Synthesis and Properties of Oligonucleotides Containing 2,4-Dihydroxycyclohexyl Nucleosides

by **Dorothée Bardiot**, **Helmut Rosemeyer**, **Eveline Lescrinier**, **Jef Rozenski**, **Arthur Van Aerschot**, and **Piet Herdewijn***

Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, K. U. Leuven, Minderbroedersstraat 10, B-3000 Leuven
(e-mail: Piet.Herdewijn@rega.kuleuven.ac.be)

Cyclohexyl nucleosides with an adenine and uracil base have been synthesized from 2-azidocyclohexane-1,5-diol. The obtained racemic nucleosides were resolved using (*R*)-*O*-methylmandelic acid. Short oligonucleotides were synthesized using phorphoramidite chemistry. However, these oligonucleotides do not show self-hybridization, and duplexes are less stable than those of ribopyranosyl-(4′ → 2′)-oligonucleotides.

1. Introduction. – DNA Hybridization has been used for the three-dimensional organization of components for nanotechnological applications. A classical experiment is the use of single-stranded DNA to induce aggregation of colloid material labelled with DNA sequences that are complementary to the single-stranded linker DNA [1]. Likewise, networks of gold particles have been obtained in the same way [2]. Disadvantages of this technology are that intramolecular folding of DNA may prevent cluster formation, that the system has a limited lifetime due to the chemical and enzymatic lability of DNA, and that the mechanical strength of the double stranded wires is limited. To avoid these three intrinsic disadvantages of natural DNA, more robust synthetic nucleic acids should be used that are less prone to folding [3]. Here, we present the synthesis of a new synthetic nucleic acid, based on a phosphorylated cyclohexane backbone. Phosphorylated internucleoside linkages are needed to keep the materials water-soluble. The expectations that these nucleic acids might form stable duplexes is based on a publication about the thermal stability of β-D-ribopyranosyl-(4′ → 2′)-oligonucleotides [4]. The difference is that the ring O-atom is replaced by a CH_2 group, and that the 3′-OH group is removed. The proposed polymers are chemically and enzymatically stable, and are less prone to intramolecular folding, given the conformational stiffness of the cyclohexane ring and the replacement of a primary OH group by a secondary OH group. The sequence-controlled polymerization can be carried out according to classical DNA synthesis chemistry, which means that the oligonucleotides are easily available. The structure of the proposed single stranded nucleic acids is given in *Fig. 1*.

To test the potential higher thermodynamic stability of dihydroxycyclohexyl nucleic acid duplexes, we decided to synthesize first the phosphoramidite building blocks with a uracil and adenine base (as these nucleoside analogues are more easily available than nucleosides with a cytosine or guanine base moiety). The uracil and adenine derivatives were prepared by construction of the heterocyclic base on the primary amine of 2-

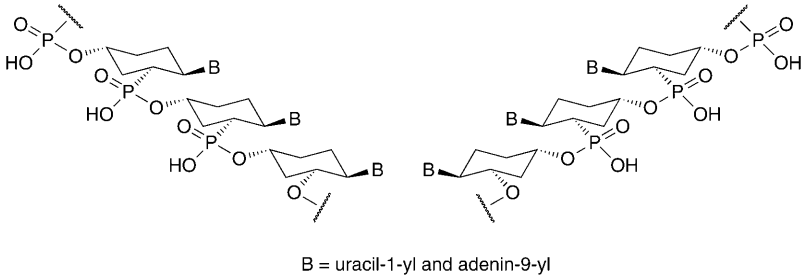


Fig. 1. *Structure of single-stranded 2,4-dihydroxycyclohexane oligonucleotides of opposite chirality*

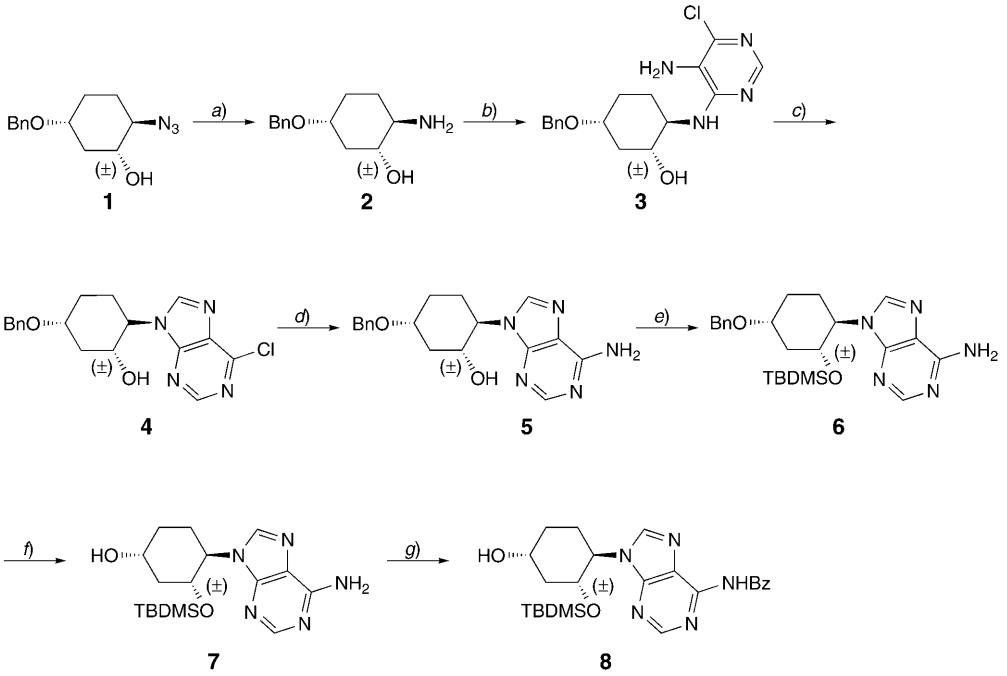
amino-5-(benzyloxy)cyclohexan-1-ol (**2**). Optically pure nucleosides were obtained *via* resolution of racemic intermediates **8** and **12** with (−)-(*R*)-methylmandelic acid. After separation of the enantiomers, phosphoramidite chemistry is used for the polymerization reaction. As compounds are meant to be used for nanotechnological purposes, the exact identification of homochirality is not needed, as long as the obtained racemates can be resolved in their enantiomers. Homochiral oligonucleotides from opposite chirality were synthesized. The sequences 4′-AUUUAUAAp-2′, its antiparallel complement 4′-UUAUAAAUp-2′, 4′-AUAUAUAUp-2′, and 4′-UAUUUUAp-2′ have been obtained. CD Measurements indicate that the oligonucleotides obtained from the (+)-series of monomers might be stereochemically related to the natural congener (*d*-DNA). Unfortunately, no duplex formation was observed with these short cyclohexyl oligonucleotides.

2. Results and Discussion. – 2.1. *Nucleoside Synthesis.* For the synthesis of cyclohexyl nucleosides, we utilized the reported (±)-(1*RS*,2*RS*,5*RS*)-2-azido-5-(benzyloxy)cyclohexan-1-ol [5] (**1**)[1]) as starting material, which was prepared in four steps from cyclohexane-1,4-diol [5–7]. *Staudinger* reduction of the N_3 group to yield the amine **2** enabled the construction of the adenine and uracil moieties according to standard methods (*Schemes 1* and *2*)[1]). Preparation of the adenine derivative involved a three-step sequence [8][9]: condensation of **2** with 5-amino-4,6-dichloropyrimidine in BuOH in the presence of Et_3N gave the substituted diaminopyrimidine **3**. Cyclization with triethyl orthoformate in acidic medium, followed by substitution of the 6-Cl atom of **4** with NH_3 provided the adenine derivative **5**. The secondary OH group of **5** was protected with a TBDMS group, followed by hydrogenolytic cleavage of the benzyl ether with 10% Pd/C and ammonium formate [10] in refluxing MeOH. A benzoyl (Bz) group was introduced at N^6 of the adenine *via* a transient protection [11] approach, to yield the racemic compound **8**.

The synthetic route to the uracil derivative **12** was based on the previously described procedures [12–14] (*Scheme 2*). Treatment of **2** with β-methoxyacryloyl isocyanate [15] gave the intermediate acryloylurea **9**, which was cyclized in refluxing diluted H_2SO_4 to provide the uracil **10**. After silylation of the secondary OH group of **10**, attempts

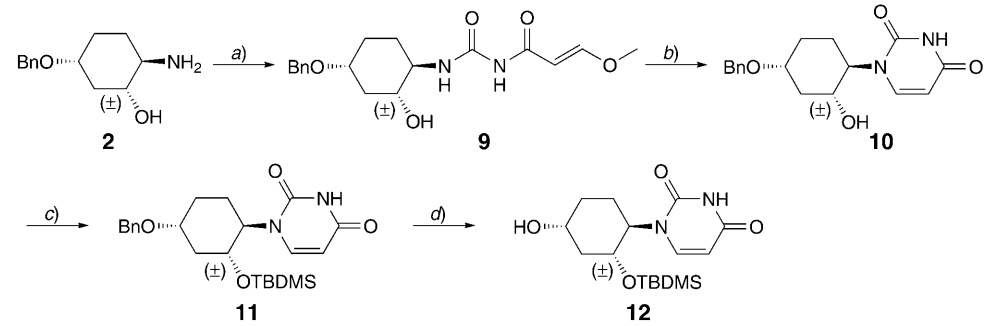
[1]) In all *Schemes*, for convenience only one enantiomer is shown; (±) below the formulae refers to racemates.

Scheme 1. *Preparation of the Adenine Derivative* **8**



a) 1. Ph_3P, dioxane; 2. NH_4OH; 97%. *b*) 5-Amino-4,6-dichloropyrimidine, Et_3N, BuOH; 83%. *c*) 1. $HC(OEt)_3$, 12N HCl; 2. 0.5N HCl, THF; 76%. *d*) NH_3/MeOH; 93%. *e*) (*t*-Bu)Me_2SiCl (TBDMSCl), 1*H*-imidazole, DMF; 81%. *f*) Pd/C, NH_4OCOH, MeOH; 96%. *g*) 1. Me_3SiCl, pyridine; 2. BzCl; 3. NH_4OH, MeOH; 73%.

Scheme 2. *Preparation of the Uracil Derivative* **12**



a) *β*-Methoxyacryloyl isocyanate, DMF; 76%. *b*) 2N H_2SO_4; 73%. *c*) TBDMSCl, 1*H*-imidazole, DMF; 95%; *d*) 1M BCl_3 in CH_2Cl_2, CH_2Cl_2; 95%.

to remove the Bn protecting group by hydrogenolysis (H_2 or $HCOONH_4$ with Pd/C) were not successful because of partial hydrogenation of the uracil double bond. Debenzylation [16] with a BCl_3 solution at $-78°$ yielded the racemic compound **12**.

The synthesis of the enantiomeric forms of the cyclohexyl-adenine and -uracil nucleosides was achieved by resolution of intermediates **8** and **12** *via* formation of diastereoisomeric esters with a chiral acid. We chose (*R*)-*O*-methylmandelic acid, which had been previously used in our laboratory to resolve diastereosiomeric esters of carbocyclic nucleosides [17][18]. Acylation of **8** was carried out with (*R*)-*O*-methylmandelic acid in the presence of DCC and DMAP (*Scheme 3*). As we cannot assign the exact configuration of the diastereoisomers, the less polar compound is called (A) and the more polar (B) by convention. Careful separation of the two diastereoisomers **13a** and **13b** by column chromatography, followed by preparative TLC gave pure compound. The diastereoisomeric purity was checked by HPLC and was found to be greater than 99% for **13a** and **13b** (*Fig. 2*). The uracil derivative **12** was resolved in a similar manner by treatment with (*R*)-*O*-methylmandelic acid. As for adenine derivatives, the less polar diastereoisomer is called (A) and the more polar (B). Isolation of **14a** and **14b** was easier than for the resolution of **8**. After purification by column chromatography, the diastereoisomers **14a** and **14b** were obtained with a diastereoisomeric purity greater than 99% (determined by HPLC; see *Fig. 2*).

Selective removal of the *O*-methylmandelate esters was accomplished by treatment with 2N NaOH in a mixture of dioxane and MeOH [19]. Tritylation of the OH group in 4′-position and desilylation with a 1N Bu_4NF solution in THF provided the enantiomerically pure cyclohexyl nucleosides **19a** and **19b**, and **20a** and **20b**. The $[\alpha]_D$ values were determined for the four compounds, showing that **19a** and **20a** (derived from the less polar mandelates **13a** and **14a**) are the (−)-isomers, and **19b** and **20b** (derived from the more polar mandelates **13b** and **14b**) are the (+)-isomers. The nucleotide building blocks **21a** and **21b**, and **22a** and **22b** were then obtained by phosphitylation of the sec-

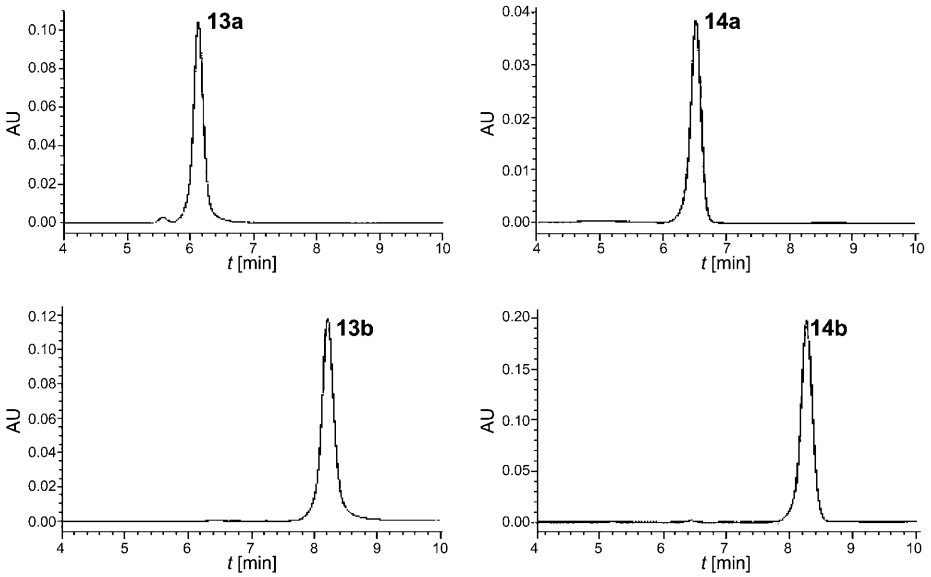
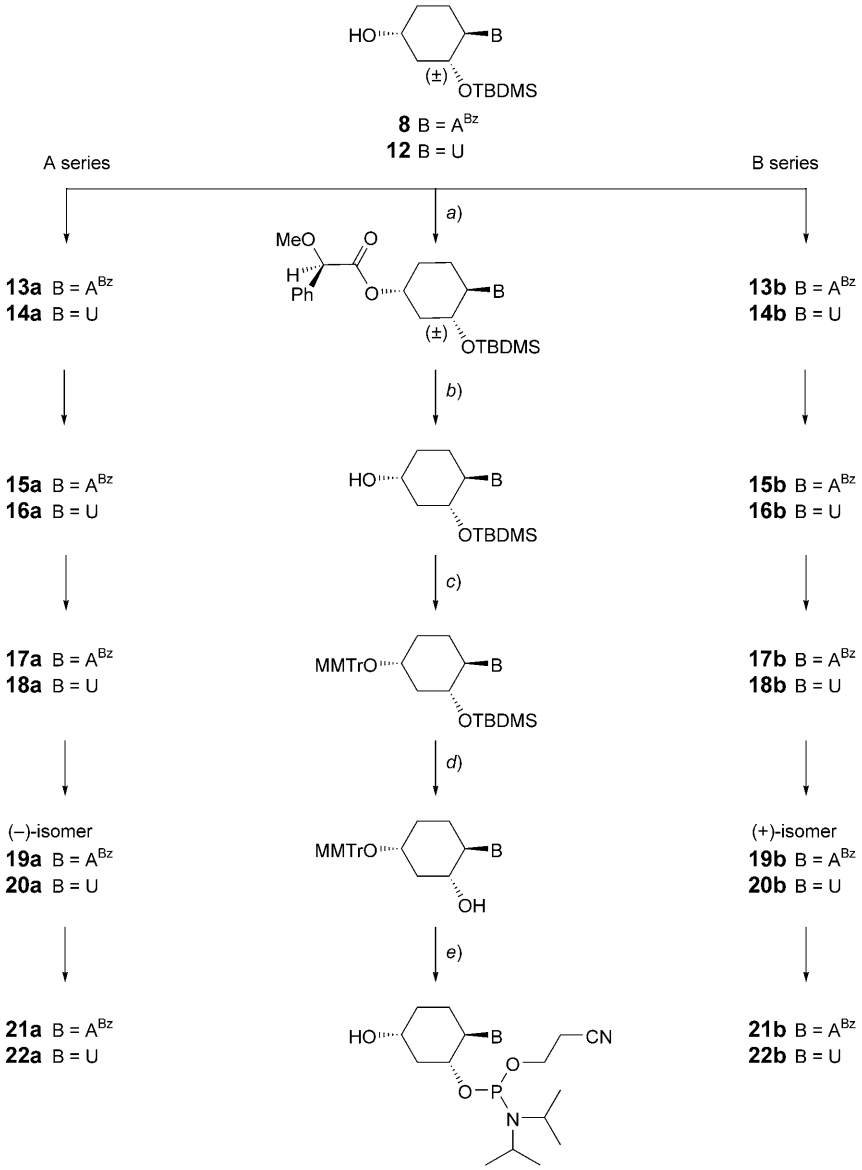


Fig. 2. *Diastereoisomeric purity of* **13a** *and* **13b**, *and* **14a** *and* **14b** *as determined by HPLC* (see *Exper. Part* for HPLC conditions)

Scheme 3. *Resolution of the Cyclohexyl Nucleosides and Preparation of the Optically Pure Building Blocks for Oligonucleotide Synthesis*



a) (*R*)-*O*-Methylmandelic acid, *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2; **13a**: 36%, **13b**: 36%, **14a**: 43%, **14b**: 39%. *b*) 2N NaOH, MeOH, dioxane; **15a**: 96%, **15b**: 66% **16a**: 94%; **16b**: 91%. *c*) Monomethoxytrityl chloride (MMTrCl), pyridine; **17a**: 88%, **17b**: 84%, **18a**: 82%, **18b**: 99%. *d*) 1N Bu_4NF in THF, THF; **19a**: 81%, **19b**: 84%, **20a**: 61%, **20b**: 70%. *e*) $EtN(i\text{-}Pr)_2$, $(i\text{-}Pr)_2NP(Cl)OCH_2CH_2CN$, CH_2Cl_2; **21a**: 83%, **21b**: 83%, **22a**: 88%, **22b**: 78%.

ondary OH group in 2′ with 2-cyano-*N*,*N*-diisopropylchlorophosphoramidite in the presence of $^{i}Pr_2NEt$.

2.2. *Oligonucleotide Synthesis.* Using the cyclohexyl-U and cyclohexanyl-A monomers (**21a** and **22a**, and **21b** and **22b**) of similar polarity and $[\alpha]_D^{20}$ values, oligonucleotides were synthesized with the sequences 4-AUUUAUAAp-2′ (*Table 1*) and its antiparallel complement 4′-UUAUAAAUp-2′. Duplexes of such sequences in the p-RNA series have been demonstrated to show a T_m of *ca.* 20° in 0.15M NaCl [4]. The mirror-image oligonucleotide 4′-AUUUAUAAp-2′ obtained from the (+)-series nucleotides and from the (−)-series nucleotides gives identical 1D-NMR spectra (data not shown). This is also the case for the mirror-image oligonucleotide 4′-UUAUAAAUp-2′.

Table 1. *Oligonucleotides Synthesized from the Dihydroxycyclohexyl Nucleosides*

Sequence		Mass spectra	
		calc.	found
From series A ((−)-series)			
I	4′-AUUUAUAA-2′-phosphate	2414.5	2414.3
II	4′-UUAUAAAU-2′-phosphate	2414.5	2414.3
III	4′-UAUUUUA-2′-phosphate	2080.4	2080.4
From series B ((+)-series)			
IV	4′-AUUUAUAA-2′-phosphate	2414.5	2414.4
V	4′-UUAUAAAU-2′-phosphate	2414.5	2414.5
VI	4′-AUAUAUAU-2′-phosphate	2414.5	2414.5

Also the self-complementary sequence 4′-AUAUAUAUp-2′ was synthesized. Unfortunately, no duplex formation was observed with the oligonucleotides at either 0.1M NaCl, 1M NaCl, 3M NaCl, or 50% formamide, demonstrating that the removal of the 3′-OH group and the replacement of the O-atom in p-RNA by a CH_2 group results in lowered duplex stability. The presence of a supplementary phosphate group at the 2′-end is considered as of minor influence on duplex stability, and was included for straightforward synthetic purposes.

2.3. C*D Analysis.* The CD spectrum of the single-stranded oligonucleotide was determined in phosphate buffer pH 7.0. The single-stranded oligonucleotides **I** and **II** show a positive *Cotton* effect with a maximum at 256 nm. The single-stranded oligonucleotides **IV** and **V** (being mirror images of the oligonucleotides **I** and **II**, resp.) show a negative *Cotton* effect with a maximum at the same wavelength (*Fig. 3*, *a* and *b*). The natural DNA oligonucleotide (5′-UUAUAAAUp-3′), likewise, demonstrates a strong negative *Cotton* effect, the maximum being located at 245 nm (*Fig. 3*, *c*), which means that it shows more similarity with the B series of dihydroxycyclohexyl oligonucleotides than with the A series.

2.4. *Mass Spectrometry.* In addition to the accurate mass determination of the building blocks, the oligonucleotides were characterized by HPLC-MS/MS. Although the fragmentation of the 2,4-dihydroxy-1-cyclohexyl-containing oligonucleotides does not follow the fragmentation rules of natural DNA [20], sequence information can be obtained from CID data. As an example, we will discuss the spectrum for oligonucleotide **I** (*Fig. 4*). The major peak in the spectrum obtained by fragmentation of the triply charged precursor (*m*/*z* 803.8) results from the loss of the phosphate group at the 2′-

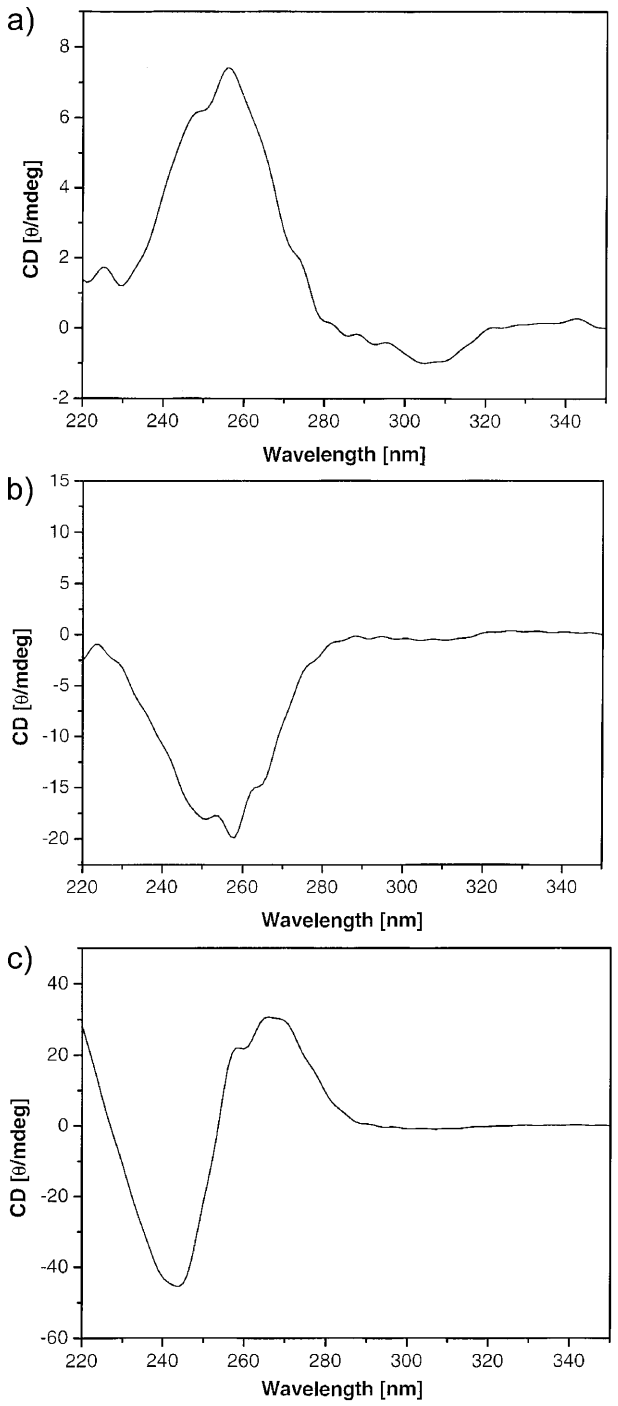


Fig. 3. a) *CD Spectrum of oligonucleotide* **II** ((−) series), b) *CD spectrum of oligonucleotide* **V** ((+) series), c) *CD spectrum of single-stranded DNA with sequence 5′-UUAUAAAUp-3′*

end. Attempts to build a sequence ladder, the classical approach for oligonucleotide sequencing from mass-spectrometry data [21], failed because of the lack of a glycosidic bond. As a consequence, some of the ions will not be found (*e.g.*, the a-B series), and ion intensities will not always relate to the sequence (*e.g.*, for the w_2 ion *m*/*z* 696 $[pUpAp]^-$ is more abundant than *m*/*z* 719 $[pApAp]^-$, making this series useless for sequencing). However, the fragmentation spectra reveal that residues are primarily lost from the 2′-end, and the sequence can be built from the 2′-terminus.

All fragments originate from backbone cleavages. Example spectra with annotations are shown in *Fig. 4*. The major ions are accompanied by a water loss (*e.g.*, *m*/*z* 390 has lost water from *m*/*z* 408). As expected, we found no differences between the spectra of the oligonucleotide isomers **I** and **IV**, nor between the spectra of **II** and **V**.

Conclusions. – 2,4-Dihydroxy-1-cyclohexyl nucleosides with an uracil and adenine base have been synthesized as racemic mixtures and resolved into their enantiomers. These nucleosides were converted to their protected phosphoramidite building blocks for oligonucleotide synthesis. The correct assembling process for oligonucleotide synthesis was verified by mass spectrometry, and the mirror-image form by CD analysis. However, we were not able to detect duplex formation with the obtained short oligomers (octamers) with U and A bases. The potential self-hybridization will have to be evaluated using longer oligomers including cytosine and guanine bases.

Experimental Part

General. All air-sensitive reactions were carried out under N_2. All solvents used for the reactions were anal. grade or freshly dried according to standard methods. Azido derivative **1** was prepared as described in [5–7]. Precoated aluminium sheets (*Fluka*, silica gel/TLC cards, 254 nm) were used for TLC, and compounds were visualized by UV fluorescence. Prep. TLC were performed on precoated TLC plates (*Macherey-Nagel* silica gel *P/UV 254*). Ecochrom silica gel (0.035–0.06 mm or 0.06–0.2 mm) was used for column chromatography (CC). CD Spectra were measured at either 5° or 20° on a *Jasco 600* spectropolarimeter with thermostatically controlled 1-cm cuvettes, connected with a *Lauda RCS* thermostate (Lauda, D-Königshofen). ^{1}H-, ^{13}C-, and ^{31}P-NMR spectra were recorded with a *Varian Unity-500* spectrometer with TMS as internal standard for ^{1}H-NMR spectra, $CDCl_3$ or (D_6)DMSO as internal standard for ^{13}C-NMR spectra, and 85% H_3PO_4 as external standard for ^{31}P-NMR. Accurate mass measurements were performed on a orthogonal acceleration quadrupole time-of-flight mass spectrometer (*Q-TOF-2*, *Micromass*, Manchester, UK) equipped with a standard electrospray ionization interface. HPLC-MS/MS was performed for sequence verification of the oligonucleotides. The mass spectrometer was coupled to a capillary HPLC (*CapLC*, *Waters*, Milford, MA). The chromatographic system was adapted from *Apffel et al.* [22]. A 500 μm × 15 mm reversed-phase *C18* column (*PepMap*, *LC Packings*, San Fransisco, CA) was used as stationary phase. Flow rate was set to 12 μl/min. Collision-induced dissociation (CID) with Ar gas in the collision cell was performed at 75 eV. All single-stranded oligomers were measured at a concentration of 5 μM in a buffer containing 10 mM Na-cacodylate, 100 mM NaCl, and 10 mM $MgCl_2$ (pH 7). Diastereoisomeric purities were determined by HPLC analysis on a *Waters 600 Controller* liquid chromatograph equipped with a *Waters 2487* UV detector, using a *Alltech Alltima Silica 5U* column (150 mm × 4.6 mm); eluent: hexane/EtOH 90:10 for **13a** and **13b** and hexane/EtOH 92:08 for **14a** and **14b**; flow rate: 1 ml/min; detection: 254 nm.

*(±)-(1*RS*,2*RS*,5*RS*)-2-Amino-5-(benzyloxy)cyclohexan-1-ol* (**2**). A soln. of **1** (1.18 g, 4.79 mmol) and Ph_3P (1.90 g, 7.23 mmol) in THF (30 ml) was stirred at r.t. for 20 min. After addition of H_2O (1.1 ml), the mixture was refluxed for 2 h. The solvent was evaporated under reduced pressure and co-evaporated with EtOH. The residue was purified by flash chromatography (FC; AcOEt/MeOH, 90:10 to 70:30) to give 1.03 g (97%) of **2**. Yellow powder. ^{1}H-NMR ($CDCl_3$): 7.26–7.34 (*m*, 5 arom. H); 4.55 (*s*, PhCH_2O); 3.39–3.47 (*m*, H–C(5)); 3.11–3.18 (*m*, H–C(1)); 2.45–2.52 (*m*, H–C(2)); 2.36–2.43 (*m*, H_a–C(6)); 2.02–2.08 (*m*, H_a–C(4)); 1.95 (br. *s*, HO–C(1),

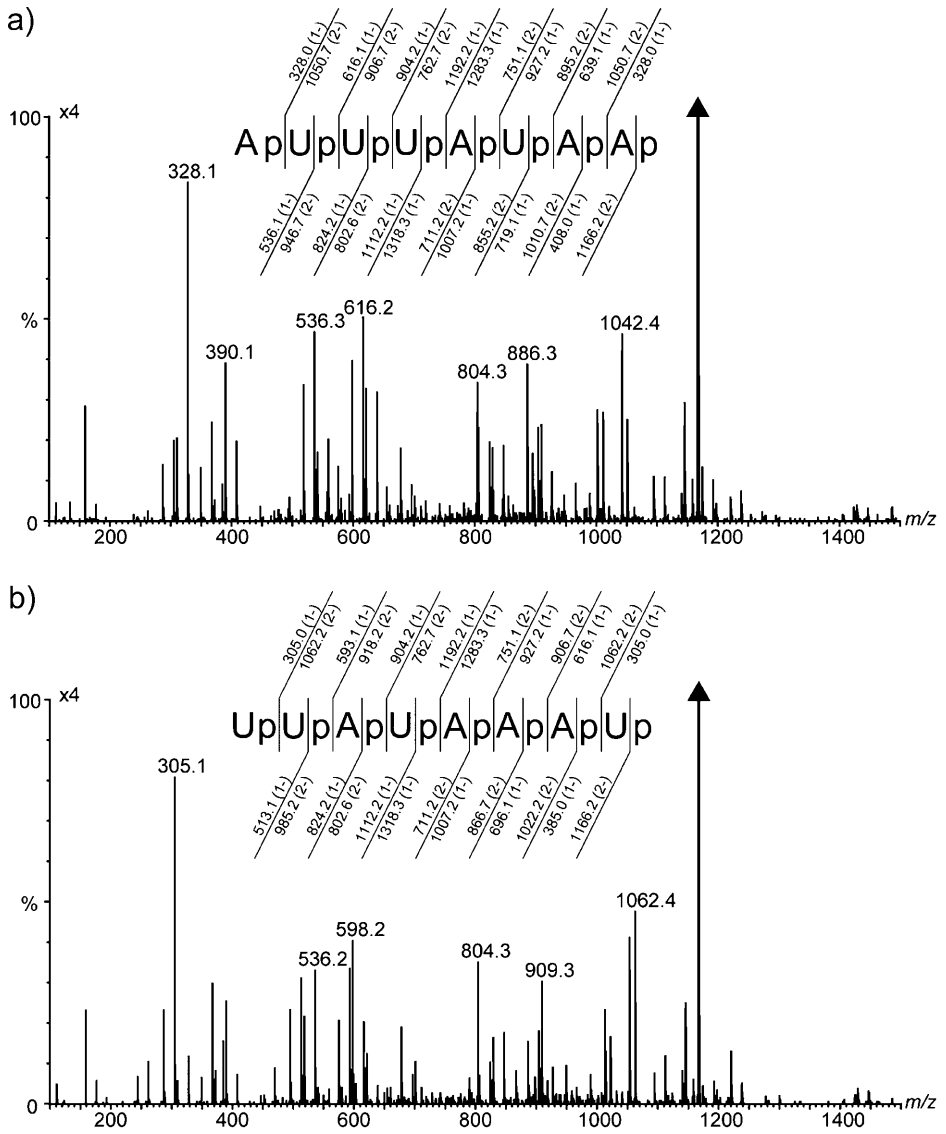


Fig. 4. a) *Fragment ion spectra of the triply charged precursor of cyclohexyl oligonucleotides AUUUAUAAp and* b) *UUAUAAAUp with the theoretical* m/z *values of the ions found in the spectra*

NH_2); 1.85–1.92 (*m*, H_a–C(3)); 1.35–1.44 (*m*, H_b–C(4), H_b–C(6)); 1.05–1.14 (*m*, H_b–C(3)). ^{13}C-NMR ($CDCl_3$): 138.55, 128.33, 127.49 (arom. C); 75.29 (C(5)); 73.04 (C(1)); 70.21 (Ph*C*H_2O); 56.09 (C(2)); 39.22 (C(6)); 30.54 (C(4)); 30.00 (C(3)). HR-MS: 244.1306 ($[M+Na]^+$, $C_{13}H_{19}NNaO_2^+$; calc. 244.1313).

*(±)-5-Amino-4-[(1′*RS*,2′*RS*,4′*RS*)-4′-(benzyloxy)-2′-hydroxycyclohexylamine]-6-chloropyrimidine* (**3**). A mixture of **2** (4.5 g, 20.35 mmol), 5-amino-4,6-dichloropyrimidine (3.51 g, 21.40 mmol), Et_3N (11.7 ml), and BuOH (70 ml) was heated under reflux for 24 h. The solvents were evaporated *in vacuo*, and the residue was purified by CC (CH_2Cl_2/MeOH, 99:1 to 85:15) to afford 5.89 g (83%) of **3**. Brown solid. ^{1}H-NMR ((D$_6$)DMSO): 7.71 (*s*, H–C(2)); 7.26–7.36 (*m*, 5 arom. H); 6.55 (*d*, *J* = 7.1, NH); 5.00 (*s*, NH_2); 4.75 (*d*,

J=5.6, HO–C(2′)); 4.49–4.55 (*m*, PhCH_2O); 3.75–3.82 (*m*, H–C(1′)); 3.39–3.48 (*m*, H–C(2′), H–C(4′)); 2.29–2.36 (*m*, H_a–C(3′)); 1.97–2.05 (*m*, H_a–C(5′), H_a–C(6′)); 1.21–1.35 (*m*, H_b–C(3′), H_b–C(5′)); 1.04–1.12 (*m*, H_b–C(6′)). ^{13}C-NMR ((D$_6$)DMSO): 152.35 (C(4)); 145.70 (C(6)); 139.18 (C(2)); 136.80, 128.25, 127.40, 127.29 (arom. C); 123.49 (C(5)); 74.99 (C(4′)); 69.57 (PhCH_2O); 69.33 (C(2′)); 56.21 (C(1′)); 40.86 (C(3′)); 30.48 (C(5′)); 27.29 (C(6′)). HR-MS: 349.1434 ([M+H]$^+$, $C_{17}H_{22}ClN_4O_2^+$; calc. 349.1431).

*(±)-9-[(1′*RS*,2′*RS*,4′*RS*)-4′-(Benzyloxy)-2′-hydroxycyclohexyl]-6-chloropurine* (**4**). Conc. HCl (1.2 ml) was added to a soln. of **3** (2 g, 5.73 mmol) in HC(OEt)$_3$ (52 ml). The mixture was stirred at r.t. overnight and concentrated to dryness. The residue was dissolved in THF (70 ml), and 0.5N HCl (70 ml) was added. After 2 h at r.t., the soln. was neutralized with 1N NaOH and diluted with CH_2Cl_2. The phases were separated, and the org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by FC (hexane/AcOEt 50:50 to 10:90) to give 1.56 g (76%) of **4**. White solid. ^{1}H-NMR ((D$_6$)DMSO): 8.75 (*s*, H–C(2)); 8.72 (*s*, H–C(8)); 7.28–7.37 (*m*, 5 arom. H); 5.09 (*d*, J=5.6, HO–C(2′)); 4.55–4.61 (*m*, PhCH_2O); 4.28–4.35 (*m*, H–C(1′)); 4.05–4.12 (*m*, H–C(2′)); 3.52–3.60 (*m*, H–C(4′)); 2.38–2.45 (*m*, H_a–C(3′)); 2.10–2.18 (*m*, H_a–C(5′), H_a–C(6′)); 1.96–2.02 (*m*, H_b–C(6′)); 1.36–1.46 (*m*, H_b–C(3′), H_b–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 152.24 (C(2)); 151.05 (C(6)); 148.90 (C(4)); 146.93 (C(8)); 139.02 (arom. C); 131.30 (C(5)); 128.29, 127.46, 127.36 (arom. C); 74.19 (C(4′)); 69.35 (PhCH_2O); 68.00 (C(2′)); 61.39 (C(1′)); 40.60 (C(3′)); 30.56 (C(5′)); 26.54 (C(6′)). HR-MS: 359.1274 ([M+H]$^+$, $C_{18}H_{20}ClN_4O_2^+$; calc. 359.1275).

*(±)-9-[(1′*RS*,2′*RS*,4′*RS*)-4′-(Benzyloxy)-2′-hydroxycyclohexyl]adenine* (**5**). A suspension of **4** (2 g, 5.6 mmol) in a MeOH soln. sat. with NH_3 (50 ml) was heated in a sealed tube at 100° overnight. NH_3 and MeOH were evaporated. The residue was purified by CC (CH_2Cl_2/MeOH 98:2 to 90:10) to afford 1.65 g (84%) of **5**. White powder. ^{1}H-NMR ((D$_6$)DMSO): 8.11 (*s*, H–C(2), H–C(8)); 7.28–7.38 (*m*, 5 arom. H); 7.12 (br. *s*, NH_2); 4.99 (*d*, J=5.6, HO–C(2′)); 4.55, 4.57 (*AB*, J=12.2, PhCH_2O); 4.05–4.16 (*m*, H–C(1′), H–C(2′)); 3.49–3.56 (*m*, H–C(4′)); 2.36–2.41 (*m*, H_a–C(3′)); 2.02–2.15 (*m*, H_a–C(5′), H_a–C(6′)); 1.87–1.91 (*m*, H_b–C(6′)); 1.32–1.42 (*m*, H_b–C(3′), H_b–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 156.01 (C(6)); 151.95 (C(2)); 149.76 (C(4)); 140.39 (C(8)); 139.09, 128.32, 127.48, 127.38 (arom. C); 119.28 (C(5)); 74.37 (C(4′)); 69.36 (PhCH_2O); 67.81 (C(2′)); 60.40 (C(1′)); 41.02 (C(3′)); 30.72 (C(5′)); 27.11 (C(6′)). HR-MS: 340.1170 ([M+H]$^+$, $C_{18}H_{22}N_5O_2^+$; calc. 340.1173).

*(±)-9-[(1′*RS*,2′*RS*,4′*RS*)-4′-(Benzyloxy)-2′-[(*tert*-butyl)dimethylsilyloxy]cyclohexyl]adenine* (**6**). To a prechilled (0°) soln. of **5** (947 mg, 2.79 mmol) and 1*H*-imidazole (1.08 g, 15.9 mmol) in DMF (28 ml) was added TBDMSCl (1.36 g, 9.02 mmol). After one night at r.t., Et_2O was added, and the mixture was washed with sat. aq. $NaHCO_3$ soln., 1N HCl, and H_2O. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by CC (CH_2Cl_2/MeOH 100:0 to 94:6) to give 1.02 g (81%) of **6**. White solid. ^{1}H-NMR ((D$_6$)DMSO): 8.10 (*s*, H–C(2)); 8.09 (*s*, H–C(8)); 7.28–7.36 (*m*, 5 arom. H); 7.08 (br. *s*, NH_2); 4.54, 4.59 (AB, J=12.1, PhCH_2O); 4.16–4.26 (*m*, H–C(1′), H–C(2′)); 3.54–3.61 (*m*, H–C(4′)); 2.27–2.34 (*m*, H_a–C(3′), H_a–C(6′)); 2.13–2.20 (*m*, H_a–C(5′)); 1.84–1.91 (*m*, H_b–C(6′)); 1.34–1.45 (*m*, H_b–C(3′), H_b–C(5′)); 0.55 (*s*, *t*-Bu); −0.18 (*s*, MeSi); −0.66 (*s*, MeSi). ^{13}C-NMR ((D$_6$)DMSO): 156.03 (C(6)); 151.87 (C(2)); 149.66 (C(4)); 140.55 (C(8)); 139.05, 128.29, 127.51, 127.39 (arom. C); 119.46 (C(5)); 74.24 (C(4′)); 70.04 (PhCH_2O); 69.57 (C(2′)); 60.43 (C(1′)); 41.38 (C(3′)); 30.55 (C(5′)); 26.19 (C(6′)); 25.32 (*Me*$_3$C); 17.19 (Me$_3$*C*); −4.73, −6.00 (Me$_2$Si). HR-MS: 454.2639 ([M+H]$^+$, $C_{24}H_{36}N_5O_2Si^+$; calc. 454.2638).

*(±)-9-[(1′*RS*,2′*RS*,4′*RS*)-2′-[(*tert*-Butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl]adenine* (**7**). A mixture of **6** (778 mg, 1.72 mmol), NH_4OCOH (922 mg, 14.6 mmol), and Pd/C (1g) in MeOH (42 ml) was refluxed for 5 h. NH_4OCOH (461 mg, 7.31 mmol) was added again, and heating was continued for 2 h. After cooling to r.t., the mixture was filtered through *Celite*, and the catalyst was washed with MeOH. The filtrate was concentrated to dryness, and the residue was purified by CC (CH_2Cl_2/MeOH 98:2 to 90:10) to yield 603 mg (96%) of **7**. White foam. ^{1}H-NMR ((D$_6$)DMSO): 8.11 (*s*, H–C(2)); 8.09 (*s*, H–C(8)); 7.06 (br. s, NH_2); 4.79 (*d*, J=4.6, HO–C(4′)); 4.17–4.23 (*m*, H–C(1′); 4.10–4.17 (*m*, H–C(2′)); 3.61–3.69 (*m*, H–C(4′)); 2.29–2.34 (*m*, H_a–C(6′)); 2.14–2.21 (*m*, H_a–C(3′)); 1.89–1.96 (*m*, H_a–C(5′)); 1.78–1.86 (*m*, H_b–C(6′)); 1.29–1.40 (*m*, H_b–C(3′), H_b–C(5′)); 0.55 (*s*, *Me*$_3$C); −0.18 (*s*, MeSi); −0.65 (*s*, MeSi). ^{13}C-NMR ((D$_6$)DMSO): 156.00 (C(6)); 151.86 (C(2)); 149.64 (C(4)); 140.51 (C(8)); 119.42 (C(5)); 70.20 (C(4′)); 66.46 (C(2′)); 60.39 (C(1′)); 44.60 (C(3′)); 33.98 (C(5′)); 26.50 (C(6′)); 25.31 (*Me*$_3$C); 17.20 (Me$_3$*C*); −4.76, −5.95 (Me$_2$Si). HR-MS: 364.2166 ([M+H]$^+$, $C_{17}H_{30}N_5O_2Si^+$; calc. 364.2169).

*(±)-*N^6*-Benzoyl-9-[(1′*RS*,2′*RS*,4′*RS*)-2′-[(*tert*-butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl]adenine* (**8**). To a prechilled (0°) soln. of **7** (552 mg, 1.52 mmol) in pyridine (8 ml) was added Me$_3$SiCl (1 ml, 7.82 mmol). After 1 h at 0°, BzCl (900 µl, 7.75 mmol) was added, and the mixture was stirred at r.t. overnight. After cooling to 0°, H_2O (2 ml) was added. The mixture was stirred for 5 min, and 25% aq. NH_4OH soln. (4 ml) was added. After 15 min at 0°, the solvents were evaporated. The residue was dissolved in MeOH (3 ml) and 25% aq. NH_4OH

soln. (25 ml). The mixture was stirred for 30 min and concentrated to dryness. The residue was partitioned between CH_2Cl_2 and 1N HCl, and washed with sat. aq. $NaHCO_3$ soln. and brine. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by CC (CH_2Cl_2/MeOH 99:1 to 92:8) to afford 521 mg (73%) of **8**. White foam. ^{1}H-NMR ((D$_6$)DMSO): 10.99 (*s*, NH); 8.68 (*s*, H–C(2)); 8.50 (*s*, H–C(8)); 8.04 (*d*, *J* = 7.4, 2 arom. H); 7.52–7.65 (*m*, 3 arom. H); 4.80 (br. *s*, HO–C(4′)); 4.30–4.36 (*m*, H–C(1′)); 4.20–4.28 (*m*, H–C(2′)); 3.65–3.73 (*m*, H–C(4′)); 2.26–2.36 (*m*, H$_a$–C(6′)); 2.19–2.25 (*m*, H$_a$–C(3′)); 1.89–2.00 (*m*, H$_a$–C(5′), H$_b$–C(6′)); 1.40 (*m*, H$_b$–C(3′), H$_b$–C(5′)); 0.54 (*s*, *Me*$_3$C); –0.18 (*s*, MeSi); –0.66 (*s*, MeSi). ^{13}C-NMR ((D$_6$)DMSO): 172.54 (NHCO); 150.82 (C(2)); 150.06 (C(6)); 148.07 (C(4)); 143.97 (C(8)); 132.20, 128.36 (arom. C); 125.86 (C(5)); 70.34 (C(4′)); 66.35 (C(2′)); 60.47 (C(1′)); 44.49 (C(3′)); 33.86 (C(5′)); 26.40 (C(6′)); 25.19 (*Me*$_3$C); 17.06 (Me$_3$*C*); –4.77, –5.91 (Me$_2$Si). HR-MS: 468.2421 ([*M* + H]$^+$, $C_{24}H_{34}N_5O_3Si^+$; calc. 468.2431).

*(±)-*N*-({[(1′*RS*,2′*RS*,4′*RS*)-4′-(Benzyloxy)-2′-hydroxycyclohexyl]amino}carbonyl)-3-methoxyprop-2-enamide* (**9**). $SOCl_2$ (160 µl, 2.19 mmol) was added to a soln. of 3-methoxyacrylic acid (129 mg, 1.26 mmol) in dry CH_2Cl_2 (1 ml). The mixture was refluxed for 3 h and concentrated to dryness. The residue was dissolved in dry toluene (1.3 ml), and AgOCN (332 mg, 2.21 mmol; dried *in vacuo* over P_2O_5 at 100° for 3 h in the dark) was added. The suspension was refluxed for 30 min and cooled to 0°. The supernatant liquor (900 µl) was added to a soln. of **2** (120 mg, 0.54 mmol) in dry DMF (2 ml) at –15°. The mixture was stirred at –15° for 2 h and then at r.t. overnight. The solvents were evaporated, and the residue was purified by FC (hexane/AcOEt 50:50 to 10:90) to afford 157 mg (83%) of **9**. White powder. ^{1}H-NMR ($CDCl_3$): 9.90 (*s*, NH); 8.81 (*d*, *J* = 7.3, NH); 7.66 (*d*, *J* = 12.2, C*H*=CHOMe); 7.26–7.36 (*m*, 5 arom. H); 5.34 (*d*, *J* = 12.2, CH=C*H*OMe); 4.55 (*s*, PhC*H*$_2$O); 3.90 (*d*, *J* = 4.6, HO–C(2′)); 3.66–3.74 (*m*, H–C(1′), MeO); 3.46–3.56 (*m*, H–C(2′), H–C(4′)); 2.32–2.38 (*m*, H$_a$–C(3′)); 2.00–2.08 (*m*, H$_a$–C(5′), H$_a$–C(6′)); 1.44–1.56 (*m*, H$_b$–C(3′), H$_b$–C(5′)); 1.30–1.38 (*m*, H$_b$–C(6′)). ^{13}C-NMR ($CDCl_3$): 168.26 (NH*C*(=O)-CH); 163.66 (NH*C*(=O)NH); 156.44 (*C*HOMe); 138.31, 128.39, 127.60, 127.52 (arom. C); 97.28 (*C*H=CHOMe); 74.51 (C(4′)); 71.97 (Ph*C*H$_2$O); 70.42 (C(2′)); 57.66 (C(1′)); 54.69 (MeO); 38.40 (C(3′)); 29.40 (C(5′)); 26.20 (C(6′)). HR-MS: 349.1768 ([*M* + H]$^+$, $C_{18}H_{25}N_2O_5^+$; calc. 349.1763).

*(±)-1-[(1′*RS*,2′*RS*,4′*RS*)-4′-(Benzyloxy)-2′-hydroxycyclohexyl]uracil* (**10**). A stirred suspension of **9** (845 mg, 2.43 mmol) in 2N H_2SO_4 (20 ml) was refluxed for 2 h. After cooling to 0°, the mixture was diluted with H_2O and neutralized with 2N NaOH. The soln. was concentrated *in vacuo* and co-evaporated with EtOH. The residual solid was extracted with EtOH, and the EtOH soln. was concentrated to dryness. The residue was purified by CC (CH_2Cl_2/MeOH 99:1 to 90:10) to give 560 mg (73%) of **10**. White powder. ^{1}H-NMR ((D$_6$)DMSO): 11.13 (*s*, NH); 7.63 (*d*, *J* = 7.8, H–C(6)); 7.26–7.37 (*m*, 5 arom. H); 5.53 (*d*, *J* = 7.8, H–C(5)); 5.02 (*d*, *J* = 5.6, HO–C(2′)); 4.50–4.54 (*m*, PhC*H*$_2$O); 4.07 (br. *s*, H–C(1′)); 3.73 (br. *s*, H–C(2′)); 3.38–3.45 (*m*, H–C(4′)); 2.29–2.35 (*m*, H$_a$–C(3′)); 2.04–2.10 (*m*, H$_a$–C(5′)); 1.66–1.72 (*m*, H$_a$–C(6′)); 1.58 (br. *s*, H$_b$–C(6′)); 1.22–1.35 (*m*, H$_b$–C(3′), H$_b$–C(5′)). ^{13}C-NMR ((D$_6$)DMSO): 163.33 (C(4)); 151.50 (C(2)); 139.07 (C(6)); 128.31, 127.45, 127.38 (5 arom. C); 100.98 (C(5)); 74.10 (C(4′)); 69.29 (O*C*H$_2$Ph); 66.59 (C(2′)); 40.85 (C(3′)); 30.71 (C(5′)); 25.72 (C(6′)). HR-MS: 317.1501 ([*M* + H]$^+$, $C_{17}H_{21}N_2O_4^+$; calc. 317.1501).

*(±)-1-{(1′*RS*,2′*RS*,4′*RS*)-4′-(Benzyloxy)-2′-[(*tert*-butyl)dimethylsilyloxy]cyclohexyl}uracil* (**11**). To a pre-chilled (0°) soln. of **10** (2.26 g, 7.14 mmol) and 1*H*-imidazole (2.43 g, 35.6 mmol) in DMF (35 ml) was added TBDMSCl (3.24 g, 21.5 mmol). After 4 h at r.t., Et_2O was added, and the mixture was washed with sat. aq. $NaHCO_3$, 1N HCl, and H_2O. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by CC (CH_2Cl_2/MeOH 100:0 to 95:5) to give 2.91 g (95%) of **11**. White solid. ^{1}H-NMR ((D$_6$)DMSO): 7.7 (br. *s*, H–C(6)); 7.27–7.34 (*m*, 5 arom. H); 5.53 (br. *d*, *J* = 5.5, H–C(5)); 4.50, 4.55 (*AB*, *J* = 12.2, PhC*H*$_2$O); 4.32 (br. *s*, H–C(1′)); 3.85 (br. *s*, H–C(2′)); 3.41–3.49 (*m*, H–C(4′)); 2.23–2.29 (*m*, H$_a$–C(3′)); 2.06–2.12 (*m*, H$_a$–C(5′)); 1.65–1.73 (*m*, 2 H–C(6′)); 1.26–1.40 (*m*, H$_b$–C(3′), H$_b$–C(5′)); 0.75 (*s*, *Me*$_3$C); 0.00 (*s*, MeSi); –0.12 (*s*, MeSi). ^{13}C-NMR ((D$_6$)DMSO): 163.11 (C(4)); 151.37 (C(2)); 139.00 (C(6)); 128.26, 127.44, 127.36 (arom. C); 101.13 (C(5)); 73.93 (C(4′)); 69.42 (Ph*C*H$_2$O); 66.55 (C(2′)); 41.32 (C(3′)); 30.43 (C(5′)); 25.45 (*Me*$_3$C, C(6′)); 17.34 (Me$_3$*C*); –4.15, –5.41 (Me$_2$Si). HR-MS: 431.2369 ([*M* + H]$^+$, $C_{23}H_{35}N_2O_4Si^+$; calc. 431.2366).

*(±)-1-{(1′*RS*,2′*RS*,4′*RS*)-2′-[(*tert*-Butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl}uracil* (**12**). To a cooled (–78°) soln. of **11** (800 mg, 1.86 mmol) in CH_2Cl_2 (54 ml) was added a 1M BCl_3 soln. in CH_2Cl_2 (19 ml). After 4 h at –78°, a mixture of pyridine and MeOH (13 ml/27 ml) was added. The mixture was allowed to warm to r.t. and concentrated to dryness. The residue was partitioned between AcOEt and H_2O. The org. phase was washed with H_2O, dried (Na_2SO_4), filtered, concentrated to dryness, and co-evaporated with toluene. The residue was purified by CC (CH_2Cl_2/MeOH 98:2 to 95:5) to provide 600 mg (95%) of **12**. White solid. ^{1}H-NMR ((D$_6$)DMSO): 11.16 (*s*, NH); 7.71 (br. *s*, H–C(6)); 5.53 (*d*, *J* = 7.6, H–C(5)); 4.17–4.29 (*m*, H–C(1′)); 3.76–3.90

(*m*, H–C(2′)); 3.40–3.61 (*m*, H–C(4′)); 2.09–2.15 (*m*, H_a–C(3′)); 1.81–1.87 (*m*, H_a–C(5′)); 1.54–1.66 (*m*, 2 H–C(6′)); 1.23–1.32 (*m*, H_b–C(3′), H_b–C(5′)); 0.74 (*s*, Me_3C); −0.01 (*s*, MeSi); −0.13 (*s*, MeSi). ^{13}C-NMR ((D_6)DMSO): 163.02 (C(4)); 151.24 (C(2)); 141.47 (C(6)); 101.09 (C(5)); 69.33 (C(4′)); 66.12 (C(2′)); 58.17(C(1′)); 44.52 (C(3′)); 33.83 (C(5′)); 25.41 (Me_3C, C(6′)); 17.29 (Me_3C); −4.25, −5.14 (Me_2Si). HR-MS: 341.1888 ($[M+H]^+$, $C_{16}H_{29}N_2O_4Si^+$; calc. 341.1896).

*(+)- and (−)-*N^6*-Benzoyl-9-{2′-[(*tert*-butyl)dimethylsilyloxy]-4′-[(*R*)-2-methoxy-2-phenylacetoxy]cyclohexyl}adenine* (**13a** and **13b**, resp.). DCC (316 mg, 1.53 mmol) was added to a soln. of **8** (590 mg, 1.26 mmol), (*R*)-*O*-methylmandelic acid (254 mg, 1.53 mmol) and DMAP (18 mg, 0.14 mmol) in CH_2Cl_2 (15 ml) at 0°. The mixture was allowed to warm to r.t. over 3 h and then washed with 1M aq. H_3PO_4 soln., sat. aq. $NaHCO_3$ soln., and H_2O. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified twice by CC (hexane/AcOEt 50:50 to 10:90) and prep. TLC (hexane/AcOEt 30:70) to afford 281 mg (36%) of **13a**, 277 mg (36%) of **13b**, and 89 mg (11%) of a mixture **13a**/**13b**.

Data of **13a**. ^{1}H-NMR ($CDCl_3$): 9.10 (br. *s*, NH); 8.75 (*s*, H–C(2)); 8.02 (*d*, *J* = 7.1, 2 arom. H); 7.91 (*s*, H–C(8)); 7.35–7.61 (*m*, 8 arom. H); 4.98–5.06 (*m*, H–C(4′)); 4.78 (*s*, C*H*(OMe)Ph); 4.30–4.37 (*m*, H–C(2′)); 4.19–4.26 (*m*, H–C(1′)); 3.43 (*s*, CH(O*Me*)Ph); 2.52–2.65 (*m*, H_a–C(6′)); 2.35–2.42 (*m*, H_a–C(3′)); 2.18–2.22 (*m*, H_b–C(6′)); 1.97–2.09 (*m*, H_a–C(5′)); 1.67–1.75 (*m*, H_b–C(3′)); 1.56–1.63 (*m*, H_b–C(5′)); 0.60 (*s*, Me_3C); −0.15 (*s*, MeSi); −0.69 (*s*, MeSi). ^{13}C-NMR ($CDCl_3$): 169.95 (COO); 164.50 (NHCO); 152.07 (C(2)); 151.85 (C(6)); 149.50 (C(4)); 143.03 (C(8)); 136.04, 132.66, 128.79, 128.62, 127.81, 127.164 (arom. C); 123.80 (C(5)); 82.45 (*C*H(OMe)Ph); 70.25 (C(4′)); 69.55 (C(2′)); 62.70 (C(1′)); 57.24 (MeO); 40.26 (C(3′)); 29.81 (C(5′)); 25.58 (C(6′)); 25.28 (Me_3C); 17.37 (Me_3C); −4.83, −5.90 (Me_2Si). HR-MS: 616.2944 ($[M+H]^+$, $C_{33}H_{42}N_5O_5Si^+$; calc. 616.2955).

Data of **13b**. ^{1}H-NMR ($CDCl_3$): 9.08 (*s*, NH); 8.76 (*s*, H–C(2)); 8.03 (*d*, *J* = 7.6, 2 arom. H); 7.91 (*s*, H–C(8)); 7.36–7.52 (*m*, 8 arom. H); 4.98–5.05 (*m*, H–C(4′)); 4.78 (*s*, C*H*(OMe)Ph); 4.26–4.33 (*m*, H–C(2′)); 4.11–4.18 (*m*, H–C(1′)); 3.43 (*s*, CH(O*Me*)Ph); 2.54–2.63 (*m*, H_a–C(6′)); 2.15–2.22 (*m*, H_a–C(3′), H_a–C(5′)); 2.04–2.10 (*m*, H_b–C(6′)); 1.47–1.58 (*m*, H_b–C(3′), H_b–C(5′)); 0.58 (*s*, Me_3C); −0.21 (*s*, MeSi); −0.70 (*s*, MeSi). ^{13}C-NMR ($CDCl_3$): 170.07 (COO); 164.48 (NHCO); 152.12 (C(2)); 151.89 (C(6)); 149.53 (C(4)); 143.03 (C(8)); 136.07, 133.77, 132.69, 128.83, 128.64, 127.84, 127.16 (arom. C); 123.48 (C(5)); 82.65 (*C*H-(OMe)Ph); 70.35 (C(4′)); 69.52 (C(2′)); 62.32 (C(1′)); 57.33 (MeO); 39.94 (C(3′)); 30.13 (C(5′)); 25.84 (C(6′)); 25.28 (Me_3C); 17.36 (Me_3C); −4.79, −5.86 (Me_2Si). HR-MS: 616.2957 ($[M+H]^+$, $C_{33}H_{42}N_5O_5Si^+$; calc. 616.2955).

*(+)- and (−)-1-{2′-[(*tert*-Butyl)dimethylsilyloxy]-4′-[(*R*)-2-methoxy-2-phenylacetoxy]cyclohexyl}uracil* (**14a** and **14b**, resp.). To a prechilled (0°) mixture of **12** (1.52 g, 5.40 mmol), DMAP (60 mg, 0.49 mmol), and (*R*)-*O*-methylmandelic acid (898 mg, 5.40 mmol) in CH_2Cl_2 (45 ml) was added DCC (1.11 g, 5.38 mmol). The mixture was then stirred at r.t. for 4 h. After dilution with CH_2Cl_2, the solid was filtered and the filtrate was washed with 1N aq. H_3PO_4 soln. sat. aq. $NaHCO_3$ soln., and H_2O. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by several CCs (hexane/AcOEt 40:60 to 10:90) to provide 938 mg (43%) of pure **14a**, 851 mg (39%) of pure **14b**, and 327 mg (15%) of a mixture **14a**/**14b**.

Data of **14a**. ^{1}H-NMR ((D_6)DMSO): 7.70 (br. *s*, H–C(6)); 7.35–7.39 (*m*, 5 arom. H); 5.58 (*d*, *J* = 7.8, H–C(5)); 4.90 (*s*, C*H*(OMe)Ph); 4.76–4.85 (*m*, H–C(4′)); 4.29 (br. *s*, H–C(1′)); 4.02 (br. *s*, H–C(2′)); 3.31 (*s*, CH(O*Me*)Ph); 2.16–2.22 (*m*, H_a–C(3′)); 1.73–1.79 (*m*, H_a–C(5′), H_a–C(6′)); 1.64–1.70 (*m*, H_b–C(6′)); 1.45–1.58 (*m*, H_b–C(3′)); 1.29–1.40 (*m*, H_b–C(5′)); 0.74 (*s*, Me_3C); −0.01 (*s*, MeSi); −0.12 (*s*, MeSi). ^{13}C-NMR ((D_6)DMSO): 169.82 (COO); 163.04 (C(4)); 151.28 (C(2)); 136.54 (C(6)); 128.57, 128.50, 127.10 (arom. C); 101.20 (C(5)); 81.42 (*C*H(OMe)Ph); 70.04 (C(4′)); 68.56 (C(2′)); 56.79 (MeO); 39.60 (C(3′) overlapped with the signals of DMSO); 29.40 (C(5′)); 25.40 (Me_3C); 24.50 (C(6′)); 17.31 (Me_3C); −4.29, −5.16 (Me_2Si). HR-MS: 489.2426 ($[M+H]^+$, $C_{25}H_{37}N_2O_6Si^+$; calc. 489.2421).

Data of **14b**. ^{1}H-NMR ((D_6)DMSO): 11.17 (*s*, NH); 7.71 (br. *s*, H–C(6)); 7.34–7.38 (*m*, 5 arom. H); 5.57 (*d*, *J* = 7.1, H–C(5)); 4.89 (*s*, C*H*(OMe)Ph); 4.75–4.86 (*m*, H–C(4′)); 4.31 (br. *s*, H–C(1′)); 3.95 (br. *s*, H–C(2′)); 3.31 (*s*, CH(O*Me*)Ph); 1.91–2.01 (*m*, H_a–C(3′), H_a–C(5′)); 1.67–1.75 (*m*, 2 H–C(6′)); 1.44–1.57 (*m*, H_b–C(5′)); 1.30–1.39 (*m*, H_b–C(3′)); 0.71 (*s*, Me_3C); −0.08 (*s*, MeSi); −0.15 (*s*, MeSi). ^{13}C-NMR ((D_6)DMSO): 169.89 (COO); 163.06 (C(4)); 151.32 (C(2)); 136.55 (C(6)); 128.59, 128.47, 127.11 (arom. C); 101.26 (C(5)); 81.50 (*C*H(OMe)Ph); 70.10 (C(4′)); 68.61 (C(2′)); 56.81 (MeO); 41.18 (C(3′)); 29.60 (C(5′)); 25.38 (Me_3C, C(6′)); 17.29 (Me_3C); −4.33, −5.17 (Me_2Si). HR-MS: 489.2426 ($[M+H]^+$, $C_{25}H_{37}N_2O_6Si^+$; calc. 489.2421).

N^6*-Benzoyl-9-{2′-[(*tert*-butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl}adenine* (**15a**). To a prechilled (0°) soln. of **13a** (180 mg, 0.29 mmol) in MeOH/dioxane (1.4 ml; 7:10 (*v*/*v*)) was added 2N NaOH (430 µl). After 5 min. of stirring, the reaction was quenched with AcOH. The solvents were partially concentrated, and the residue was partitioned between CH_2Cl_2 and H_2O. The org. phase was washed with H_2O, sat. aq. $NaHCO_3$ soln.

(2×) and brine, dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by CC (CH_2Cl_2/MeOH 98:2 to 90:10) to give 132 mg (96%) of **15a**. Yellow oil. HR-MS: 468.2430 ($[M+H]^+$, $C_{25}H_{34}N_5O_3Si^+$; calc. 468.2431).

N^6-*Benzoyl-9-{2′-[(*tert-*butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl}adenine* (**15b**). Compound **15b** was obtained by reaction of **13b** with 2N NaOH as described for **15a** in 66% yield as a yellow oil. HR-MS: 468.2426 ($[M+H]^+$, $C_{25}H_{34}N_5O_3Si^+$; calc. 468.2431).

*1-{2′-[(*tert-*Butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl}uracil* (**16a**). Compound **16a** was obtained by reaction of **14a** with 2N NaOH as described for **15a** in 94% yield as a white solid. HR-MS: 341.1888 ($[M+H]^+$, $C_{16}H_{29}N_2O_4Si^+$; calc. 341.1896).

*1-{2′-[(*tert-*Butyl)dimethylsilyloxy]-4′-hydroxycyclohexyl}uracil* (**16b**). Compound **16b** was obtained by reaction of **14b** with 2N NaOH as described for **15a** in 91% yield as a white solid. HR-MS: 341.1891 ($[M+H]^+$, $C_{16}H_{29}N_2O_4Si^+$; calc. 341.1896).

N^6-*Benzoyl-9-{2′-[(*tert-*butyl)dimethylsilyloxy]-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl}adenine* (**17a**). A soln. of **15a** (250 mg, 0.53 mmol) and MMTrCl (518 mg, 1.68 mmol) in pyridine (6.6 ml) was heated at 50° overnight. MMTrCl (173 mg, 0.56 mmol) was added again, and the same amount of MMTrCl was added after 7 h at 50°. The mixture was then stirred at r.t. for 2.5 days and concentrated to dryness. The residue was dissolved in CH_2Cl_2, and washed with 0.1N HCl and brine. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by CC (hexane/AcOEt 70:30 to 10:90) to give 350 mg (88%) of **17a**. White foam. ^{1}H-NMR ($CDCl_3$): 8.99 (*s*, NH); 8.75 (*s*, H–C(2)); 8.00 (*d*, *J* = 7.6, 2 arom. H); 7.85 (*s*, H–C(8)); 7.21–7.60 (*m*, 15 arom. H); 6.85 (*d*, *J* = 8.8, 2 arom. H); 4.02–4.10 (*m*, H–C(1′)); 3.87–3.95 (*m*, H–C(2′)); 3.79 (*s*, MeO); 3.70–3.78 (*m*, H–C(4′)); 2.19–2.29 (*m*, H_a–C(6′)); 1.81–1.90 (*m*, H_b–C(6′)); 1.55–1.68 (*m*, H_a–C(3′), H_a–C(5′)); 1.42–1.56 (*m*, H_b–C(3′), H_b–C(5′)); 0.52 (*s*, *Me*$_3$C); −0.35 (*s*, MeSi); −0.79 (*s*, MeSi). ^{13}C-NMR ($CDCl_3$): 164.39 (NHCO); 158.63 (*C*OMe); 151.99 (C(2)); 151.76 (C(6)); 149.37 (C(4)); 145.59, 145.36 (arom. C); 143.31 (C(8)); 136.46, 132.65, 130.67, 128.83, 128.59, 128.49, 127.78, 127.74, 127.01 (arom. C); 123.42 (C(5)); 113.12 (arom. C); 86.65 (Ar$_3$*C*O); 70.06 (C(4′)); 69.74 (C(2′)); 62.83 (C(1′)); 55.15 (MeO); 42.78 (C(3′)); 32.60 (C(5′)); 26.39 (C(6′)); 25.30 (*Me*$_3$C); 17.33 (Me$_3$*C*); −4.73, −5.95 (Me$_2$Si). HR-MS: 740.3631 ($[M+H]^+$, $C_{44}H_{50}N_5O_4Si^+$; calc. 740.3632).

N^6-*Benzoyl-9-{2′-[(*tert-*butyl)dimethylsilyloxy]-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl}adenine* (**17b**). Compound **17b** was obtained from **15b** by reaction with MMTrCl in pyridine as described for **17a** in 84% yield as a yellow oil. Spectroscopic data are the same as for **17a**.

*1-{2′-[(*tert-*Butyl)dimethylsilyloxy]-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl}uracil* (**18a**). A soln. of **16a** (600 mg, 1.76 mmol) and MMTrCl (1.63 g, 5.28 mmol) in pyridine (22 ml) was heated at 50° overnight. MMTrCl (543 mg, 1.76 mmol) was added again, and heating was continued for 24 h. The mixture was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed with 0.1N HCl, sat. aq. $NaHCO_3$ soln., and brine. The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness. The residue was purified by FC (hexane/AcOEt 80:20 to 10:90) to afford 881 mg (82%) of **18a**. White foam. ^{1}H-NMR ((D$_6$)DMSO): 11.10 (*s*, NH); 7.65 (br. *s*, H–C(6)); 7.29–7.46 (*m*, 12 arom. H); 6.90 (*d*, *J* = 8.8, 2 arom. H); 5.49 (br. *d*, *J* = 7.1, H–C(5)); 4.14–4.25 (*m*, H–C(1′)); 3.79 (*s*, MeO); 3.54–3.63 (*m*, H–C(4′)); 3.47–3.54 (*m*, H–C(2′)); 1.56–1.63 (*m*, H_a–C(5′)); 1.49–1.55 (*m*, H_a–C(6′)); 1.37–1.46 (*m*, H_b–C(5′), H_b–C(6′)); 1.26–1.32 (*m*, H_a–C(3′)); 1.20–1.25 (*m*, H_b–C(3′)); 0.66 (*s*, *Me*$_3$C); −0.23 (*s*, MeSi); −0.26 (*s*, MeSi). ^{13}C-NMR ((D$_6$)DMSO): 163.04 (C(4)); 158.37 (*C*OMe); 151.38 (C(2)); 145.69, 145.27 (arom. C); 141.72 (C(6)); 135.92, 130.43, 128.24, 128.04, 127.88, 127.80, 126.91, 113.12 (arom. C); 101.08 (C(5)); 86.03 (Ar$_3$*C*O); 69.34 (C(2′), C(4′)); 57.83 (C(1′)); 55.04 (MeO); 42.43 (C(3′)); 32.23 (C(5′)); 25.40 (*Me*$_3$C, C(6′)); 17.25 (Me$_3$*C*); −4.26, −5.33 (Me$_2$Si). HR-MS: 613.3093 ($[M+H]^+$, $C_{36}H_{45}N_2O_5Si^+$; calc. 613.3098).

*1-{2′-[(*tert-*Butyl)dimethylsilyloxy]-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl}uracil* (**18b**). Compound **18b** was obtained by reaction of **16b** with MMTrCl in pyridine as described for **18a** in quant. yield as a yellow foam. Spectroscopic data are the same as for **18a**.

N^6-*Benzoyl-9-{2′-hydroxy-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl}adenine* (**19a**). 1M Bu_4NF in THF (910 µl) was added to a soln. of **17a** (330 mg, 0.45 mmol) in THF (3 ml). After 24 h at r.t., 1M Bu_4NF (450 µl) was added again, and stirring was continued overnight. The solvent was evaporated. The residue was dissolved in Et_2O, and washed with H_2O and brine. The org. phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified twice by FC (CH_2Cl_2/MeOH 99:1 to 98:02) to give 226 mg (81%) of **19a**. White powder. $[\alpha]_D^{20} = -20.6$ (*c* = 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 9.10 (br. *s*, NH); 8.52 (*s*, H–C(2)); 8.00 (*d*, *J* = 7.6, 2 arom. H); 7.72 (*s*, H–C(8)); 7.22–7.59 (*m*, 15 arom. H); 6.84 (*d*, *J* = 8.8, 2 arom. H); 4.11–4.17 (*m*, H–C(1′)); 3.93–4.00 (*m*, H–C(2′)); 3.79 (*s*, MeO); 3.62–3.69 (*m*, H–C(4′)); 1.98–2.05 (*m*, H_a–C(3′)); 1.74–1.81 (*m*, 2 H–C(6′); 1.61 (*q*, *J* = 11.5, H_b–C(3′)); 1.31–1.41 (*m*, H_a–C(5′)); 1.22–1.29 (*m*,

H_b–C(5′)). ^{13}C-NMR ($CDCl_3$): 164.69 (NHCO); 158.68 (*C*OMe); 152.09 (C(2)); 151.83 (C(6)); 148.88 (C(4)); 145.48, 145.38 (arom. C); 143.24 (C(8)); 136.56, 133.66, 132.71, 130.52, 128.77, 128.57, 127.97, 127.82, 126.99 (arom. C); 122.61 (C(5)); 88.68 (Ar_3*C*O); 69.92 (C(4′)); 69.61 (C(2′)); 61.98 (C(1′)); 55.23 (MeO); 41.84 (C(3′)); 32.30 (C(5′)); 27.43 (C(6′)). HR-MS: 626.2768 ($[M+H]^+$, $C_{38}H_{36}N_5O_4^+$; calc. 626.2767).

N^6-*Benzoyl-9-[2′-hydroxy-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl]adenine* (**19b**). Compound **19b** was obtained from **17b** by reaction with 1M Bu_4NF in THF as described for **19a** in 84% yield as a white solid. Spectroscopic data are the same as for **19a**. $[\alpha]_D^{20} = +22.4$ ($c = 0.5$, $CHCl_3$).

1-[2′-Hydroxy-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl]uracil (**20a**). Compound **20a** was obtained by reaction of **18a** with 1M Bu_4NF in THF as described for **19a** in 61% yield as a white foam. $[\alpha]_D^{20} = -18.8$ ($c = 0.5$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 10.00 (br. *s*, NH); 7.20–7.52 (*m*, 12 arom. H); 6.98 (*d*, $J = 8.1$, H–C(6)); 6.81 (*d*, $J = 8.5$, 2 arom. H); 5.57 (*d*, $J = 8.1$, H–C(5)); 4.15–4.26 (*m*, H–C(1′)); 3.77 (*s*, MeO); 3.70 (br. *s*, HO–C(2′)); 3.43–3.51 (*m*, H–C(4′)); 3.34–3.42 (*m*, H–C(2′)); 1.97–2.03 (*m*, H_a–C(3′)); 1.57–1.66 (*m*, H_b–C(3′), H_a–C(6′)); 1.33–1.44 (*m*, H_a–C(5′)); 1.12–1.29 (*m*, H_b–C(5′), H_b–C(6′)). ^{13}C-NMR ($CDCl_3$): 163.62 (C(4)); 158.59 (*C*OMe); 152.01 (C(2)); 145.43, 145.35 (arom. C); 141.17 (C(6)); 136.51, 130.49, 129.19, 128.55, 127.82, 127.73, 126.91, 113.07 (arom. C); 102.34 (C(5)); 86.60 (Ar_3*C*O); 70.05 (C(4′)); 69.07 (C(2′)); 61.38 (C(1′)); 55.17 (O*C*H$_3$); 42.03 (C(3′)); 32.16 (C(5′)); 26.34 (C(6′)). HR-MS: 521.2058 ($[M+Na]^+$, $C_{30}H_{30}N_2NaO_5^+$; calc. 521.2052).

1-[2′-Hydroxy-4′-[(4-methoxyphenyl)diphenylmethoxy]cyclohexyl]uracil (**20b**). Compound **20b** was obtained by reaction of **18b** with 1M Bu_4NF in THF as described for **19a** in 70% yield as a white powder. Spectroscopic data are the same as for **20a**. $[\alpha]_D^{20} = +19.8$ ($c = 0.5$, $CHCl_3$).

General Procedure for the Synthesis of Phosphoramidite Building Blocks. A soln. of modified nucleoside (see *Table 1*) in CH_2Cl_2 was treated with dry EtN(i-Pr)$_2$ (3 equiv.) and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (1.5 equiv.). After 45 min. at r.t., the mixture was cooled to 0°, and the reaction was quenched by addition of cold 5% aq. $NaHCO_3$ soln. The mixture was stirred further for 10 min., and then diluted with CH_2Cl_2, and washed with cold 5% aq. $NaHCO_3$ soln. and brine (3×). The org. phase was dried (Na_2SO_4), filtered, concentrated to dryness and purified by CC (hexane/acetone/1% pyridine). Appropriate fractions were combined, concentrated to dryness, and co-evaporated with toluene (3×) and CH_2Cl_2 to give the phosphoramidite as an oil. Dissolution in 1.5 ml of CH_2Cl_2 and double precipitation in 160 ml of cooled (−70°) hexane afforded the desired product as a powder. Starting quantities, yields, mass analysis, and ^{31}P-NMR data are given in *Table 2*.

Table 2. *Analytical Data for the Phosphoramidite Derivatives*

Compound	mmol of starting materials	Yield (%)	HR-MS $[M+H]^+$		^{31}P-NMR in $CDCN_3$
			Calc.	Found	
19a	0.29	83	for $C_{47}H_{53}N_7O_5P$		148.47, 147.48
			826.3845	826.3843	
19b	0.19	83	for $C_{47}H_{53}N_7O_5P$		148.46, 147.46
			826.3845	826.3835	
20a	0.36	88	for $C_{39}H_{48}N_4O_6P$		148.66, 146.96
			699.3311	699.3317	
20b	0.36	78	for $C_{39}H_{48}N_4O_6P$		148.65, 146.96
			699.3311	699.3322	

Solid-Phase Oligonucleotide Synthesis and Analysis. Oligonucleotide synthesis was performed on an *Expedite*™ DNA synthesizer (*Applied Biosystems*) by using the phosphoramidite approach. The standard DNA assembly protocol was used with 0.25M ETT as the activator and a 5-min coupling time using 0.07M of the newly synthesized amidites. A universal 3′-phosphate CPG-support was used, prepared according to *Kumar* [23], to afford 3′-phosphorylated oligonucleotides. The oligomers were deprotected and cleaved from the solid support by treatement with conc. aq. NH_3 (55°, 16 h). After gel filtration on a *NAP-10*® column (*Sephadex G25-DNA* grade; *Pharmacia*) with H_2O as eluent, the crude was analyzed on a *Mono-Q*® *HR 5/5* anion-exchange column, after which purification was achieved on a *Mono-Q*® *HR 10/10 column* (*Pharmacia*) with the following gradient system ($A = 10$ mM NaOH, pH 12.0, 0.1M NaCl; $B = 10$ mM NaOH, pH 12.0, 0.9M NaCl; gradient used depended on the oligomers; flow rate: 2 ml/min). The low-pressure liquid chromatography system

consisted of a *Merck-Hitachi L 6200 A* intelligent pump, a *Mono Q® HR 10/10* column (*Pharmacia*), a *Uvicord SII 2138* UV detector (*Pharmacia-LKB*), and a recorder. The product-containing fraction was desalted on a *NAP-10®* column and lyophilized.

Oligonucleotides were characterized, and their purity was checked by HPLC/MS on a cap. chromatograph (*CapLC*, *Waters*, Milford, MA). Columns of 150 mm × 0.3 mm length (*LCPackings*, San Francisco, CA) were used. Oligonucleotides were eluted with a triethylammonium-1,1,1,3,3,3-hexafluoropropan-2-ol MeCN solvent system. Flow rate was 5 µl/min. Electrospray spectra were acquired on an orthogonal acceleration/time-of-flight mass spectrometer (*Q-TOF-2*, *Micromass*, Manchester, UK) in negative-ion mode. Scan time used was 2 s. The combined spectra from a chromatographic peak were deconvoluted using the *MaxEnt* algorithm of the software (Masslynx 3.4, *Micromass*, Manchester, UK). Theoretical oligonucleotide masses were calculated using the monoisotopic element masses.

The authors thank Dr. *Marie-Jesús Pérez-Pérez* for $[\alpha]_D$ determinations, and the K.U. Leuven for financial support (*I.D.O.*).

REFERENCES

[1] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607.
[2] R. C. Mucic, J. J. Storhoff, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 12674.
[3] E. Lescrinier, M. Froeyen, P. Herdewijn, *Nucl. Acids. Res.* **2003**, *31*, 2975.
[4] S. Pitsch, S. Wendeborn, R. Krishnamurthy, A. Holzner, M. Minton, M. Bolli, C. Miculka, N. Windhab, R. Micura, M. Stanek, B. Jaun, A. Eschenmoser, *Helv. Chim. Acta* **2003**, *86*, 4270.
[5] M. Chini, P. Crotti, L. A. Flippin, F. Macchia, *J. Org. Chem.* **1991**, *56*, 7043.
[6] D. A. Prins, *Helv. Chim. Acta* **1957**, *40*, 1621.
[7] M. Chini, P. Crotti, L. A. Flippin, F. Macchia, *J. Org. Chem.* **1990**, *55*, 4265.
[8] R. Vince, J. Donovan, *J. Am. Chem. Soc.* **1969**, *12*, 175.
[9] R. G. Bhushan, R. Vince, *Bioorg. Med. Chem.* **2002**, *10*, 2325.
[10] P. Wang, B. Gullen, M. G. Newton, Y.-C. Cheng, R. F. Schinazi, C. K. Chu, *J. Med. Chem.* **1999**, *42*, 3390.
[11] G. S. Ti, B. L. Gaffney, R. A. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 1316.
[12] G. Shaw, R. N. Warrener, *J. Chem. Soc.* **1958**, 153.
[13] G. Shaw, R. N. Warrener, *J. Chem. Soc.* **1958**, 157.
[14] Y. F. Shealy, C. A. O'Dell, *J. Heterocycl. Chem.* **1976**, *13*, 1015.
[15] N. Gauvry, F. Huet, *J. Org. Chem.* **2001**, *66*, 583.
[16] H. Ohrui, S. Kohgo, K. Kitano, S. Sakata, E. Kodama, K. Yoshimura, M. Matsuoka, S. Shigeta, H. Mitsuya, *J. Med. Chem.* **2000**, *43*, 4516.
[17] Y. Maurinsh, H. Rosemeyer, R. Esnouf, A. Medvedovici, J. Wang, G. Ceulemans, E. Lescrinier, C. Hendrix, R. Busson, P. Sandra, F. Seela, A. Van Aerschot, P. Herdewijn, *Chem.–Eur. J.* **1999**, *5*, 2139.
[18] J. Wang, J. Morral, C. Hendrix, P. Herdewijn, *J. Org. Chem.* **2001**, *66*, 8478.
[19] J. S. Nelson, K. L. Fearon, M. Q. Nguyen, S. N. McCurdy, J. E. Frediani, M. F. Foy, B. L. Hirschbein, *J. Org. Chem.* **1997**, *62*, 7278.
[20] S. A. McCluckey, S. Habibi-Goudarzi, *J. Am. Chem. Soc.* **1993**, *115*, 12085.
[21] J. Ni, S. C. Pomerantz, J. Rozenski, Y. Zhang, J. A. McCloskey, *Anal. Chem.* **1996**, *68*, 1989.
[22] A. Apffel, J. A. Chakel, S. Fischer, K. Lichtenwalter, W. S. Hancock, *Anal. Chem.* **1997**, *69*, 1320.
[23] A. Kumar, *Nucleosides Nucleotides* **1993**, *12*, 441.

Received September 1, 2005