3.62 (m, 2 CH2), 3.71–3.79 (m, 2 ArH), 3.80 (2, OMe), 4.14–4.21 (m, H-2′, H-4′), 4.66–4.69 (m, H-3′), 5.13 and 5.23 (2 d, J 5.1, OCH2O), 6.01 (d, J 2.8, H-1′), 6.84–6.86 (m, 4 ArH), 7.25–7.63 (m, 12 ArH, H-5), 7.82–7.85 (m, 2 ArH), 7.88 (d, J 7.2, H-6); m/z 1313 (26, MH+), 1312 (36, M+), 303 (100).

SUPPLEMENTARY MATERIAL The 13C NMR and IR data of compounds 2–17, the CD spectra of all duplexes and a table of all concentration-dependent transition temperatures are available upon request.

ACKNOWLEDGEMENTS We thank Prof. A. Vasella for continuously supporting this work. We also thank Patrick A. Weiss (Xeragon AG, Switzerland) for providing us with numerous reagents and TOM-phosphoramidites and T. Vivlemore and A. Ernst for helpful suggestions. The ETH Zürich Research Council and the Alfred Werner Foundation are gratefully acknowledged for financial support.
REFERENCES