The 5-Methylisocytosine β-D-Ribopyranosyl (4′ → 2′)-Oligonucleotide

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In the context of *Eschenmoser*'s work on pyranosyl-RNA ('p-RNA'), we investigated the synthesis and base-pairing properties of the 5-methylisocytidine derivative. The previously determined clear-cut restrictions of base-pairing modes of p-RNA had led to the expectation that a 5-methylisocytosine β-D-ribopyranosyl (=D-pr(^{Me}isoC)) based (4′ → 2′)-oligonucleotide would pair *inter alia* with D-pr(isoG) and L-pr(G) based oligonucleotides (D-pr and L-pr = pyranose form of D- and L-ribose, resp.). Remarkably, we could not observe pairing with the D-pr(isoG) oligonucleotide but only with the L-pr(G) oligonucleotide. Our interpretation concludes that this – at first hand surprising – observation is caused by a change in the nucleosidic torsion angle specific for isoC.

1. Introduction. – More than 40 years ago, the noncanonical *Watson–Crick* pair of isocytidine with isoguanosine *(Fig. 1)* was proposed as an additional component of an early genetic system [1]. In RNA, isocytosine (isoC) pairing with isoguanosine [2–6], purine-2,6-diamine ribofuranoside [7], and guanosine [8][9] and functional consequences for the isoC-containing RNA have been investigated. In the context of broad experimental work led by *Eschenmoser* on the synthesis and determination of base-pairing properties of β-D-ribopyranosyl (4′ → 2′)-oligonucleotides (='pyranosyl-RNA' or 'p-RNA') [10], this paper describes the synthesis of the corresponding 5-methylisocytosine β-D-ribopyranosyl (4′ → 2′)-oligonucleotide. The switch from isoC to the derivative ^{Me}isoC was done for synthetic reasons since the 5-methyl-substituted moiety had been shown to be chemically more stable [3]. The preparation of the β-D-ribopyranosyl nucleoside D-pr(^{Me}isoC)[2]) containing the protected 5-methylisocytosine was performed based on extensive experiences from the syntheses of other nucleobases [10]. The results of the determination of the base-pairing properties of this oligonucleotide have been reported previously in [11] and will be complemented by a broader interpretation.

2. Results. – Synthesis of the ^{Me}isoC building block **9** amenable to automated oligonucleotide synthesis followed the previously established route [10] for the cytosine building block *(Scheme)*. The commercially not available 5-methylisocytosine had to

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[2]) The abbreviations 'D-pr' and 'L-pr' stand for the pyranose form of D- and L-ribose, respectively.

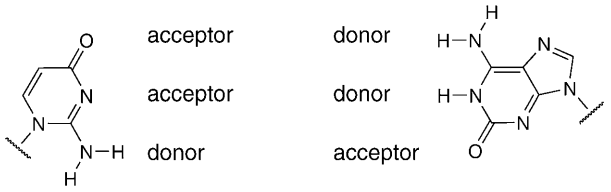


Fig. 1. *Complementary* Watson–Crick-*like donor/acceptor pattern of isoC and isoG*

be synthesized by basic condensation from methyl formate, ethyl propanoate, and guanidine with sodium methanolate following [12]. Then 5-methylisocytosine was benzoylated to the previously unknown N^2-benzoyl-5-methylisocytosine (**1**), the solubility of which – in comparison to N^4-benzoylcytosine – in organic solvents was significantly lower, requiring a change of the usual workup procedure [13]. Nucleosidation following a modified *Vorbrüggen* approach [14] by using an α/β-mixture of tetra-*O*-benzoyl-D-ribopyranose as starting material furnished mainly β-D-pyranoside **2**, containing only minor amounts of furanoside. Both the silylation of the free nucleobase with bis(trimethylsilyl)acetamide and the *Lewis* acid catalyzed nucleosidation with trimethylsilyl trifluoromethanesulfonate proceeded very fast in comparison to other nucleosidations in the p-RNA series. Regioselective cleavage of the sugar benzoyl groups left the N^2-benzoyl group intact and led to sugar-unprotected nucleoside **3**.

Assignment of the configuration at the anomeric center of **3** is based on the characteristic value of the ^{1}H-NMR coupling constant of 9.4 Hz between the anomeric proton H–C(1′) and the H-atom at C(2′) of **3**, both occupying axial positions at the pyranose chairs. Additional NOE experiments established the constitution of **3** with respect to the anomeric bond: As shown in *Fig. 2*, the NOE signal between H–C(6) and H–C(2′) confirms the N^1-constitution of the nucleoside; the additional NOE signal between H–C(1′) and H–C(5′) supports the $^4C_1$ conformation of the pyranose chair.

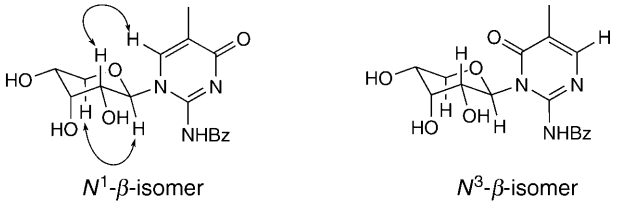
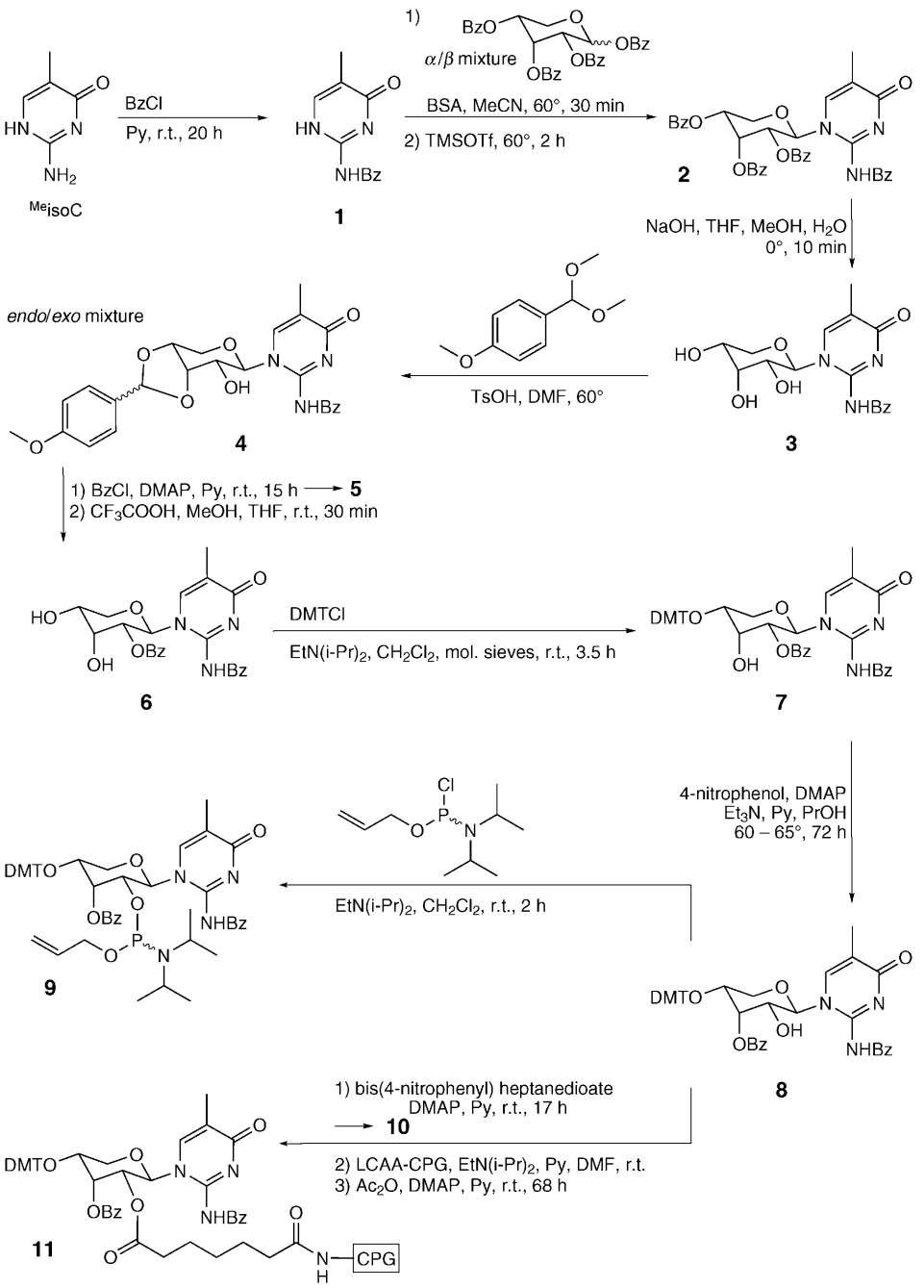


Fig. 2. *Interpretation of NOE signals of* **3**

Taking into account the necessity to cleave the N^2-benzoyl group after synthesis of the oligonucleotide, we tested the anticipated cleavage methods: Both the treatment with aqueous hydrazine at 0° or conc. aqueous ammonia at 60° afforded de-benzoylated **3**. As expected, the ammonia procedure also led to formation of the thymine derivative as by-product by concomitant deamination of the nucleobase. The subsequent reaction steps followed the procedures worked out for the other nucleobases [10], *i.e.*, formation of the 3′,4′-acetal **4** as a diastereoisomer mixture, 2′-benzoylation (→ **5**), deacetalation (→ **6**), and regioselective dimethoxytritylation at O–C(4′) (→ **7**). The 2′ → 3′ benzoyl

Scheme. *Preparation of isoC Building Block* **9** *and Solid-Phase-Bound Unit* **11** *for Automated Synthesis*



BSA = *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf = $CF_3SO_3SiMe_3$, DMAP = *N*,*N*-dimethylpyridin-4-amine, Py = pyridine, DMT = 4,4′-dimethoxytrityl = $(MeO)_2Tr$, LCAA-CPG = long-chain aminoalkyl controlled pore glass.

migration was slower than in the cytosine series: The reaction mixture was worked up after 72 h and delivered almost equal amounts of the desired 3′-benzoate **8** and starting material **7**. Separation could be achieved easily by chromatography as **7** and **8** showed distinct differences in retention in comparison to the cytosine derivative. Constitutional uniformity was confirmed by the shift of the characteristic *dd*-signal of H–C(5′) from δ 3.39 (2′-benzoate **7**) to 2.82 (3′-benzoate **8**). The 3′-benzoate **8** served as educt for the building blocks for the automated synthesis: phosphitylation with allyl diisopropylphosphoramidochloridite led to **9** as a diastereoisomer mixture, and conversion with bi-activated heptanedioic acid (→ **10**) and long chain alkylamino controlled pore glass led to **11**, the protected isoC unit coupled to the solid support.

The main diastereoisomer of the mixture **4** happened to crystallize and was subjected to X-ray structure analysis; it established the '*endo*'-configuration of the 3′,4′-acetal moiety (see below).

Automated synthesis of the β-D-ribopyranosyl (4′ → 2′)-oligonucleotide followed previous experiences (Scheme 4 in [10]) and was performed on a 1-μmol scale in the 'trityl on' mode with a DNA/RNA synthesizer applying modifications to the manufacturer's protocol for DNA synthesis, mainly with respect to extending the coupling step, the capping step, and the detritylation step because of the reactions being performed at the secondary 4′-OH rather than at a primary 5′-OH in DNA. Not optimized coupling efficiencies in the synthesis of protected D-pr(^{Me}isoC$_8$) were 85–87%, these values being lower than in the other p-RNA-syntheses. The allyl protecting groups were removed by treatment with tetrakis(triphenylphosphine)palladium. Cleavage of the dimethoxytritylated oligonucleotide from the glass support with concomitant debenzoylation was achieved by a comparably long treatment with aqueous hydrazine solution at 4°, which suggests low reactivity of the benzoyl group at the isoC nucleobase. This reaction had to be monitored carefully by HPLC: After 15 h, no free oligonucleotide could be detected, after 65 h, the oligonucleotide was fully deprotected, after 115 h, the HPLC showed substantial strand cleavage. The still dimethoxytritylated oligonucleotide was purified by prep. reversed-phase HPLC, detritylated, resubjected to prep. HPLC, and desalted. The calculated molecular mass of D-pr(^{Me}isoC$_8$) was confirmed by MALDI-TOF-MS.

Base-pairing properties of D-pr(^{Me}isoC$_8$) alone (10 μM) and with D-pr(isoG$_8$), L-pr(G$_8$), D-pr(C$_8$), and D-pr(G$_8$) (5 μM each) were determined under previously established standard conditions (0.15M NaCl, 0.01M *Tris*·HCl, pH 7.0 in H_2O) by observing UV absorption as a function of temperature[3]). D-pr(^{Me}isoC$_8$) alone and in an equimolar mixture with D-pr(G$_8$) did not show any cooperative behavior. Also, we could not observe any pairing between D-pr(^{Me}isoC$_8$) and D-pr(C$_8$) both under neutral and weakly acid conditions. D-pr(^{Me}isoC$_8$) did not pair with D-pr(isoG$_8$), as was confirmed by measuring the absorption at three different wavelengths (290, 280, and 245 nm), at three different concentrations, *i.e.*, 5 μM, 0.5 μM, and 0.25 μM each, and at pH 5.5 additionally. As expected, D-pr(^{Me}isoC$_8$) paired with L-pr(G$_8$), T_m 24° [11].

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[3]) The oligonucleotides for base-pairing experiments with D-pr(^{Me}isoC$_8$) were kindly provided by colleagues from *Eschenmoser*'s ETH group. Originally planned base pairing with partner L-pr(isoG$_8$) was not performed since this partner was not available anymore when this work was carried out.

3. Discussion. – In our hands, the nucleobase ^{Me}isoC behaved very much like C in the synthetic workflow. Main differences were the significantly lower solubility of N^2-benzoyl-5-methylisocytosine (**1**) in comparison to N^4-benzoylcytosine in organic solvents, the faster nucleosidation (**1** → **2**), the slower 2′ → 3′-benzoyl migration (**7** → **8**), and the slower N^2-debenzoylation of the octameric oligonucleotide in comparison to other nucleobases in the p-RNA series. The chemical shift of the anomeric proton H−C(1′) of **2** to lower fields (δ 6.99) in comparison to the other nucleosides in the p-RNA series (from [10], δ 6.38 to 6.67) seems noticeable but can be explained by the influence of the benzoyl group at the exocyclic N-atom.

The molecular conformation of the '*endo*'-isomer of **4** observed in the crystal structure is shown in *Fig. 3*. The six-membered ribopyranose ring approximately has a boat conformation with atoms C(2) and C(5) about 0.61 Å outside the best plane C(3)−C(4)−O(2)−C(1) (arbitrary atom numbering, see *Fig. 3*). The boat conformation is stabilized by the acetal ring, which forces the C(3)−C(4) bond to be almost eclipsed (torsion angle C(2)−C(3)−C(4)−C(5) 6.9(2)°). The five-membered acetal ring has a conformation intermediate between a C6 envelope and a C6,O3 twist. The proton expected at the *N*-benzoyl group (N(3)) has migrated to the isocytosine group (N(2)). It is involved in an intramolecular H-bond (N(2)−H(02)···O(7) with N(2)−H(02) 0.87(2) Å, H(02)···O7 1.84(2) Å, N(2)···O(7) 2.558(2) Å, and N(2)−H(02)−O(7) 139(2)°). The C(17)−N(3) bond length of 1.315(2) Å corresponds to a double bond. The *N*-benzoyl-5-methylisocytosine group is almost planar (mean deviation from plane: 0.032 Å). It has a *syn*-periplanar conformation with respect to the C(1)−H(1) bond, resulting in a short intramolecular contact distance of only 2.27(2) Å between H(1) and N(3) (torsion angle H(1)−C(1)−N(1)−C(17) 12°). The OH group at C(2) donates an intermolecular H-bond to the MeO group of a neighboring molecule.

The pairing properties of p-RNA and the restrictions on pairing modes have been discussed *in extenso* [10] and will not be repeated here. Of all base-pairing experiments with D-pr(^{Me}isoC$_8$), only pairing between D-pr(^{Me}isoC$_8$) and L-pr(G$_8$) was observed, albeit this pairing was significantly weaker than in the corresponding D-pr(C$_8$)·L-pr(isoG$_8$) duplex. The absence of pairing of D-pr(^{Me}isoC$_8$) alone and of D-pr(^{Me}isoC$_8$) with D-pr(G$_8$) was in line with the expected inability of the nucleobases to meet the constitutional requirements of a *Watson–Crick* pairing. The absence of pairing between D-pr(^{Me}isoC$_8$) and D-pr(C$_8$) also under weakly acidic conditions can be explained by the lack of interstrand stacking between pyrimidine bases, which would contribute to the stability of the duplex.

The surprise that D-pr(^{Me}isoC$_8$) did not pair with D-pr(isoG$_8$) was based on expectations arising from knowledge of one of the strongest pairings in the p-RNA series, namely D-pr(C$_8$) with D-pr(G$_8$) (T_m 82° at 2.5 μM each). Here we would like to elaborate on the original assumption (see comment [32] in [11]) that a sterically induced propeller twist could hinder the duplex formation of D-pr(^{Me}isoC$_8$) and D-pr(isoG$_8$): *Fig. 4* illustrates the interdependency of the nucleosidic torsion angle χ and pairing of isochiral (left) and heterochiral (right) strands. In the idealized case of $\chi = -120°$ for the D-configured strand ($+120°$ for the L-configured strand), base planes of opposite strands are perfectly aligned for *Watson–Crick* pairing (isochiral case, D/D, left column) or reverse *Watson–Crick* pairing (heterochiral case, D/L, right column). Inclination of the nucleobases' upper rim towards the pyranose O-atom in one strand by changing the nucleo-

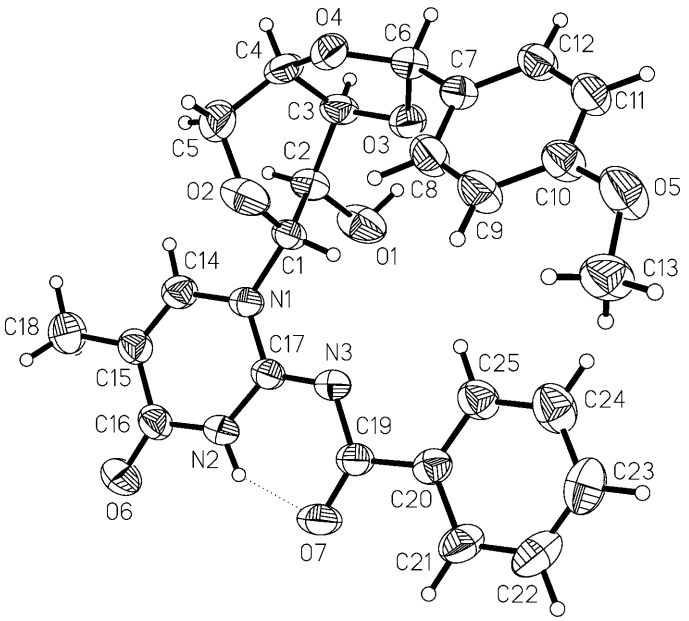

Fig. 3. *Molecular conformation of the* 'endo'-*isomer of* **4** *observed in the X-ray crystal structure.* 50% Probability displacement ellipsoids. The H atoms are drawn as small spheres of arbitrary radius. Arbitrary atom numbering.

sidic torsion angle χ to 180° (middle and lower row, left strand in each case) needs an *opposite* change of the nucleosidic torsion angles to $-60°$ in the isochiral pairing partner (middle row, left case, right strand), and the *same* change of the nucleosidic torsion angles to 180° in the heterochiral pairing partner (middle row, right case, right strand) to keep nucleobases in plane for pairing. *Vice versa*, the *same* change of the nucleosidic torsion angles to 180° in the isochiral pairing partner (lower row, left case, right strand), and the *opposite* change of the nucleosidic torsion angles to $+60°$ in the heterochiral pairing partner (lower row, right case, right strand) would bring the nucleobases out of their pairing planes and would constitute the propeller twist. In other words, changes of nucleosidic torsion angles in the *same* sense favor pairing of heterochiral strands and disfavour pairing of isochiral strands, under the condition that all other requirements for pairing in p-RNA [10] be fulfilled. We assume that our experimental observation that D-pr(^{Me}isoC$_8$) pairs with L-pr(G$_8$) (heterochiral case) but not with D-pr(isoG$_8$) (isochiral case) can be interpreted accordingly: the main structural difference between the isocytosine and the canonical nucleobases of relevance for the interpretation of this observation is the 2-amino group replacing the O-atom. When incorporated into a ribopyranosyl nucleotide, the protons of the isocytosine amino group and the anomeric proton sustain a steric strain, which can be relieved by a change of the nucleosidic torsion angle χ, presumably specific for the isocytosine moiety in direction and/or extent in relation to the other canonical pyrimidine nucleobases (see [15] for the furanose-based nucleotide). In *Fig. 4*, the pairing of D-pr(^{Me}isoC$_8$) with L-pr(G$_8$) would correspond to the heterochiral *anti/anti*-case (middle row, right), and the respective isochiral

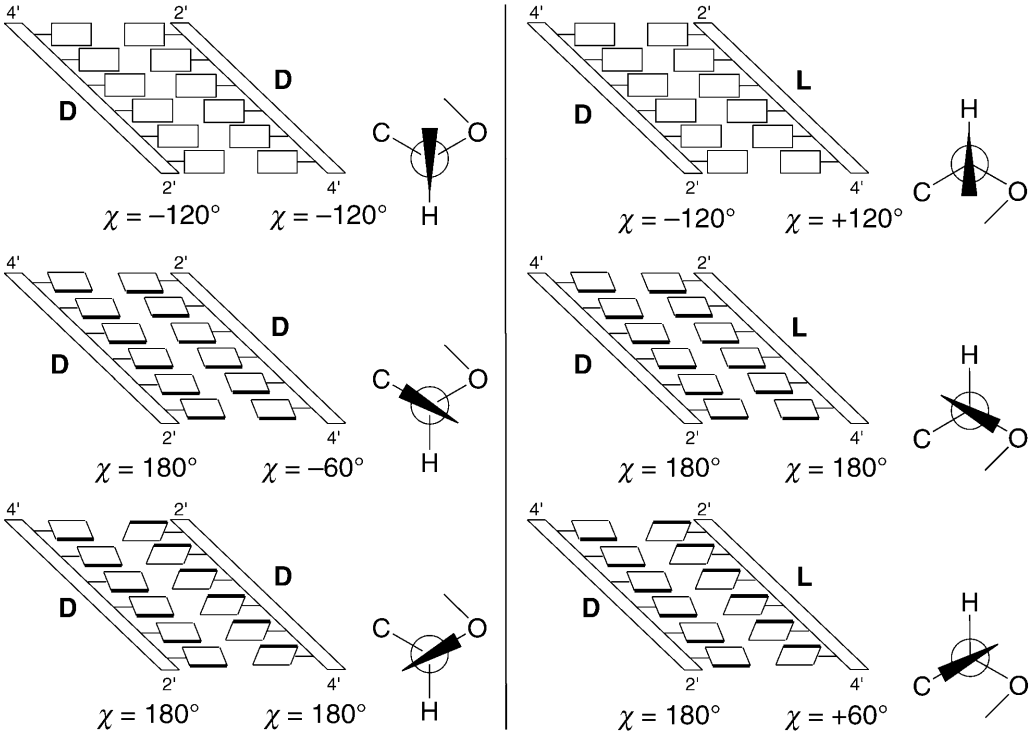

Fig. 4. *Changes of nucleosidic torsion angle* χ *and their consequences for pairing of isochiral* (left) *and heterochiral* (right) *strands.* View for *Newman* projections is from the duplex centre outwards, in plane with bases (black wedge).

anti/anti-case (lower row, left) would correspond to the at first hand surprising nonpairing of D-pr(^{Me}isoC$_8$) with D-pr(isoG$_8$). With this interpretation, we would like to complement the findings on base pairing between heterochiral p-RNA strands [11].

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Experimental Part

1. *General.* Solvents: distilled, synthesis grade. Reagents: unless otherwise noted, from *Merck*, *Roth*, *Fluka*, *Riedel-de Haën*, *Aldrich*, *Sterling*, *MWG-Biotech*, or *Sigma*, various grades. TLC: silica gel *60* F_{254} aluminium plates (*Merck*); visualization by UV absorption and/or spraying with $CeSO_4$ (1.05 g), ammonium molybdate (2.1 g), and H_2SO_4 (conc., 6 ml) in 90 ml H_2O followed by heating. Flash column chromatography (CC): silica gel *60* (0.40 ± 0.63 mm, 230 ± 440 mesh; *Merck*) at low pressure (max. 2 bar). Oligonucleotides were synthesized on an *Applied-Biosystems 392* DNA/RNA synthesizer. HPLC: *Beck-*

man-Gold® chromatography system, equipped with a diode array detector; anal. and prep., reversed-phase column, *Spherisorb-S10X RP-C18* (10 μm, 300 Å, packed by Dr. *J. Schreiber*, ETH Zürich; 220×12 mm); flow 3 ml/min; buffer *A*: 0.1M Et_3N, 0.1M AcOH, H_2O, pH 7.0; buffer *B*: 0.1M Et_3N, 0.1M AcOH, H_2O/MeCN 1:4. Concentrations of oligonucleotide solns. were calculated from the UV absorbance of the solns. at 260 nm (pH 7) at *ca.* 80° with the following molar extinction coefficients: ε(pr(C))=7600 l·mol^{-1}·cm^{-1}, ε (pr(G))=11700 l·mol^{-1}·cm^{-1}, ε(pr(isoC))=6300 l·mol^{-1}·cm^{-1} (taken from the furanoid isomer [8]). M.p. uncorrected. NMR: δ values in ppm rel. to $SiMe_4$ as internal standard, *J* in Hz; ^{1}H assignments, in some cases, based on ^{1}H,^{1}H-COSY. ^{13}C assignments and multiplicities based on ^{1}H,^{13}C-COSY. FAB-MS: positive-ion mode, *VG-ZAB-SEQ* double-focusing high-resolution mass spectrometer, with 3-nitrobenzyl alcohol (3-NBA) as matrix, or alternatively, EI mass spectrometer; in *m*/*z* (intensity in%). Matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS). *Voyager-Elite* mass spectrometer (*Perseptive Biosystems*) with delayed extraction with 2,4,6-trihydroxyacetophenone (THAP) or 2,5-dihydroxybenzoic acid (DHB) as the matrix with ammonium citrate added to the sample.

2. *Synthesis of* **11**. N^2-*Benzoyl-5-methylisocytosine* (**1**). Benzoyl chloride (49.4 g, 0.352 mol, 1.1 equiv.) in abs. pyridine (50 ml) was added within 30 min to a suspension of 5-methylisocytosine (40 g, 0.32 mol, 1.0 equiv.; prepared following the procedure in [12]) under mechanic stirring. Additional benzoyl chloride (4.9 g, 0.035 mol, 0.11 equiv.) was added after 4 h. After 16 h of stirring, 2N HCl (100 ml) was added, the mixture adjusted to pH 4.5 by dropwise addition of conc. HCl soln., and the precipitate filtered off after 15 min. The precipitate was washed with hot EtOH (250 ml) and dried *in vacuo* overnight: 62 g (90%) of **1**. Colorless solid, suitable for the next step. Anal. data were obtained from a sample recrystallized from $CHCl_3$. Colorless crystals. M.p. 277–279°. TLC (CH_2Cl_2/MeOH 8:1): R_f 0.70. UV (CH_2Cl_2): 237 (10600), 298 (9200). IR (KBr): 3190*m*, 3070*m*, 2970*w*, 2940*w*, 2920*w*, 2880*w*, 2340*w*, 1680*m*, 1650*s*, 1620*s*, 1590*s*, 1560*m*, 1508*m*, 1484*m*, 1448*w*, 1425*w*, 1368*w*, 1290*m*, 1265*m*, 1230*m*, 1161*w*, 1107*w*, 1024*m*, 992*w*, 959*w*, 935*w*, 892*w*, 827*w*, 802*m*, 784*m*, 745*w*, 718*w*, 698*m*, 676*w*, 581*w*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.89 (*s*, Me); 7.49–7.64 (*m*, arom. H); 7.68 (*s*, H–C(6)); 8.06 (*d*, *J*=7.2, arom. H); 12.15 (br. *s*, NH). ^{13}C-NMR[4]) (50 MHz, (D_6)DMSO): 12.54 (*q*, Me); 117.15 (*s*, C(5)); 128.37, 128.53, 132.62 (3*d*, arom. C); 133.94 (*s*, arom. C); 151.57, 162.32, 171.45 (3*s*, C(2), C(4), CO). EI-MS: 229.1 (14, M^+), 228.1 (100, $[M-1]^+$), 113 (33), 69 (17). Anal. calc. for $C_{12}H_{11}N_3O_2$: C 62.87, H 4.84, N 18.33; found: C 62.78, H 4.96, N 18.18.

N^2-*Benzoyl-5-methyl-1-(2,3,4-tri*-O-*benzoyl-β*-D-*ribopyranosyl)isocytosine* (**2**). Bis(trimethylsilyl)-acetamide (33.5 g, 130 mmol, 2.0 equiv.) was added to a suspension of **1** (14.2 g, 65 mmol, 1.0 equiv.) and tetra-*O*-benzoyl-D-ribopyranose (*α*/*β* mixture [16]; 37 g, 65 mmol, 1.0 equiv.) in MeCN (350 ml). After 30 min stirring at 60° (→ clear soln.), trimethylsilyl trifluoromethanesulfonate (43.3 g, 195 mmol, 3.0 equiv.) was added in one portion. After 2 h, the mixture was cooled down to r.t., poured on a stirred mixture of ice (300 g) and solid $NaHCO_3$ (50 g), and extracted with AcOEt (3×400 ml). The combined org. phase was dried (Na_2SO_4) and evaporated and the residue filtered over silica gel (250 g, 10×6 cm, AcOEt/hexanes 1:1): 41.4 g of **2**. Slightly yellow oil containing impurities (TLC), suitable for the next step. Anal. data were obtained from a sample after CC (silica gel, AcOEt/hexanes 2:3), containing 5% of furanoid nucleosides (as determined by ^{1}H-NMR). Colorless foam. TLC (AcOEt/hexanes 1:1): R_f 0.54. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.04 (*s*, Me); 4.38 (*t*, *J*=10.9, H_{ax}–C(5′)); 4.44 (*dd*, *J*=5.3, 10.9, H_{eq}–C(5′)); 5.50 (*dd*, *J*=2.8, 9.5, H–C(2′)); 5.61 (*ddd*, *J*=2.7, 5.7, 8.5, H–C(4′)); 6.40 (br. *t*, *J*=2.4, H–C(3′)); 6.99 (*m*, H–C(1′), arom. H); 7.2–7.4 (*m*, arom. H); 7.45–7.6 (*m*, arom. H); 7.65–7.8 (*m*, arom. H); 7.8–7.9 (*m*, arom. H); 8.05–8.15 (*m*, arom. H); 8.15–8.25 (*m*, arom. H); 13.34 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.10 (*q*, Me); 64.43 (*t*, C(5′)); 66.81, 69.20, 69.30 (3*d*, C(2′), C(3′), C(4′)); 80.62 (*d*, C(1′)); 115.79 (*s*, C(5)); 127.89, 128.25, 128.42, 128.50, 129.04, 129.30, 129.60, 129.82, 130.14, 132.06, 133.63, 133.82, 134.26 (13*d*, arom. C); 136.78 (*s*, arom. C); 153.73, 160.18 (2*s*, C(2), C(4)); 164.88, 165.06 (2*s*, CO); 177.23 (*s*, Ph*C*ONH). EI-MS: 673 (11, M^+), 672 (24, $[M-1]^+$), 281 (10), 255 (29), 253 (12), 228 (11), 227 (11), 121 (16), 97 (14), 77 (30), 69 (100), 61 (15), 60 (18), 59 (11). Anal. calc. for $C_{38}H_{30}N_3O_9$: C 67.85, H 4.50, N 6.25; found: C 67.52, H 4.80, N 6.21.

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[4]) C(6) could not be determined.

N^2-*Benzoyl-5-methyl-1-(β-*D*-ribopyranosyl)isocytosine* (**3**). Slightly impure **2** (39 g, *ca.* 58 mmol, 1.0 equiv.) was dissolved in THF/MeOH/H_2O 6:3:1 (800 ml) and cooled to 0°. NaOH (13.9 g, 348 mmol, 6.0 equiv.) was dissolved in H_2O/MeOH 3:6 (90 ml), and added up to 150 ml with THF. The basic soln. was added to the soln. of **2** within 5 min, and left between −2 and +2° for 10 min. Solid NH_4Cl (37.2 g) was added, the mixture evaporated (bath temp. <40°), the residue taken up in toluene (150 ml), and the mixture evaporated. The residue was suspended in CH_2Cl_2/MeOH 15:1 (200 ml) and adsorbed on silica gel (100 g). CC (silica gel (350 g), 13×5.5 cm, CH_2Cl_2/MeOH 15:1 → 5:1) within 5.5 l gave fractions from which **3** precipitated on evaporation as colorless crystals, which were collected and dried *in vacuo*: 9.21 g of **3** (used for anal. data). Additional material was obtained from the CC by eluting with CH_2Cl_2/MeOH 1:1 (1 l), evaporation, dissolution of the residue in MeOH (80 ml), addition of H_2O (80 ml), separation of the precipitate by filtration, washing with MeOH, and drying *in vacuo*: 5.17 g of **3**. Combined yield: 14.38 g (64%) of **3**. Colorless crystals. M.p. 221–222°. TLC (AcOEt/hexanes 1:1): R_f 0.17. $[\alpha]_D^{20°} = +11$ ($c = 0.55$, H_2O). UV (H_2O): 209 (7000), 248 (5100), 298 (9900). IR (KBr): 3375*m* (br.), 3075*w*, 2930*w*, 1680*s*, 1655*s*, 1595*s*, 1580*s*, 1555*s*, 1510*w*, 1490*w*, 1475*w*, 1460*w*, 1450*w*, 1400*m*, 1380*s*, 1360*s*, 1350*s*, 1310*w*, 1270*m*, 1235*m*, 1165*w*, 1135*w*, 1095*s*, 1050*s*, 1025*m*, 1000*m*, 980*w*, 930*w*, 910*w*, 885*w*, 815*w*, 800*w*, 790*w*, 740*w*, 710*s*, 685*w*, 670*w*. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.04 (*s*, Me); 3.6–3.75 (*m*, H–C(4′)); 2 H–C(5′)); 3.86 (*m*, H–C(2′)); 4.05 (br. *s*, H–C(3′)); 4.88, 5.21 (2*m*, OH); 6.42 (*d*, $J = 9.4$, H–C(1′)); 7.4–7.65 (*m*, arom. H); 7.94 (*s*, H–C(6)); 8.15 (*d*, $J = 7.0$, arom. H); 13.41 (br. *s*, NH). ^{13}C-NMR (50 MHz, (D_6)DMSO): 12.29 (*q*, Me); 65.51 (*t*, C(5′)); 66.45, 67.94, 71.24 (3*d*, C(2′), C(3′), C(4′)); 80.75 (*d*, C(1′)); 113.96 (*s*, C(5)); 128.18, 128.99, 132.28 (3*d*, arom. C); 136.83 (*s*, arom. C); 137.63 (*d*, C(6)); 154.35, 159.95 (2*s*, C(2), C(4)); 176.54 (*s*, Ph*C*ONH). EI-MS: 396 (17), 361 (19, M^+), 360 (100, $[M-1]^+$), 228 (20), 113 (41), 79 (47), 69 (19). Anal. calc. for $C_{17}H_{19}N_3O_6$: C 56.51, H 5.30, N 11.63; found: C 56.71, H 5.39, N 11.57.

'endo'/'exo'-N^2-*Benzoyl-1-[3,4-*O*-(4-methoxybenzylidene)-β-*D*-ribopyranosyl]-5-methylisocytosine* ('*endo*'/'*exo*'-**4**). A soln. of **3** (10.2 g, 28.2 mmol, 1.0 equiv.), anisaldehyde dimethyl acetal (12.9 g, 70.6 mmol, 2.5 equiv.), and $TsOH \cdot H_2O$ (536 mg, 2.82 mmol, 0.1 equiv.) in DMF (155 ml) was heated at 60° under vacuum (20–30 mbar) until no **3** could be detected. After cooling to 0°, the mixture was treated with solid $NaHCO_3$ (1 g) and then with sat. $NaHCO_3$ soln. (100 ml), and then diluted with CH_2Cl_2 (500 ml). The org. phase was washed with H_2O, dried (Na_2SO_4), and evaporated: 21.3 g of '*endo*'/'*exo*'-**4** as brown oil containing impurities (mainly anisaldehyde dimethyl acetal; TLC), suitable for the next step. Upon standing, the main diastereoisomer '*endo*'-**4** precipitated as crystals. Anal. data and X-ray analysis (see below) of '*endo*'-**4** were obtained from a sample recrystallized from THF. Colorless crystals. M.p. 233–234°. TLC (CH_2Cl_2/MeOH 15:1): R_f 0.48. UV (CH_2Cl_2): 232 (17000), 248 (14000), 297 (21000). IR (KBr): 3460*m* (br.), 3070*w*, 2900*w* (br.), 1680*s*, 1570*s*, 1520*m*, 1450*m*, 1400*m*, 1340*s*, 1280*m*, 1250*s*, 1220*m*, 1170*m*, 1110*m*, 1090*s*, 1070*s*, 1030*m*, 1010*m*, 920*w*, 890*m*, 880*m*, 860*m*, 840*m*, 820*m*, 770*w*, 760*w*, 740*m*, 710*m*, 680*m*, 630*w*, 580*w*, 540*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.01 (*s*, Me); 3.80 (*s*, MeO); 4.01 (*dd*, $J = 5.8, 12.4$, ^{1}H–C(5′)); 4.08 (*dd*, $J = 3.2, 9.2$, H–C(2′)); 4.19 (*dd*, $J = 4.7, 12.5$, ^{1}H–C(5′)); 4.58 (*dd*, $J = 5.8, 11.2$, H–C(4′)); 4.77 (*dd*, $J = 3.5, 6.7$, H–C(3′)); 6.01 (*s*); 6.75 (*d*, $J = 9.6$, H–C(1′)); 6.75 (*d*, $J = 8.7$, arom. H); 7.21 (*d*, $J = 7.6$, arom. H); 7.34 (*d*, $J = 1$, arom. H); 7.44 (*t*, $J = 7.4$, arom. H); 7.56 (*d*, $J = 8.7$, arom. H); 8.06 (*d*, $J = 7.2$, arom. H); 13.49 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.24 (*q*, Me); 55.37 (*q*, MeO); 67.25 (*t*, C(5′)); 69.52, 72.36, 76.77 (3*d*, C(2′), C(3′), C(4′)); 81.34 (*d*, C(1′)); 105.04 (*s*, C(5)); 114.12 (*d*); 115.73 (*s*); 127.97, 128.20, 129.33, 132.25, 134.38 (5*d*, arom. C); 136.63 (*s*, arom. C); 154.63, 160.27, 160.85 (3*s*, C(2), arom. C, C(4)); 177.15 (*s*, Ph*C*ONH). EI-MS: 479 (15, M^+), 478 (59, $[M-1]^+$), 396 (11), 360 (24), 229 (15), 228 (100), 113 (35), 69 (77). Anal. calc. for $C_{25}H_{25}N_3O_7$: C 62.62, H 5.26, N 8.76; found: C 62.13, H 5.44, N 8.63.

Crystal-Structure Determination of 'endo'-**4**[5]). $C_{25}H_{25}N_3O_7$, M_r 479.49; monoclinic, $P2_1$; $a = 6.2960(17)$, $b = 16.9340(11)$, $c = 10.7640(10)$ Å, $\beta = 98.598(15)°$, $V = 1134.7(3)$ Å^3, $Z = 2$, $D_x = 1.403$ g·cm^{-3}; $\mu = 0.87$ mm^{-1}, r.t. (293 K). A colorless block with dimensions 0.10×0.32×0.50 mm^3 was mea-

[5]) CCDC-282785 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* http://www.ccdc.cam.ac.uk/data_request/cif.

sured on an *Enraf-Nonius-CAD4* diffractometer with Cu-$K\alpha$ radiation (graphite monochromator). A hemisphere of reciprocal space was measured up to $2\Theta = 130°$ with ω-scans. Three reference reflections every 100 min showed no intensity variations. Empirical absorption correction by using ψ scans of 6 reflections; transmission range: 0.813 to 0.917. *Friedel* opposites were not merged. Structure determination by direct methods (SHELXS-97 [17]). H-Atoms were taken from a difference synthesis and were refined with individual isotropic thermal parameters. Structure refinement on F^2 values (SHELXL-97 [17]), number of reflections: 3862, observed reflections with $I > 2\sigma(I)$: 3806, number of parameters 394, $wR(F^2) = 0.084$, $R(F) = 0.031$, $S = 1.13$, final difference density between -0.10 and $+0.12$ e·Å^{-3}. The absolute configuration was established by anomalous dispersion effects, mainly of the O atoms (*Flack-x* $= -0.06(13)$).

N^2-*Benzoyl-1-(2*-O-*benzoyl-β*-D-*ribopyranosyl)-5-methylisocytosine* (**6**). To a soln. of impure **4** (20.71 g) from the acetalization step (containing max. 11.9 g (24.8 mmol, 1.0 equiv.) of pure **4**) and DMAP (0.303 g, 2.48 mmol, 0.10 equiv.) in pyridine (120 ml) cooled to 0°, BzCl (8.72 g, 62 mmol, 2.5 equiv.) was added within 10 min. After stirring at r.t. for 15 h, the mixture was cooled again, and sat., aq. $NaHCO_3$ soln. (150 ml) was added. After stirring at r.t. for 30 min, the mixture was extracted with CH_2Cl_2 (500 ml), the org. phase extracted with H_2O (100 ml), dried (Na_2SO_4), and evaporated. Residual H_2O was removed by addition of toluene (2×50 ml) and evaporation: 22.41 g of **5** containing impurities (TLC), which was immediately used for the next step. TLC (CH_2Cl_2/MeOH 25 : 1): R_f 0.57.

CF_3COOH (22.6 g, 198 mmol, 8 equiv.) was added to a soln. of impure **5** (22.41 g) in MeOH/THF 10 : 1 (275 ml), and the mixture was stirred for 30 min at r.t. After cooling (0°), solid $NaHCO_3$ (18.3 g, 218 mmol, 8.8 equiv.) was added. The mixture was left stirring for 30 min at r.t., then evaporated to *ca.* 50 ml, and adsorbed on silica gel (50 g) by addition of CH_2Cl_2 (200 ml) and careful evaporation. CC silica gel (500 g), 26×7 cm, linear gradient CH_2Cl_2 → CH_2Cl_2/MeOH 5 : 1 in 5 l) furnished 7.69 g (67% based on employed **3**) of **6**. Colorless foam. TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.20. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.91 (*s*, Me); 3.45–3.6, 3.95–4.1, 4.1–4.3 (3*m*, 2 OH, H–C(4′), 2 H–C(5′)); 4.57 (br. *s*, H–C(3′)); 5.09 (*dd*, *J* = 2.4, 9.4, H–C(2′)); 6.9–7.1 (*m*, arom. H, H–C(1′)); 7.25–7.5, 7.75–7.85 (2*m*, arom. H, H–C(6)); 8.25 (*d*, *J* = 7.1, arom. H); 13.38 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 12.96 (*q*, Me); 66.51 (*t*, C(5′)); 66.51, 69.62, 71.55 (3*d*, C(2′), C(3′), C(4′)); 78.65 (*d*, C(1′)); 115.18 (*s*, C(5)); 128.12, 128.29, 129.59, 129.87, 132.32, 133.61, 135.16 (7*d*, arom. C, C(6)); 136.75 (*s*, arom. C); 153.41, 160.90 (2*s*, C(2), C(4)); 165.15 (*s*, CO); 177.36 (*s*, Ph*C*ONH). EI-MS: 510 (26), 500 (25), 465 (25, M^+), 464 (88, $[M-1]^+$), 228 (50), 121 (100), 113 (17), 69 (21).

N^2-*Benzoyl-1-{2*-O-*benzoyl-4*-O-*[bis(4-methoxyphenyl)phenylmethyl]-β*-D-*ribopyranosyl}-5-methylisocytosine* (**7**). A mixture of **6** (7.53 g, 16.2 mmol, 1.0 equiv.), $(MeO)_2TrCl$ (7.13 g, 21.0 mmol, 1.3 equiv.) and 4-Å molecular sieves (5 g) was suspended in CH_2Cl_2 (70 ml), and $^i Pr_2NEt$ (5.44 g, 42.1 mmol, 2.6 equiv.) was added with stirring. After 2.5 h, additional $(MeO)_2TrCl$ (1.64 g, 4.85 mmol, 0.3 equiv.), 4-Å molecular sieves (1 g), and $^i Pr_2NEt$ (1.05 g, 8.1 mmol, 0.5 equiv.) were added. After a total reaction time of 3.5 h, CH_2Cl_2 (200 ml) and sat. $NaHCO_3$ soln. (250 ml) were added. The org. phase was extracted with 20% aq. citric acid soln. (200 ml) and sat. $NaHCO_3$ soln. (100 ml), dried (Na_2SO_4), and evaporated: 15.0 g of impure (TLC) **7** as slightly yellow foam, suitable for the next step. A small part of this mixture was separated by CC (silica gel, AcOEt/hexanes 2 : 3) for characterization purposes. Slightly yellow foam. TLC (CH_2Cl_2/MeOH 20 : 1): R_f 0.64. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.92 (*s*, Me); 2.60 (*s*, OH); 3.39 (*dd*, *J* = 4.5, 10.3, 1 H–C(5′)); 3.70 (br. *s*, H–C(3′)); 3.82 (*s*, MeO); 3.9–4.15 (*m*, H–C(4′), 1 H–C(5′)); 4.82 (*dd*, *J* = 2.3, 9.4, H–C(2′)); 6.91 (*dd*, *J* = 1.5, 8.8, arom H.); 6.95–7.6 (*m*, arom. H, therein H–C(1′)); 7.80 (*d*, *J* = 7.2, arom. H); 8.32 (*d*, *J* = 7.0, arom. H); 13.28 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.01 (*q*, Me); 55.29 (*q*, MeO); 65.21 (*t*, C(5′)); 68.73, 69.00, 70.78 (3*d*, C(2′), C(3′), C(4′)); 78.31 (*d*, C(1′)); 87.53 (*s*); 113.65 (*d*); 115.18 (*s*, C(5)); 127.40, 127.81, 128.15, 128.22, 128.34, 128.50, 129.62, 129.82, 129.95, 132.21, 133.53 (11*d*, arom. C, therein C(6)); 135.61, 135.65, 137.05, 144.75 (4*s*, arom. C); 153.56, 159.10, 160.31 (3*s*, C(2), C(4), arom. C); 165.06 (*s*, CO); 177.28 (*s*, Ph*C*ONH). EI-MS: 768 (1.3, M^+), 767 (2.5, $[M-1]^+$), 465 (13), 464 (45), 372 (33), 272 (13), 229 (15), 228 (100), 215 (17), 121 (28).

N^2-*Benzoyl-1-{3*-O-*benzoyl-4*-O-*[bis(4-methoxyphenyl)phenylmethyl]-β*-D-*ribopyranosyl}-5-methylisocytosine* (**8**). A mixture of impure **7** (13.76 g) from the last step (max. 11.4 g (14.9 mmol, 1.0 equiv.) of pure **7**), 4-nitrophenol (4.13 g, 29.7 mmol, 2.0 equiv.) and DMAP (2.0 g, 16.4 mmol, 1.1 equiv.) in pyridine

(160 ml), PrOH (17.9 g, 297 mmol, 20 equiv.), and Et_3N (4.5 g, 44.6 mmol, 3.0 equiv.) was stirred at 60–65° for 72 h. The mixture was evaporated and co-evaporated with toluene (50 ml). The residue was dissolved in CH_2Cl_2 (400 ml), the soln. washed with 20% aq. citric acid soln. (2×100 ml) and sat. aq. $NaHCO_3$ soln. (6×200 ml), dried (Na_2SO_4), and evaporated after addition of silica gel (35 g). The residue was subjected to CC (silica gel (620 g), 25×8 cm, linear gradient AcOEt/hexanes 1:20 → 1:1 in 4 l, then isocratic 1:1): 3.67 g (25%) of starting **7** and 3.78 g (32%) **8**. Slightly yellowish foam. TLC (AcOEt/hexanes 1:1): R_f 0.31. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.97 (*s*, Me); 2.82 (*dd*, *J*=5.1, 11.2, ^{1}H–C(5′)); 3.65–3.9, 3.95–4.05 (2*m*, H–C(2′), H–C(4′), 1 H–C(5′)); 3.79 (*s*, MeO); 5.95 (br. *s*, H–C(3′)); 6.56 (*d*, *J*=9.3, H–C(1′)); 6.75–6.85, 7.1–7.45, 7.5–7.65, 7.7–7.8 (4*m*, arom. H); 7.97 (*d*, *J*=7.2, arom. H); 8.37 (*d*, *J*=7.2, arom. H); 13.48 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.06 (*q*, Me); 55.25 (*q*, MeO); 66.36 (*t*, C(5′)); 67.77, 71.28, 73.95 (3*d*, C(2′), C(3′), C(4′)); 81.63 (*d*, C(1′)); 87.45 (*s*); 113.41 (*d*); 115.59 (*s*, C(5)); 127.13, 127.98, 128.93, 129.05, 129.95, 130.11, 132.18, 133.64, 134.24 (9*d*, arom. C, therein C(6)); 135.76, 135.92, 136.27, 144.83 (4*s*, arom. C); 154.68, 158.86, 160.09 (3*s*, C(2), C(4), arom. C); 166.15 (*s*, CO); 176.96 (*s*, Ph*C*ONH). EI-MS: 768 (16, M^+), 767 (48, $[M-1]^+$), 766 (96), 372 (31), 283 (14), 281 (19), 255 (36), 253 (13), 229 (16), 228 (100), 216 (16), 215 (88), 146 (19), 145 (12), 144 (13), 121 (40), 97 (34), 77 (10), 60 (13).

N^2-*Benzoyl-1-[3*-O-*benzoyl-4*-O-*[bis(4-methoxyphenyl)phenylmethyl]-β*-D-*ribopyranosyl}-5-methylisocytosine 2′-[Prop-2-enyl Diisopropylphosphoramidite]* (diastereoisomer mixture; **9**). A soln. of **8** (1.92 g, 2.5 mmol, 1.0 equiv.) in abs. CH_2Cl_2 (10 ml) was stirred at r.t. in the presence of ^{i}Pr$_2$NEt (1.29 g, 10 mmol, 4.0 equiv.) and prop-2-enyl diisopropylphosphoramidochloridite (0.70 g, 3.75 mmol, 1.5 equiv.) for 2 h. The mixture was then concentrated to 5 ml and directly subjected to CC (silica gel (80 g); 14×4.5 cm, linear gradient hexanes/AcOEt 10:1 → 1:1 in 1 l; all eluents contained 2% of Et_3N). Product fractions were evaporated (<40°), then co-evaporated with CCl_4 (4 ml): 1.90 g (79%) of **9**. Colorless foam. TLC (AcOEt/hexanes/Et_3N 39:59:2): R_f 0.45. ^{1}H-NMR (300 MHz, $CDCl_3$): 0.68 (*d*, *J*=6.7, *Me*$_2$CH); 0.87 (*d*, *J*=6.7, *Me*$_2$CH); 0.96 (*t*, *J*=6.4); 1.27 (*dd*, *J*=3.0, 6.8); 1.95, 1.98 (2*s*, Me); 2.41, 2.63 (2 *dd*, *J*=5.0, 11.0, H–C(5′)); 3.2–3.45, 3.45–3.85, 3.85–4.2 (3*m*); 3.78, 3.79 (2*s*, MeO); 4.4–4.6, 4.95–5.1, 5.15–5.3, 5.3–5.5, 5.55–5.7, 5.85–6.05 (6*m*); 6.07, 6.29 (br. *s*, H–C(3′)); 6.75–6.9, 7.1–7.55, 7.62–7.72 (3*m*, arom. H, therein H–C(1′)); 8.13 (*d*, *J*=8.0, arom. H); 8.29 (*t*, *J*=7.3, arom. H); 13.46 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.01, 13.08, 22.90, 22.95, 24.06, 24.14, 24.24, 24.32, 42.78, 43.02, 45.15, 45.27 (12*q*, Me); 55.21 (*q*, MeO); 63.11, 63.44, 63.84, 63.98, 64.15, 66.01 (6*t*, C(5′)); 68.02, 68.11, 70.35, 70.44, 70.62, 73.00, 73.73 (7*d*); 80.33, 80.49 (2*d*, C(1′)); 87.38, 87.44, 87.53 (3*s*); 113.37, 113.45 (2*d*); 114.69, 114.99 (2*s*); 115.41, 116.07, 117.32 (3*t*); 127.02, 127.90, 128.01, 128.71, 128.94, 129.40, 129.74, 130.17, 130.65, 130.79, 131.79, 131.86, 132.99, 133.05, 134.70, 134.86, 135.00, 135.11, 135.17, 135.90 (20*d*, arom. C); 136.00, 136.06, 136.22, 137.15, 137.24, 145.01, 145.05 (7*s*, arom. C); 154.14, 154.22, 160.41, 160.54 (4*s*, C(2), C(4)); 158.81, 158.84 (2*s*); 165.59 (*s*, CO); 177.46, 177.56 (*s*, Ph*C*ONH). FAB-MS: 955 (10, M^+), 856 (20), 855 (57), 854 (100), 850 (22), 849 (42), 750 (14), 304 (33), 303 (90), 188 (25), 146 (11), 105 (10), 104 (50), 102 (16).

N^2-*Benzoyl-1-[3*-O-*benzoyl-4*-O-*[bis(4-methoxyphenyl)phenylmethyl]-β*-D-*ribopyranosyl}-5-methylisocytosine 2′-[(4-Nitrophenyl) Heptanedioate]* (**10**). A soln. of DMAP (52 mg, 0.422 mmol, 1.0 equiv.), bis(4-nitrophenyl) heptanedioate (1.24 g, 3.08 mmol, 7.3 equiv.), and **9** (324 mg, 0.422 mmol, 1.0 equiv.) in pyridine (3 ml) was stirred for 17 h. The mixture was evaporated, treated with toluene (6 ml), and evaporated. The residue was subjected to CC (silica gel (30 g), 12×2.5 cm, linear gradient AcOEt/hexanes 1:6 → 1:1 in 0.8 l): 264 mg (61%) of **10**. Colorless foam. TLC (AcOEt/hexanes 1:1): R_f 0.49. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.05–1.2, 1.3–1.55 (2*m*); 1.97 (*s*, Me); 1.95–2.2 (*m*); 2.41 (*t*, *J*=7.4); 2.85 (*dd*, *J*=5.0, 11.1, H–C(5′)); 3.79 (*s*, MeO); 3.87 (*t*, *J*=11.0); 3.95–4.1 (*m*); 4.95 (*dd*, *J*=2.5, 9.5, H–C(2′)); 5.89 (br. *s*, H–C(3′)); 6.75–6.85, 6.85–7.05, 7.2–7.3, 7.3–7.45 (4*m*, arom. H, therein H–C(1′)); 7.59 (*t*, *J*=7.7, arom. H); 7.76 (*t*, *J*=7.4, arom. H); 8.07 (*d*, *J*=7.2, arom. H); 8.15 (*d*, *J*=9.1, arom. H); 8.23 (*d*, *J*=9.1, arom. H); 8.33 (*d*, *J*=7.2, arom. H); 13.37 (br. *s*, NH). ^{13}C-NMR (50 MHz, $CDCl_3$): 13.04 (*q*, Me); 24.00, 24.02, 28.12, 33.58, 33.79 (5*t*); 55.26 (*q*, MeO); 66.31 (*t*, C(5′)); 67.31, 67.54, 68.70 (3*d*, C(2′), C(3′), C(4′)); 79.13 (*d*, C(1′)); 87.48 (*s*); 113.47 (*d*); 113.54 (*d*); 115.28 (*s*, C(5)); 115.63 (*d*); 122.44, 125.12, 126.17, 127.23, 127.96, 128.07, 128.98, 129.31, 129.90, 130.06, 132.14, 133.64, 134.60 (13*d*, arom. C, therein C(6)); 135.57, 135.74, 136.75, 144.71, 145.23 (5*s*, arom. C); 153.58, 160.30

(2*s*, C(2), C(4)); 155.51, 158.96 (2*s*); 161.69, 165.59, 170.84, 171.89 (4*s*); 177.33 (*s*, Ph*C*ONH). FAB-MS: 1033 (21), 1032 (51), 1031 (78, $[M+H]^+$), 307 (12), 304 (37), 303 (100), 289 (12), 230 (14), 139 (13), 138 (19), 137 (28), 136 (31), 121 (10), 107 (14), 104 (42).

N^2-*Benzoyl-1-{3-*O*-[bis(4-methoxyphenyl)phenylmethyl]-β-*D*-ribopyranosyl}-5-methylisocytosine 2′-{7-[(LCAA-CPG)amino]-7-oxoheptanoate}* (**11**). A suspension of **10** (80 mg, 0.078 mmol, 1.0 equiv.), LCAA-CPG (390 mg), $^iPr_2NEt$ (101 mg, 0.78 mmol, 10 equiv.), and pyridine (31 mg, 0.39 mmol, 5 equiv.) in DMF (1.2 ml) was shaken overnight. The solid support was filtered off, washed consecutively with DMF (30 ml), MeOH (30 ml), and Et_2O (30 ml), dried under high vacuum, suspended in pyridine (1.5 ml), and treated with Ac_2O (0.25 ml) and DMAP (12 mg). After being shaken for 68 h at r.t., the solid support was filtered off and washed consecutively with DMF (30 ml), MeOH (30 ml), and Et_2O (30 ml). After drying (→ 310 mg of **11**), the loading density was determined by detritylation of **11** (6.3 mg) with 0.1M TsOH in MeCN (10 ml) and measuring the absorption at 498 nm (ε ($(MeO)_2Tr^+$ ion) $= 7 \cdot 10^5\ l \cdot mol^{-1} \cdot cm^{-1}$). The loading density was 27 μmol isoC nucleoside/g solid support.

3. *Automated Solid-Phase Synthesis and Purification of the Oligonucleotide* D-*pr(*^{Me}*isoC*$_8$*)* (see Scheme 4 in [10]). Oligonucleotide synthesis was carried out on a 1-μmol scale on an *Applied Biosystems-392* DNA/RNA synthesizer.

Pre-automation Procedures. The DNA/RNA synthesizer column was filled with **11** (27 mg, 1 μmol). A soln. of phosphoramidite **9** (112 mg, 0.1175 mmol) in MeCN (1.26 ml; → 0.1M soln.) was dried (4-Å molecular sieves, 8–12 mesh, freshly activated) for 3 h at r.t. prior to use.

Activator soln.: A mixture of 0.15M 5-(4-nitrophenyl)-1*H*-tetrazole and 0.35M 1*H*-tetrazole in MeCN was dried over freshly activated 4-Å molecular sieves.

Capping A (THF/Ac_2O/lutidine), capping B (THF/1-methyl-1*H*-imidazol), and oxidizing soln. (THF/pyridine/H_2O/I_2) as purchased from *MWG-Biotech*.

Detritylation reagent: 6% $Cl_2CHCOOH$ in CH_2Cl_2.

The automated 'trityl on' synthesis of the oligonucleotide with the DNA/RNA synthesizer was accomplished with the following modifications to the manufacturer's protocol for DNA synthesis: *1*) the delivery step of **9** and activator was done within 2.5 s, *2*) the coupling step (20 equiv. of **9** each) was extended to 225 s, *3*) the delivery step of activator alone was done within 4 s, *4*) the capping soln. was pumped for 90 s, *5*) the detritylation step was conducted within 253 s, and *6*) the oxidizer soln. was pumped within 8 s.

Post-automation Procedure. For allyl deprotection, following *Method B* in [10], a soln. of Et_2NH (50 μl), $[Pd^0(Ph_3P)_4]$ (20 mg), and Ph_3P (20 mg) in CH_2Cl_2 (1.5 ml) was added to the CPG solid support, and the resulting suspension was vigorously shaken at r.t. for 5 h. The suspension was filtered, the CPG solid support carefully washed sequentially with CH_2Cl_2 (10 ml) and acetone (15 ml), treated with 0.1M aq. $NaCS_2NEt_2$ (4.5 ml) at r.t. for 30 min, filtered, and washed again sequentially with H_2O (10 ml), acetone (15 ml), and EtOH (10 ml), and dried under high vacuum. For the detachment from CPG and acyl deprotection, the CPG-attached oligonucleotide was suspended in 24% aq. $NH_2NH_2 \cdot H_2O$ soln. (1.5 ml) and vigorously shaken at 4° for 70 h. The mixture was diluted with 0.1M aq. $Et_3NH \cdot HCO_3$ buffer to a total volume of 10 ml. A *Sep-Pak-C18* cartridge (*Waters)* was washed three times sequentially with MeCN (5 ml) and 0.1M aq. $Et_3NH \cdot HCO_3$ buffer (5 ml). Alternatingly, the oligonucleotide soln. (1 ml) and 0.1M aq. $Et_3NH \cdot HCO_3$ buffer (1 ml) were applied to the cartridge, and subsequently, 0.1M aq. $Et_3NH \cdot HCO_3$ buffer (10 ml) and H_2O (10 ml) were used to elute excess hydrazine (UV monitoring). The oligonucleotide was eluted by applying an H_2O/MeCN gradient (5, 10, 25, and 50% MeCN, 10 ml each). Product fractions were evaporated, and the oligonucleotide was purified by reversed-phase HPLC (gradient 0–100% eluent *B* in 100 min; t_R of $(MeO)_2$Tr-D-pr(^{Me}isoC$_8$) 47 min). The combined product fractions from HPLC purification were evaporated and then detritylated by treatment with 80% HCOOH (3 ml; immediate orange color) for 10 min at r.t. The mixture was evaporated, taken up in H_2O (5 ml), evaporated again, taken up in H_2O (10 ml) again, extracted with CH_2Cl_2 (3 × 2 ml) to remove the $(MeO)_2$TrOH, evaporated and subjected to prep. reversed-phase HPLC (gradient 0–100% eluent *B* in 100 min; t_R of D-pr(^{Me}isoC$_8$) 21 min). The oligonucleotide was desalted by using the published method (see [10], Exper. Part, Sect. 7). MALDI-TOF-MS: 2489.3 ($C_{80}H_{113}N_{24}O_{54}P_7^+$; calc. 2490).

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