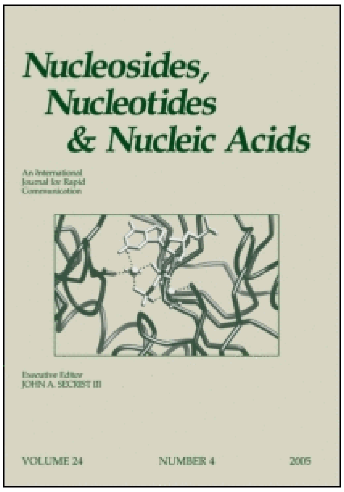

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Synthesis of D-Altritol Nucleosides with a 3′-*O-Tert*-Butyldimethylsilyl Protecting Group[†]

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ABSTRACT

Four D-altritol nucleosides with a 3′-*O-tert*-butyldimethylsilyl protecting group are synthesized (base moieties are adenine, guanine, thymine and 5-methylcytosine). The nucleosides are obtained by ring opening reaction of 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol. Optimal reaction circumstances (NaH, LiH, DBU, phase transfer, microwave irridation) for the introduction of the heterocycles are base-specific. For the introduction of the 3′-*O*-silyl protecting group, long reaction times and several equivalents of *tert*-butyldimethylsilyl chloride are needed.

Key Words: D-altritol nucleosides; *3′-O-tert*-butyldimethylsilyl protecting group; 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol.

[†] In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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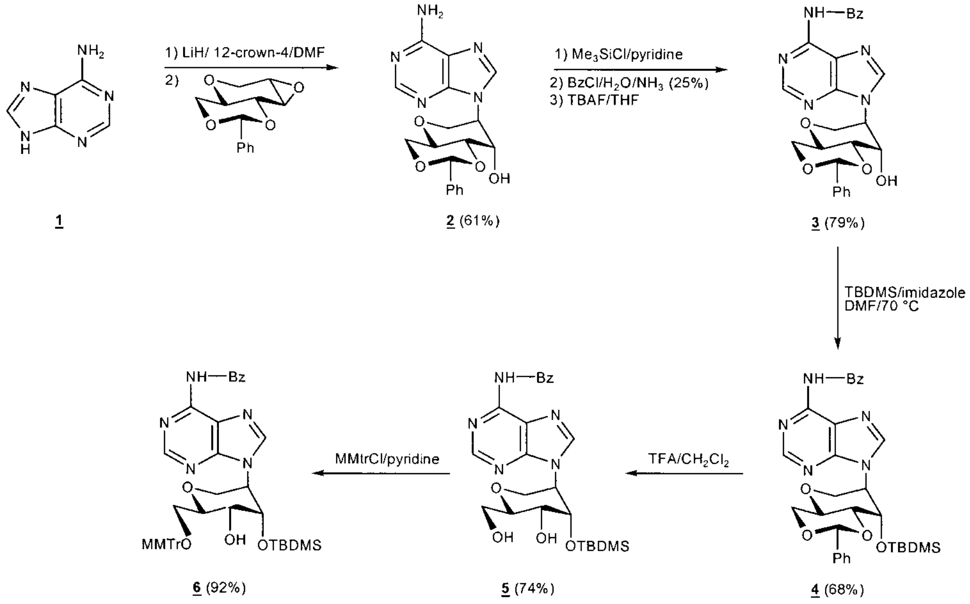
INTRODUCTION

D-altritol Nucleic Acids (ANA) are oligonucleotides with a phosphorylated D-altritol backbone and nucleobases introduced in the 2-position of the hexitol ring. ANA hybridize strongly and sequence selective with RNA in an antiparallel way.[1] These oligonucleotides were previously synthesized from 3-*O*-benzoyl-2-deoxy-4-*O*-[N,N-diisopropyl(2-cyanoethyl)]phosphoramidite-6-*O*-monomethoxytrityl-D-altritol building blocks.[1,2] The disadvantage of this approach is the instability of the 3-*O*-benzoyl group due to migration reaction of the benzoyl protecting group from the 3-position to the 4-position. This migration reaction is very fast due to the axial orientation of the 3-OH group and the equatorial orientation of the 4-OH group. A similar migration reaction (from the 2′-OH to the 3′-OH group) in the case of ribonucleosides[3] was one of the reasons for the introduction of the *tert*-butyldimethylsilyl protecting group for the 2′-OH function in RNA synthesis.[4] The introduction of a *tert*-butyldimethylsilyl group in an axially oriented hydroxyl group of a pyranosyl nucleoside is, however, more difficult than the introduction of the same protecting group on a secondary hydroxyl group of a ribofuranose nucleoside. More vigorous reaction conditions are needed. Here we describe the synthesis of four new building blocks, with a 3-*O*-*tert*-butyldimethylsilyl protecting group, that will be used for ANA synthesis.

CHEMISTRY

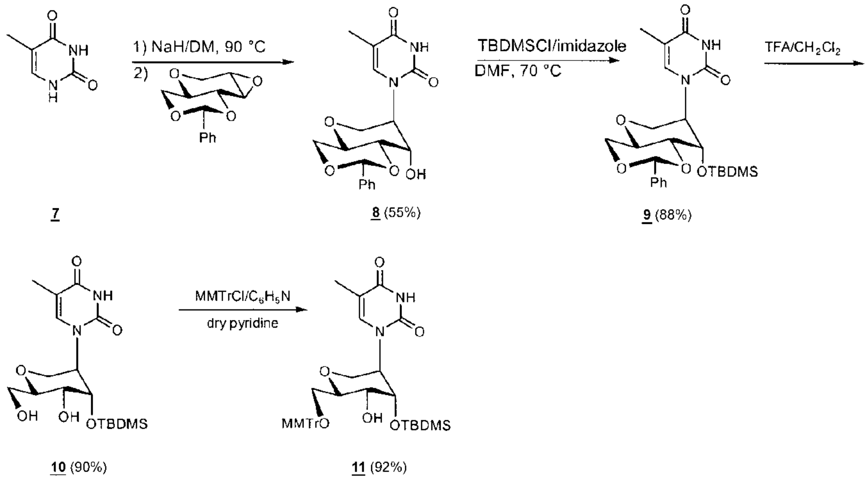
The easiest way to obtain D-altritol nucleosides, is by epoxide opening of 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol using nucleobases. This sugar compound can be prepared from commercially available tetraacetyl-α-D-bromoglucose in 5 steps (54% overall yield).[5] The advantage of this approach is that a D-altritol nucleoside is obtained with a free 3′-OH group and a protected 4′-OH and 6′-OH group, which avoid problems with the regioselective introduction of a protecting group in the 3′-position. Different conditions were evaluated for the nucleophilic opening of the epoxide by the salts of nucleobases. As well classical sodium and lithium salts, as a more soft base (DBU) and new technologies (phase transfer catalyst using tricaprylylmethylammonium chloride or microwave assisted synthesis) were explored.

Reaction of the sodium salt of adenine 1 (3 equiv.) with 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol in DMF at 120°C for 18 h. yielded 70% of 2-(adenin-9-yl)-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol **2**.[2] When using the lithium salt of adenine in the presence of 12-crown-4-ether the reaction could be speeded up (1 h.) and somewhat higher yields (80%) were obtained (Scheme 1). The use of DBU as base or a phase transfer catalyst or microwave circumstances didn't improve the yield (see further, for the observed differences when using the 2-amino-6-chloropurine base). Monobenzoylation at the N^6-position of the adenine base was carried out with benzoyl chloride using the transient protection strategy.[6] Previously, we reported the difficulties obtained for introduction of a *tert*-butyldimethylsilyl protecting group in the 3-*O*-position of 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol nucleosides (base moiety in the 2-position).[2] Due to steric hindrance, low amounts of materials were obtained. In order to force the reaction to completion, we tried more vigorous reaction conditions using a 5-fold excess of *tert*-butyldimethylsilyl chloride and a 7-fold excess of imidazole (both reagents were added in three portions) and a reaction

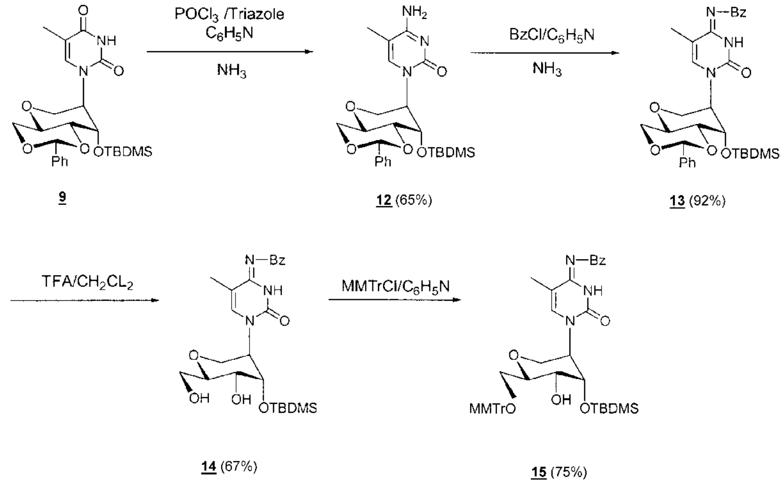


Scheme 1. Synthesis of 1,5-anhydro-2-(N^5-benzoyladenin-9-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-6-*O*-monomethoxytrityl-D-*altro*-hexitol.

time of 38 h at 70°C in DMF as solvent. In these circumstances, 1,5-anhydro-4,6-*O*-benzylidene-2-(N^6-benzoyladenin-9-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-*altro*-hexitol **4** was obtained in 68% yield. Removal of the benzylidene protecting group could be done with trifluoroacetic acid in dichloromethane without migration of the 3-*O*-silyl protecting group. Likewise, the 6-*O*-monomethoxytrityl group can be



Scheme 2. Synthesis of 1,5-anhydro-2-deoxy-3-*O*-*tert*-butyldimethylsilyl-6-*O*-monomethoxytrityl-2-(thymlin-1-yl)-D-*altro*-hexitol.



Scheme 3. Synthesis of 1,5-anhydro-2-(N^4-benzoyl-5-methylcytosin-1-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-6-*O*-monomethoxytrityl-D-*altro*-hexitol.

introduced under common reaction circumstances (pyridine, room temperature) without (3′→4′) *tert*-butyldimethylsilyl migration, which was difficult to perform using a 3-*O*-benzoyl protecting group.[2] Overall, this reaction scheme offers a better yield of the individual steps and the migration problems of the protecting group could be avoided.

The compound with the thymine and 5-methylcytosine base is described here for the first time (Schemes 2 and 3). The sodium salt of thymine **7** was reacted with 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-altritol in DMF at 130°C for 18 h yielding 55% of 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-2-(thymin-1-yl)-D-*altro*-hexitol **8**. Introduction of the 3-*O*-*tert*-butyldimethylsilyl protecting group was carried out with *tert*-butyldimethylsilyl chloride and imidazole in DMF (yield 88%). After removal of the benzylidene protecting group (90% yield), the primary hydroxyl group was protected with a monomethoxytrityl group (92% yield). These reactions occur without any problems dealing with protecting group migration from the 3′-*O*-axial position to the

Table 1. Nucleophilic opening of epoxide in the presence of nucleoside bases in DMF.[a]

	NaH	Aliquat 336 K_2CO_3[c]	DBU	MW irradiation
Guanine	–	0	0	10
iso-Butyrylguanine	–	traces	–	traces
2-Amino-6-chloropurine	45	70[b]	45[b]	35
Thymine	55	0	75	75
Uracil	86	–	96	–
Adenine	70	55	70	50

[a] For conditions, see experimental section.

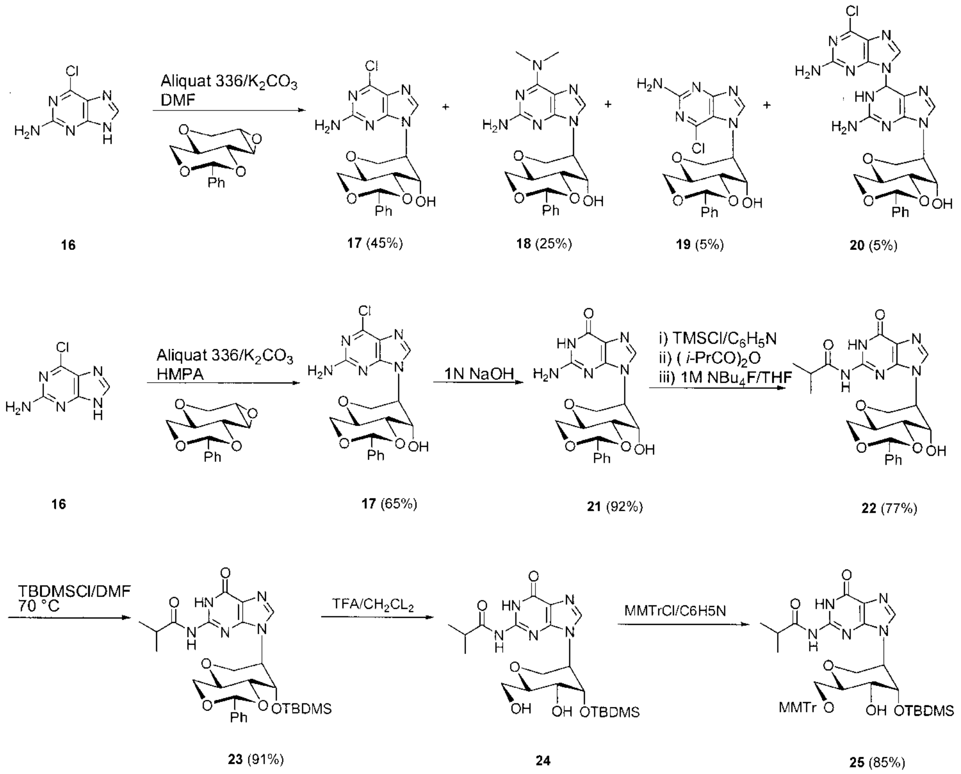
[b] HMPA.

[c] Aliquat 336/K_2CO_3. tricaprylylmethylammonium chloride.

4′-*O*-equatorial position. The crucial reaction is the epoxide opening using the pyrimidine base. The approach we followed here was the same as previously used for the synthesis of the uracil congener.[2] Yields were higher with the uracil base (88%) than with the thymine base (55%). Later on, we found out that the reaction using DBU instead of NaH or the use of microwave irridation, gives higher yields of the pyrimidine nucleosides (Table 1) i.e. 75% and 96% respectively (which was not the case with the adenine base).

1,5-Anhydroxy-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-(thymin-1-yl)-D-*altro*-hexitol **19** was used as starting material for the synthesis of the protected 5-methylcytosine congener (Scheme 3). The method used is 1,2,4-triazolyl activation of the 4-position of the thymine base, followed by substitution with ammonia (65% yield).[7] The N^4-position is protected with a benzoyl group, followed by benzylidene removal and 6-*O*-monomethoxytritylation. For all cases investigated, it seems that the conversion of the uracil/thymine base in the cytosine/methylcytosine base is a better way (higher yield) to obtain the 4-aminopyrimidine nucleosides than the direct opening reaction of the epoxide ring with the salts of the respective nucleobases (cytosine and 5-methylcytosine).

As could be expected, the guanine nucleoside is a particular case. Previously, we described the epoxide opening using the sodium salt of 2-amino-6-chloropurine in DMF



Scheme 4. Synthesis of 1,5-anhydro-3-*O*-*tert*-butyldimethylsilyl-2-(N^2-*iso*-butylguanin-9-yl)-2-deoxy-6-*O*-monomethoxytrityl-D-*altro*-hexitol.

in 40% yield (Scheme 4). Besides the major product, two side compounds were identified, i.e. the N-7-substituted compound and the bis-purinyl nucleoside. The same reaction using the lithium salt of N_2-acetyl-2-amino-6-[2-(trimethylsilyl)ethoxy)purine] afforded the protected guanine nucleoside in 45% yield (after deacetylation).[2] These results are unsatisfactory for large scale synthesis of the altritol nucleosides. The reaction in the presence of aliquat 336/K_2 CO_3 in DMF gives 45% of the described compound together with three side compounds. The additional side compound, observed here, is the N-9-substituted 2-amino-4-dimethylaminopurine nucleoside (Scheme 4). By utilizing the same phase transfer catalyst but in HMPA as solvent, side product formation could be avoided and the desired compound was obtained in 70% yield. Reactions with related bases (guanine and N_2-*iso*-butyrylguanine) did not lead to the condensation product. The 6-chloro-2-aminopurine base was converted into the guanine base, followed by transient protection procedure, to introduce the isobutyryl group and 3-*O*-silylation. After removal of the benzylidene group, the primary hydroxyl group was protected with monomethoxytrityl chloride. All the deprotection and protection reactions could be carried out in satisfactory yields (77%–92%).

CONCLUSION

The four building blocks for the synthesis of altritol nucleic acids were obtained from 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol. The optimal circumstances for ring opening of the oxirane is base specific. Uracil and thymine are used as DBU salt, or introduced by microwave irridation. Adenine is introduced as lithium salt, but the sodium and DBU salt of adenine base are useful alternatives. For the introduction of the 2-amino-6-chloropurine base, phase transfer conditions and HMPA as solvent is the method of choice. Introduction of the 3-*O*-*tert*-butyldimethylsilyl protecting group needs more vigorous reaction conditions than described for ribofuranose nucleosides. These building blocks will be used for oligonucleotide synthesis after conversion in the phosphoramidites.

EXPERIMENTAL SECTION

Tetra-*O*-acetyl-α-D-bromoglucose was provided by Fluka; adenine, cytosine, guanine and uracil were from ACROS. All other chemicals were provided by Aldrich or ACROS and were of the highest quality. ^{1}H NMR and ^{13}C NMR spectra were determined with a 200 MHz Varian Gemini apparatus with tetramethylsilane as internal standard for the ^{1}H NMR spectra (s = singlet, d = doublet, dd = double doublet, t = triplet, br s = broad signal, br d = broad doublet, m = multiplet) and the solvent signal DMSO-*d6* (39.6 ppm) or $CDCl_3$ (76.9 ppm) for the ^{13}C NMR spectra. For some products a Varian Unity-500 spectrometer (500 MHz for ^{1}H) was used. Coupling constant values were derived by first-order spectral analysis. Exact mass measurements were performed on a quadrupole/orthogonal acceleration time-of-flight tandem mass spectrometer (qTOF2, Micromass, Manchester, UK) equiped with a standard electrospray ionization interface. Precoated Machery-Nagel Alugram SILG/UV_{254} plates were used for TLC, and the spots were examined with UV light and sulfuric acid/anisaldehyde spray. Column chromatography was performed on ACROS silica gel (0.060–0.200 mm or 0.035–0.060 mm). Anhydrous solvents were obtained as follows: dichloromethane was stored over calcium hydride, refluxed and distilled. Pyridine was refluxed over potassium hydroxide pellets and distilled. Dimethylformamide was dried over 4 Å activated molecular sieves. HMPA was dried by azeotropic distilation using toluene. Absolute methanol was refluxed overnight over magnesium iodide and distilled. Methanolic ammonia was prepared by bubbling NH_3 gas through absolute methanol at 0°C and was stored at – 20°C. All microwave irradiation experiments were carried out in a dedicated CEM-Discover monomode microwave apparatus,[8] operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W utilizing the standard absorbance level (300 W maximum power). The reactions were carried out in 10 mL glass tubes, sealed with an aluminum/Teflon crimp top, which can be exposed to 250°C and 20 bar internal pressure. The temperature was measured with an IR sensor on the outer surface of the process vial. After the irradiation period, the reaction vessel was cooled rapidly (60–120 s) to ambient temperature by gas jet cooling.

2-(Adenin-9-yl)-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol (2). Lithium hydride (0.716 g, 90.00 mmol) was added to a stirred suspension of adenine (12.57 g, 93.00 mmol) and 12-crown-4 ether (0.145 ml, 0.90 mmol) in anhydrous DMF (560 ml) at room temperature under nitrogen. The mixture was stirred at 120°C for 1.5 hours (clear solution) and the epoxide (7.03 g, 30.00 mmol) was added. The stirring was continued for 1 hour at 120°C and the reaction mixture was cooled and evaporated to dryness. The residue was dissolved in ethyl acetate (1000 ml), and the organic layer was washed with a saturated solution of sodium hydrogencarbonate (3 × 1000 ml) and brine (1000 ml). The organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was dissolved in a mixture of dichloromethane and methanol (1/1: 75 ml) and precipitated with hexane. Two crops were obtained, collected and precipitated one more time to give the title compound **2** as a pure white solid (6.78 g, 61%). The filtrate can be purified on silica gel chromatography using a stepwise gradient of methanol (2–5%) in dichloromethane to afford 2.11 g of pure material (total yield: 80%). Physical and chemical data are in accord with the literature.[2]

HRMS calcd for $C_{18}H_{20}N_5O_4$(MH) + 370,1515, found 370,1507.

1-5-Anhydro-4,6-*O*-benzylidene-2-(N^6-benzoyladenin-9-yl)-2-deoxy-D-*altro*-hexitol (3). Trimethylsilyl chloride (2.57 ml, 20 mmol) was added dropwise to a cold suspension (0°C) of **2** (3.69 g, 10 mmol) in dry pyridine (50 ml) under nitrogen. The reaction mixture was stirred at room temperature, for 1 hour, and cooled at 0°C. Then, benzoyl chloride (5.81 ml, 50 mmol) was added dropwise, and the reaction mixture was stirred at room temperature, for 2 hours. The solution was cooled in an ice-bath, and water (10 ml) was added, followed 5 min later by 10 ml of aqueous ammonia (25%). The reaction mixture was stirred at room temperature for 30 minutes and the volatiles were removed. The residue was dissolved in ethyl acetate (150 ml) and the organic layer was extracted with a saturated solution of sodium hydrogencarbonate (3 × 150 ml). The solvent was evaporated and the residue was dissolved in THF (50 ml). Then, a 1M TBAF solution in THF was added (10 ml). After 5 min stirring at room temperature, the reaction mixture was evaporated to dryness, dissolved in dichloromethane (100 ml) and washed with water (3 × 100 ml). The organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was subjected to silica gel column chromatography using a mixture of ethyl acetate/n-hexane (1/9) as eluent, to afford the title compound **3** as a white solid (3.72 g, 79%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.64 (1H, dd, J = 2.6 Hz, J = 9.7 Hz, 4′-H); 3.74 (1H,t, J = 10.4 Hz, 6′ax-H); 4.17 (1H, dd, J = 5.1 Hz, J = 10.0 Hz, 5′-H); 4.21 (1H, br d, J = 14.4 Hz, 1′ax-H); 4.42 (1H, dd, J = 4.9 Hz, J = 10.5 Hz, 6′eq-H); 4.48–4.52 (2H, m, 3′-H and 1′eq-H); 4.87 (1H, br s, 2′-H); 5.51 (1H, s, PhC*H*); 7.26–7.60 (8H, m, H *arom*); 8.05 (2H, d, J = 7.5 Hz, II *orto*Bz); 8.55 (1H, s, 8-H); 8.74 (1H, s, 2-H); 9.46 (1H, br. s, NH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ □55.36 (2′-C); 64.99 (1′-C); 66.21 (3′-C); 67.12 (5′-C); 68.91 (6′-C); 76.63 (4′-C); 102.15 (Ph*C*H); 122.68 (5-C); 125.97–136.74 (8 × C *arom*), 141.96 (8-C); 149.76 (**4**-C); 152.12 (2-C); 152.68 (6-C); 164.87 (*C*ONH).

HRMS calcd for $C_{25}H_{24}N_5O_5(MH)^+$ 474,1777, found 474.1774.

1,5-Anhydro-2-(*N*^{6}-benzoyladenin-9-yl)-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-*altro*-hexitol (4). *Tert*-butydimethylsilyl chloride (1.81 g, 12 mmol) was added to a solution of **3** (1.89 g, 4 mmol) and imidazole (1.36 g, 20 mmol) in dry DMF (20 ml). The reaction mixture was stirred at 70°C, under nitrogen. After 15 hours, imidazole (0.272 g; 4 mmol) and TBDMSCl (0.603 g; 4 mmol) were added and stirring was continued for 15 hours. Then, imidazole (0.272 g; 4 mmol) and TBDMSCl (0.603 g; 4 mmol) were added and stirring was continued for 8 hours at 70°C. DMF was removed under reduced pressure and the residue was dissolved in dichloromethane (70 ml) and washed with water (2 × 70 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The brownish residue was subjected to a silica gel column chromatography using a stepwise gradient of methanol (0–0.5%) in dichloromethane, to afford the title compound **4** as a white foam (1.48 g, 68%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.13 (3H, s, C*H*$_3$ Si); 0.28 (3H, s, C*H*$_3$ Si); 0.99 (9H, s, (C*H*$_3$)$_3$C); 3.55 (1H, dd, J = 2.5 Hz, J = 9.6 Hz, 4′-H); 3.73 (1H, **t**, J = 10.5 Hz, 6′ax-H); 4.16–4.21 (1H, m, 5′-H); 4.29 (1H, br d, J = 13.2 Hz, 1′ax-H); 4.39 (1H, dd, J = 5.1 Hz, J = 10.5 Hz, 6′eq-H); 4.51 (1H, t, J = 2.9 Hz, 3′-H); 4.57 (1H, dd, J = 2.93 Hz, J = 13.2 Hz, 1′eq-H); 4.68 (1H, **t**, J = 2.7 Hz, 2′-H); 5.45 (1H, s, PhC*H*); 7.26–7.61 (8H, m, H *arom*); 8.06 (2H, d, J = 7.0 Hz, H *orto*Bz); 8.59 (1H, s, 8-H); 8.79 (1H, s, 2-H); 9.33 (1H, br s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ□4.90 (*C*H$_3$Si); − 4.63 (*C*H$_3$Si); 18.08 ((CH$_3$)$_3$*C*); 25.77 ((*C*H$_3$)$_3$C); 56.84 (2′-C); 64.74 (1′-C); 66.95 (5′-C); 66.99 (3′-C); 69.00 (6′-C); 76.63 (4′-C); 102.31 (Ph*C*H); 122.72 (5-C); 126.15-137.19 (11 × C *arom*), 141.99 (8-C); 149.61 (4-C); 152.52 (2-C); 152.70 (6-C); 164.80 (*C*ONH).

HRMS calcd for $C_{31}H_{38}N_5O_5Si$ $(MH)^+$ 588.2642, found 588.2645.

1,5-Anhydro-2-(*N*^{6}-benzoyladenin-9-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-*altro*-hexitol (5). The compound **4** (6.23 g, 10.60 mmol) was dissolved in dichloromethane (112 ml) and TFA was added (48 ml). After 1 hour stirring at room temperature, the volatiles were removed under reduced pressure and the residue was purified on silica gel column chromatography using a stepwise gradient of methanol (0–4%) in dichloromethane to afford the title compound **5** as a white solid (3.14 g, 74%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.20 (3H, s, CH_3Si); 0.36 (3H, s, CH_3Si); 0.98 (9H, s, $(CH_3)_3C$); 3.67–3.73 (2H, m, 4′-H and 5′-H); 3.77 (1H, dd, J = 3.7 Hz, J = 12.2 Hz, 6′ax-H); 3.87 (1H, br d, J = 11.2 Hz, 6′eq-H); 4.21 (1H, br d, J = 12.5 Hz, 1′ax-H); 4.34–4.37 (2H, m, 3′-H and 1′eq-H); 4.72 (1H, br s, 2′-H); 7.46–7.49 (2H, m, H *arom*); 7.54–7.57 (1H, m, H *arom*); 8.00–8.03 (2H, m, H *arom*); 8.65 (1H, s, 8-H); 8.77 (1H, s, 2-H); 9.76 (1H, br. s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-4.85 (CH_3Si); -4.54 (CH_3Si);18.01 ($(CH_3)_3C$); 25.79 ($(CH_3)_3C$); 55.90 (2′-C); 61.94 (1′-C); 64.02 (5′-C); 64.25 (3′-C); 69.29 (6′-C); 77.13 (4′-C); 121.98 (5-C); 128.02–133.77 (4 × C *arom*); 142.25 (8-C); 149.66 (4-C); 152.21 (2-C); 152.77 (6-C); 165.22 (*C*ONH).

HRMS calcd for $C_{24}H_{34}N_5O_5Si$ $(MH)^-$ 500.2329, found 500.2324.

1,5-Anhydro-2-(N^6-benzoyladenin-9-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-6-*O*-monomethoxytrityl-D-*altro*-hexitol (6). Monomethoxytrityl chloride (1.94 g, 6.20 mmol) was added to a stirred solution of 5 (2.82 g, 5.64 mmol) in dry pyridine (30 ml) at room temperature under nitrogen. After 16 hours, methanol (3 ml) and solid sodium hydrogencarbonate were added. The volatiles were removed and the residue was dissolved in ethyl acetate (100 ml). The organic phase was washed with a 5% solution of sodium hydrogencarbonate (2 × 150 ml), dried over anhydrous sodium sulfate, evaporated and coevaporated with toluene. The residue was subjected to silica gel column chromatography using a mixture of ethyl acetate/hexane (9/1), (containing 0.2% triethylamine) to afford the title compound 6 as a white solid (4.00 g, 92%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.18 (3H, s, CH_3Si); 0.32 (3H, s, CH_3Si); 0.97 (9H, s, $(CH_3)_3C$); 1.94 (1H, d, J = 8.5 Hz, 4′-OH); 3.38–3.46 (1H, m, 4′-H); 3.49 (1H, dd, J = 2.7 Hz, J = 10.3 Hz, 5′-H); 3.72–3.90 (5H, m, 6′ax-H, 6′cq-H and CH_3O); 4.26–4.36 (3H, m, 1′ax-H, 3′-H and 1′eq-H); 4.75 (1H, m, 2′-H); 6.84 (2H, d, J = 9.0 Hz, H *arom*); 7.23–7.63 (15H, m, H *arom*); 8.03 (2H, d, J = 7.0 Hz, H *arom*); 8.70 (1H, s, 8-H); 8.81 (1H, s,2-H); 9.11 (1H, br s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-4.85 (CH_3Si); – 4.57 (CH_3Si);18.00 ($(CH_3)_3C$); 25.79 ($(CH_3)_3C$); 55.18 (CH_3O); 55.77 (2′-C); 63.44 (1′-C); 63.97 (5′-C); 65.23 (3′-C); 69.19 (6′-C); 76.39 (4′-C); 86.62 (CPh_3); 113.18 (C*meta*-$PhOCH_3$); 122.43 (5-C); 126.94-135.56 (9 × C *arom*), 142.67 (8-C); 144.25 (C *arom*); 149.47 (4-C); 152.25 (2-C); 152.77 (6-C); 158.59 (C *arom*); 164.47 (*C*ONH).

HRMS calcd for $C_{44}H_{50}N_5O_6Si$ $(MH)^+$ 772.3530, found 772.3519.

1.5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(thymin-1-yl)-D-*altro*-hexitol (8).

a) A mixture of thymine (6.31 g, 50.00 mmol) and sodium hydride 60% in mineral oil (1.80 g, 48.00 mmol) in anhydrous DMF (200 ml) was stirred at 90°C for 30 minutes under nitrogen. Then, the mixture was cooled down to 60°C and the epoxide (4.69 g, 20.00 mmol) was added. The temperature was raised up to 130°C and stirring was continued for 18 hours. The reaction mixture was then cooled and evaporated to dryness. The pink residue was dissolved in ethyl acetate (250 ml), and the organic layer was washed with a saturated solution of sodium hydrogencarbonate (3 × 250 ml). The organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The crude material was subjected to silica gel column chromatography, using a stepwise gradient of methanol (1–2.5%) in dichloromethane to afford the title compound (**8**) after crystallization from toluene (3.98 g, 55%).

b) A mixture of epoxide (3,04 g, 13 mmol), thymine (3.78 g, 30 mmol), and DBU (6.0 mL, 39 mmol) in dry DMF (50 mL) was stirred at 80–90 C under argon for 3 h. The reaction was monitored with TLC. When starting epoxide has disappeared the reaction mixture was cooled, the volatiles were removed, and the residue was powdered into ice-water (250 mL). The pH was adjusted to neutral with 1N HCl at 0°C and solid formed was filtered off, washed with water, and dissolved in dichloromethane (50 mL). The solution was dried over magnesium sulfate, concentrated and crude material was purified by column chromatography on silica gel using 2% MeOH in dichloromethane. Yield 3.5 g (75%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.93 (3H, s, CH_3); 3.64 (1H, dd, $J = 2.9$ Hz, $J = 9.7$ Hz, 4′-H); 3.69 (1H, t, $J = 10.5$ Hz, 6′ax-H); 3.88 (1H, br d, $J = 13.7$ Hz, 1′ax-H); 4.11 (1H, td, $J = 5.1$ Hz, $J = 10.0$ Hz, 5′-H); 4.30-4.38 (3H, m, 1′eq-H, 3′-H and 6′eq-H); 4.42 (2H, m, 2′-H and 3′-OH); 5.57 (1H, s, PhC*H*); 7.29–7.33 (3H, m, H *arom*); 7.43–7.45 (2H, m, H *arom*); 7.77 (1H, s, 6-H); 10.36 (1H, br s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ□2.69 (CH_3); 57.01 (2′-C); 63.91 (1′-C); 65.76 (3′-C); 66.07 (5′-C); 68.91 (6′-C); 76.60 (4′-C); 102.06 (Ph*C*H); 111.34 (5-C); 126.13–137.03 (4 × C *arom*), 137.74 (6-C); 151.45 (2-C); 164.04 (4-C).

HMRS calcd for $C_{18}H_{21}N_2O_6$ $(MH)^+$ 361.1399, found 361.1408.

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsylil-2 deoxy-2-(thymin-1-yl)-D-*altro*-hexitol (9). *Tert*-butyldimethylsilyl chloride (9.04 g, 60 mmol) was added to a solution of **8** (7.21 g, 20 mmol) and imidazole (6.68 g, 100 mmol) in dry DMF (60 ml). The reaction mixture was stirred at 70°C, under nitrogen. After 15 hours, 1 equivalent of imidazole (1.36 g) and 1 equivalent of TBDMSCl (3.00 g) were added and stirring was continued for 24 hours. The DMF was removed under reduced pressure and the residue was dissolved in ethylacetate (100 ml) and washed with brine (2 × 100 ml). The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. The white solid was subjected to silica gel column chromatography using a stepwise gradient of methanol (0–2%) in dichloromethane, to afford the title compound **9** as a white foam (3.88 g, 88%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.08 (3H, s, CH_3Si); 0.18 (3H, s, CH_3Si); 0.94 (9H, s, $(CH_3)_3C$); 1.98 (3H, s, CH_3); 3.56 (1H, dd, $J = 2.2$ Hz, $J = 9.5$Hz, 4′-H); 3.71 (1H, t, $J = 10.4$ Hz, 6′ax-H); 4.02 (1H, br d, $J = 13.4$ Hz, 1′ax-H); 4.11 (1H, td, $J = 5.1$ Hz, $J = 10.0$ Hz, 5′-H); 4.27–4.29 (2H, m, 2′-H and 3′-H); 4.35 (1H, dd, $J = 5.1$ Hz, $J = 10.3$ Hz, 6′eq-H); 4.39 (1H, dd, $J = 2.9$ Hz, $J = 13.4$ Hz, 1′eq-H); 5.55 (1H, s, PhC*H*); 7.33–7.35 (3H, m, H *arom*); 7.43–7.45 (2H, m, H *arom*); 7.86 (1H, s, 6-H); 9.00 (1H, br. s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-5.0 1 (CH_3Si); -4.74 (CH_3Si); 12.69 (CH_3); 18.03 ($(CH_3)_3C$); 25.73 ($(CH_3)_3C$); 57.85 (2′-C); 64.00 (1′-C); 66.22 (5′-C); 66.77 (3′-C); 68.98 (6′-C); 76.95 (4′-C); 102.32 (Ph*C*H); 110.96 (5-C); 126.18-137.27 (4 × C *arom*), 137.84 (6-C); 150.95 (2-C); 163.65 (4-C).

HMRS calcd for $C_{24}H_{35}N_2O_6Si$ $(MH)^+$ 475.2264, found 475.2263.

1,5-Anhydro-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-(thymin-1-yl)-D-*altro*-hexitol (10). The compound **9** (2.37 g, 5.00 mmol) was dissolved in dichloromethane (52.5 ml) and TFA was added (22.5 ml). After 1 hour stirring at room temperature, the volatiles were removed under reduced pressure (water bath < 30°C) and the residue was purified on silica gel column chromatography using a stepwise gradient of methanol (2–4%) in dichloromethane to afford the title compound **10** as a white solid (1.74 g, 90%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.18 (3H, s, $C\mathit{H}_3Si$); 0.28 (3H, s, $C\mathit{H}_3Si$); 0.95 (9H, s,$(C\mathit{H}_3)_3C$); 1.91 (3H, s, CH_3); 2.11 (2H, br s, 6′-OH and 4′-OH); 3.61 (1H,dd, *J* = 2.9 Hz, *J* = 9.8 Hz, 4′-H); 3.71 (1H, ddd, *J* = 2.9 Hz, *J* = 5.2 Hz, *J* = 9.6 Hz, 5′-H); 3.85 (1H, dd, *J* = 5.4 Hz, *J* = 11.9 Hz, 6′ax-H); 3.96 (1H, dd, *J* = 2.8 Hz, *J* = 11.8 Hz, 6′eq-H); 4.06 (1H, br d, *J* = 13.4 Hz, 1′ax-H); 4.14 (1H, t, *J* = 2.9 Hz, 3′-H); 4.23 (1H, dd, *J* = 3.4 Hz, *J* = 13.4 Hz, 1′eq-H); 4.36 (1H, td, *J* = 3.4 Hz, *J* = 13.4 Hz, 2′-H); 7.91 (1H, s, 6-H); 9.13 (1H, br. s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-5.09 ($\mathit{C}H_3Si$); − 4.57 ($\mathit{C}H_3Si$);12.63 (CH_3); 17.98 ($(CH_3)_3\mathit{C}$); 25.75 ($(\mathit{CH}_3)_3C$); 56.45 (2′-C); 63.00 (6′-C); 63.23 (1′-C); 65.26 (4′-C); 69.14 (3′-C); 76.37 (5′-C); 110.95 (5-C); 138.33 (6-C); 151.21 (2-C); 163.69 (4-C).

HMRS calcd for $C_{17}H_{31}N_2O_6Si$ $(MH)^+$ 387.1951, found 387.1956.

1,5-Anhydro-3-*O*-*tert*-butyldimethylsilyl-2 deoxy-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-D-*altro*-hexitol (11). Monomethoxytrityl chloride (2.36 g, 7.64 mmol) was added to a stirred solution of **10** (2.69 g, 6.95 mmol) in dry pyridine (35 ml) at room temperature under nitrogen. After 2 hours, methanol (5 ml) and solid sodium hydrogencarbonate were added. The volatiles were removed and the residue was dissolved in dichloromethane (150 ml). The organic phase was washed with a 5% solution of sodium hydrogencarbonate (2 × 100 ml), dried over anhydrous sodium sulfate, evaporated and coevaporated with toluene. The residue was subjected to silica gel column chromatography using a stepwise gradient of ethylacetate (20–40%) in hexane (containing 0.2% triethylamine), to afford the title compound **11** as a white solid (4.23 g, 92%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.16 (3H, s, $C\mathit{H}_3Si$); 0.27 (3H, s, $C\mathit{H}_3Si$); 0.92 (9H, s, $(C\mathit{H}_3)_3C$); 1.78 (3H, s, CH_3); 1.85 (1H, br s, 4′-OH); 3.34 (1H, dd, *J* = 3.7 Hz, *J* = 10.3 Hz, 4′-H); 3.44 (1H, dd, *J* = 2.2 Hz, *J* = 10.3 Hz, 5′-H); 3.67 (1H, td, *J* = 2.8 Hz, *J* = 9.3 Hz, 6′ax-H); 3.79 (3H, s, OC *H3*); 3.97 (1H, dd, *J* = 2.9 Hz, *J* = 9.6 Hz, 6′eq-H); 4.05 (1H, br d, *J* = 13.4 Hz, 1′ax-H); 4.18–4.22 (2H, m, 3′-H and 1′eq-H); 4.36 (1H, t, *J* = 2.4 Hz, 2′-H); 6.83 (2H, d, *J* = 9.0 Hz, H *arom*); 7.22–7.48 (12H, m, H *arom*); 8.16 (1H, s, 6-H); 8.79 (1H, br. s, NH).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-5.06 ($\mathit{C}H_3Si$); -4.64 ($\mathit{C}H_3Si$);12.81 (CH_3); 17.97 ($(CH_3)_3\mathit{C}$); 25.76 ($(\mathit{CH}_3)_3C$); 55.19 ($O\mathit{C}H_3$); 56.53 (2′-C); 63.07 (1′-C); 63.48 (6′-C); 64.88 (4′-C); 69.27 (3′-C); 75.79 (5′-C); 86.36 ($\mathit{C}Ph_3$); 110.87 (5-C); 113.13 (C*meta*-$PhOCH_3$); 126.94–135.46 (5 × C *arom*), 138.65 (6-C); 144.38 (2 × C *arom*); 151.16 (2-C); 158.59 (C *arom*); 163.62 (4-C).

HMRS calcd for $C_{37}H_{46}N_2O_7SiNa$ $(MNa)^+$ 681.2972, found 681.2996.

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-2 deoxy-2-(5-methylcytosin-1-yl)-D-*altro*-hexitol (12). Phosphorous oxychloride freshly distilled (0.700 ml, 7.5 mmol) was added to a stirred solution of 1,2,4-1H-triazole (1.73 g, 25.0 mmol) in dry pyridine (17.5 ml) under nitrogen. After 20 minutes stirring, **9**

(1.19 g, 2.50 mmol) was added, and stirring was continued for 3 hours. The volatiles were removed and the residue was coevaporated with toluene (2 times) and partitioned between water and ethyl acetate (strong emulsion). The organic layer was washed with water (2 times), brine (2 times), and evaporated to dryness to afford a yellow foam. This crude intermediate was dissolved in dioxane (17 ml), and 8 ml aqueous ammonia (25%) were added. After 45 minutes stirring, the volatiles were evaporated and the solid was coevaporated with toluene (2 times). The residue was subjected to silica gel column chromatography, using a stepwise gradient of methanol (2–4%) in dichloromethane, to afford the title compound **12** as a white powder (0.770 g, 65%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.08 (3H, s, CH_3Si); 0.22 (3H, s, CH_3Si); 0.94 (9H, s, $(CH_3)_3$C); 1.97 (3H, s, CH_3); 3.53 (1H, dd, J = 2.4 Hz, J = 9.5 Hz, 4′-H); 3.69 (1H, t, J = 10.4 Hz, 6′ax-H); 4.01 (1H, br d, J = 13.7 Hz, 1′ax-H); 4.11 (1H, td, J = 5.1 Hz, J = 10.0 Hz, 5′-H); 4.34 (1H, dd, J = 5.1 Hz, J = 10.3 Hz, 6′eq-H); 4.36–4.41 (3H, m, 3′-H, 1′ax-H and 2′-H); 5.52 (1H, s, PhC*H*); 7.32–7.36 (3H, m, H *arom*); 7.42–7.45 (2H, m, H *arom*); 7.88 (1H, s, 6-H).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-4.80 (CH_3Si); -4.74 (CH_3Si); 13.51 (CH_3); 18.04 ($(CH_3)_3C$); 25.80 ($(CH_3)_3$C); 58.24 (2′-C); 64.49 (1′-C); 66.19 (5′-C); 66.51 (3′-C); 69.08 (6′-C); 76.97 (4′-C); 101.60 (5-C); 102.21 (Ph*C*H); 126.18–137.44 (4 × C *arom*), 140.76 (6-C); 156.32 (2-C); 165.48 (4-C).

HMRS calcd for $C_{24}H_{36}N_3O_5Si$ $(MH)^+$ 474.2424, found 474.2421.

1,5-Anhydro-4,6-*O*-benzylidene-2-(N^4-benzoyl-5-methylcytosin-1-yl)-3-*O*-*tert*-butyldimethylsylil-2 deoxy-D-*altro*-hexitol (13). Benzoyl chloride (3.20 ml, 27.55 mmol) was added to a stirred solution of **12** (2.61 g, 5.51 mmol) in dry pyridine (40 ml) at 0°C under nitrogen. The reaction mixture was stirred at room temperature overnight and the pyridine was removed. The residue was suspended in dichloromethane (50 ml) and the organic phase was washed with a saturated solution of sodium hydrogencarbonate (2 × 50 ml). Aqueous ammonia (25%), 20 ml was added at 0°C to the organic phase, and the mixture was stirred at room temperature for 1 hour. The volatiles were removed and the crude material was subjected to silica gel column chromatography using a mixture of hexane/ethyl acetate (7/3) as eluent, to afford the title compound **13**(1.83 g, 92%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.09 (3H, s, CH_3Si); 0.20 (3H, s, CH_3Si); 0.94 (9H, s, $(CH_3)_3$C); 2.16 (3H, br d, J = 1.0 Hz, CH_3); 3.59 (1H, dd, J = 2.4 Hz, J = 9.5 Hz, 4′-H); 3.73 (1H, t, J = 10.4 Hz, 6′ax-H); 4.07 (1H, br d, J = 13.7 Hz, 1′ax-H); 4.13 (1H, td, J = 5.1 Hz, J = 10.0 Hz, 5′-H); 4.31 (1H, t, J = 2.4 Hz, 2′-H); 4.35 (1H, t, J = 2.9 Hz, 3′-H); 4.37 (1H, dd, J = 5.5 Hz, J = 10.5 Hz, 6′eq-H); 4.43 (1H, dd, J = 3.4 Hz, J = 13.4 Hz, 1′eq-H); 5.55 (1H, s, PhC*H*); 7.32–7.35 (3H, m, H *arom*); 7.42–7.45 (4H, m, H *arom*); 7.51–7.54 (1H, m, H *arom*); 8.02 (1H, s, 6-H); 8.31–8.33 (2H, m, H *arom*).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ-5.01 (CH_3Si); -4.72 (CH_3Si); 13.89 (CH_3); 18.04 ($(CH_3)_3C$); 25.74 ($(CH_3)_3$C); 58.32 (2′-C); 64.00 (1′-C); 66.27 (5′-C); 66.60 (3′-C); 69.00 (6′-C); 76.74 (4′-C); 102.32 (Ph*C*H); 112.00 (5-C); 126.17–137.25 (8 × C *arom*), 139.13 (6-C); 148.57 (2-C); 159.65 (4-C); 179.65 (Ph*C*O).

HMRS calcd for $C_{31}H_{40}N_3O_6Si$ $(MH)^4$ 578.2686, found 578.2687.

1,5-Anhydro-2-(N^4-benzoyl-5-methylcytosin-1-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-D-*altro*-hexitol (14). Compound **13** (1.83 g, 3.17 mmol) was dissolved in dichloromethane (34 ml) and cooled to 0°C. Trifluoroacetic acid (15 ml) was then added, and the reaction mixture was stirred at room temperature for 45 min. Solvents were then removed and the residue was coevaporated with toluene (water bath < 30°C). The crude material was subjected to silica gel column chromatography, using a stepwise gradient of methanol (0–2%) in dichloromethane, to afford the title compound **14** as a white foam (1.05 g, 67%), which was directly used for the next step because of its poor stability.

1,5-anhydro-2-(N^4-benzoyl-5-methylcytosin-1-yl)-3-*O*-*tert*-butyldimethylsilyl-2-deoxy-6-*O*-monomethoxytrityl-D-*altro*-hexitol (15). Monomethoxytrityl chloride (0.46 g, 1.42 mmol) was added to a solution of **14** (0.63 g, 1.29 mmol) in dry pyridine (6.45 ml) at room temperature under nitrogen. After 16 hours, methanol (1 ml) and solid sodium hydrogencarbonate were added. The volatiles were removed and the residue was dissolved in dichloromethane (50 ml). The organic phase was washed with a 5% solution of sodium hydrogencarbonate (2 × 50 ml), dried over anhydrous sodium sulfate, evaporated and coevaporated with toluene. The residue was subjected to silica gel column chromatography using a stepwise gradient of ethyl acetate (0–10%) in hexane (containing 0.2% triethylamine), to afford the title compound **15** as a white solid (0.74 g, 75%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.16 (3H, s, CH_3Si); 0.28 (3H, s, CH_3Si); 0.93 (9H, s, (CH_3)$_3$C); 1.97 (3H, s, CH_3); 3.37 (2H, dd, J = 3.4 Hz, J = 10.5 Hz, 4′-H and 4′-OH); 3.46 (1H, dd, J = 2.2 Hz, J = 10.4 Hz, 5′-H); 3.69 (1H, td, J = 2.8 Hz, J = 9.8 Hz, 6′ax-H); 3.79 (3H, s, OCH_3); 3.98 (1H, dd, J-2.2 Hz, J = 9.0 Hz, 6′eq-H); 4.11 (1H, br d, J = 13.4 Hz, 1′ax-H); 4.20–4.24 (2H, m, 3′-H and 1′eq-H); 4.43 (1H, t, J = 2.4 Hz, 2′-H); 6.84 (2H, d, J = 9.0 Hz, H *arom*); 7.21–7.54 (12H, m, H *arom*); 8.30–8.33 (3H, m, H *arom*and 6-H).

^{13}C-NMR ($CDCl_3$, 125 MHz) δ – 5.03 L (*C*H_3Si); -4.63 (*C*H_3Si); 13.85 (CH_3); 18.00 ((CH$_3$)$_3$*C*); 25.78 ((*C*H_3)$_3$C); 55.21 (O*C*H_3); 57.06 (2′-C); 63.05 (1′-C); 63.55 (6′-C); 64.87 (4′-C); 69.27 (3′-C); 75.86 (5′-C); 86.39 (*C*Ph$_3$); 111.94 (5-C); 113.16 (C*meta*-PhOCH$_3$); 126.95–137.26 (10 × C *arom*), 140.04 (6-C); 144.38 (2 × C *arom*); 148.80 (2-C); 158.62 (C *arom*); 159.77 (4-C); 179.65 (Ph*C*O).

HMRS calcd for $C_{44}H_{52}N_3O_7Si$ $(MH)^+$ 762.3574, found 762.3576.

2-(2-Amino-6-chloropurin-9-yl)-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol (17).

a) A mixture of epoxide (304 mg, 1.3 mmol), 2-amino-6-chloropurin (450 mg, 2.65 mmol), aliquat 336 (343 mg, 1.3 mmol), and potassium carbonate (360 mg, 2,6 mmol) in dry HMPA (5 mL) was stirred at 80–90 C under argon for 3 h. The reaction was monitored with TLC and, when starting epoxide has disappeared, the mixture was cooled immediately and powdered into ice-water (50 mL). The suspension was stirred for 1 h, solid was filtered off, washed with water, and dissolved in dichloromethane (50 mL). Solution was dried over magnesium sulfate, evaporated to dryness and crude material was purified by column chromatography on silica gel using 2% MeOH in dichloromethane. Yield 335 mg (64%).

b) General procedure for purine nucleosides: a mixture of purine base (1.5 mmol), epoxide (0.5 mmol), and DBU (0.5 mL) dry DMF (1.5 mL) in a 10 mL glass tube, which was tightly sealed with an aluminum/Teflon crimp, was irradiated at 150 W, 110°C with gas jet cooling for 15 min in a CEM-Discover monomode microwave apparatus. The vessel was cooled down to 60°C and the residue was powdered into ice-water (10 mL). The pH was adjusted to neutral with 1N HCl at 0°C and solid formed was filtered off, washed with water, and dissolved in dichloromethane (50 mL). The solution was dried over magnesium sulfate, concentrated and crude material was purified by column chromatography on silica gel (35% yield).

^{1}H NMR: (DMSO-d_6, 500 MHz, δ): 3.69 (1H, dd, $J = 2.4$ Hz, $J = 9.5$ Hz, 4′-H); 3.80 (1H, t, $J = 10.2$ Hz, 6′ax-H), 4.00 (1H, td, $J = 5.1$ Hz, $J = 10.2$ Hz, 5′-H); 4.23–4.30 (4H, m, 1′eq-H, 1′ax-H, 3′-H, and 6′eq-H); 4.46 (1H, br s, 2′-H); 5.64 (1H, s, PhC*H*); 5.88 (1H, d, $J = 4.5$ Hz, 3′-OH), 6.98 (2H, s, NH_2); 7.32–7.34 (3H, m, H *arom*); 7.38–7.40 (2H, m, H *arom*); 8.23 (1H, s, 8–11).

^{13}C NMR: (DMSO-d_6, 125 MHz, δ): 55.87 (2′-C); 64.29 (1′-C); 64.77 (3′-C); 66.46 (5′-C); 68.13 (6′-C); 76.27 (4′-C); 101.06 (Ph*C*H); 123.02 (5-C); 126.41-137.75 (4 × C *arom*); 141.37 (8-C); 149.73 (2-C); 154.26 (4-C); 159.93 (6-C).

HRMS: calcd for $C_{18}H_{19}ClN_5O_4$(M + H) ^{1}404.1125, found 404.1119.

c) When epoxidation of 2-amino-6-chloropurin was carried out in DMF the formation of next by-products have been observed.

2-(2-Amino-6-*N,N*-dimethylaminopurin-9-yl)-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol. Yield 25% (18). ^{1}H NMR: (DMSO-d_6, 500 MHz, δ): 3.57 (6H, br s, N(CH_3)$_2$); 3.67 (1H, dd, $J = 2.4$ Hz, $J = 9.5$ Hz, 4′-H); 3.77 (1H, t, $J = 10.2$ Hz, 6′ax-H), 3.99 (1H, td, $J = 5.1$ Hz, $J = 10.2$ Hz, 5′-H); 4.16–4.29 (4H, m, 1′eq-H, 1′ax-H, 3′-H, and 6′eq-H); 4.39 (1H, br s, 2′-H); 5.64 (1H, s, PhC*H*); 5.80 (1H, br s, 3′-OH), 5.88 (2H, s, NH_2); 7.32–7.34 (3H, m, H *arom*); 7.40–7.42 (2H, m, H *arom*); 7.92 (1H, s,8-H).

^{13}C NMR: (DMSO-d_6, 125 MHz, δ): 37.70 (N(CH_3)$_2$); 55.16 (2′-C); 64.59 (1′-C); 64.96 (3′-C); 66.34 (5′-C); 68.21 (6′-C); 76.42 (4′-C); 101.06 (Ph*C*H); 113.24 (5-C); 126.41–137.75 (4 × C *arom*); 134.74 (8-C); 152.87 (4-C); 154.88 (4-C) and 159.66 (2-C and 6-C).

HRMS: calcd for $C_{20}H_{25}N_6O_4$$(M + H)^+$ 413.1937, found 413.1916.

2-(2-Amino-6-chloropurin-7-yl)-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol (19). Yield 5%. ^{1}H NMR: ^{1}H NMR: (DMSO-d_6, 500 MHz, δ): 3.62 (1H, dd, $J = 2.4$ Hz, $J = 9.5$ Hz, 4′-H); 3.78 (1H, t, $J = 10.2$ Hz, 6′ax-H), 3.99 (1H, td, $J = 5.1$ Hz, $J = 10.2$ Hz, 5′-H); 4.24–4.38 (4H, m, 1′eq-H, 1′ax-H, 3′-H, and 6′eq-H); 4.74 (1H, br s, 2′-H); 5.61 (1H, s, PhC*H*); 6.00 (1H, d, $J = 4.4$ Hz, 3′-OH), 6.65 (2H, s, NH_2); 7.32–7.34 (3H, m, H *arom*); 7.38–7.40 (2H, m, H *arom*); 8.46 (1H, s, 8-H).

^{13}C NMR: (DMSO-d_6, 125 MHz, δ): 57.90 (2′-C); 64.45 (1′-C); 66.23 (3′-C); 66.50 (5′-C); 68.11 (6′-C); 75.69 (4′-C); 101.00 (Ph*C*H); 115.22 (5-C); 126.35–137.77 (4 × C *arom*); 142.49 (8-C); 147.54 (2-C); 160.11 (6-C); 163.86 (4-C).

HRMS: calcd for $C_{18}H_{19}ClN_5O_4$$(M + H)^+$ 404.1125, found 404.1119.

[2-Amino-6-(2-amino-6-chloropurin)purin-9-yl]-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol (20). Yield 5%. ^{1}H NMR: (DMSO-d_6, 500 MHz, δ): 3.72 (1H, dd, $J = 2.4$ Hz, $J = 9.5$ Hz, 4′-H); 3.83 (1H, t, $J = 10.2$ Hz, 6′ax-H), 4.02 (1H, td, $J = 5.1$ Hz, $J = 10.2$ Hz, 5′-H); 4.25–4.32 (4H, m, 1′eq-H, 1′ax-H, 3′-H, and 6′eq-H); 4.56 (1H, br s, 2′-H); 5.67 (1H, s, PHC*H*); 5.92 (1H, br s, 3′-OH); 6.98 (2H, s, NH_2); 7.05 (2H, s, NH_2); 7.32–7.35 (3H, m, H *arom*); 7.39–7.43 (2H, m, H *arom*); 8.37 (1H, s, 8″-H); 8.63 (1H, s, 8-H).

^{13}C NMR: (DMSO-d_6, 125 MHz, δ): 55.75 (2′-C); 64.42 (1′-C); 64.87 (3′-C); 66.47 (5′-C); 68.08 (6′-C); 76.31 (4′-C); 101.07 (Ph*C*H); 118.86; 123.35; 126.33-137.75 (4 × C *arom*); 142.03; 145.42; 150.14; 153.83; 155.96; 160.08; 160.41; 160.55.

HRMS: calcd for $C_{23}H_{22}N_{10}O_4$ $(M + H)^+$ 537.1513, found 537.1514.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(guanin-9-yl)-D-*altro*-hexitol (21). A suspension of 2-(2-amino-6-chloropurin-9-yl)-1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*altro*-hexitol (2.5 g, 6.2 mmol) and DABCO (200 mg) in 1 N NaOH (100 ml) was stirred at 90 C for 2 h. The resulting clear colorless solution was cooled with ice-bath. The pH was adjusted to neutral with 1N HCl at 0 C and solid formed was filtered off, washed with water and dried under reduced pressure. Yield 2.2 g (92% of **21**) (**2**).

1,5-Anhydro-4,6-*O*-benzylidene-2-(N^2-*iso*-butyrylguanin-9-yl)-2-deoxy-D-*altro*-hexitol (22). 1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-(guanin-9-yl)-D-*altro*-hexitol (3.4 g, 8.8 mmol) was co-evaporated with with pyridine (2 × 100 ml) and to a resulting suspension in pyridine (150 mL) was added TMSCl (5.5 mL) dropwise at 0°C under argon. The resulting clear solution was stirred overnight at room temperature, after that extra TMSCl (8 mL) was added and stirring was continued for 8 h. *iso*-Butyric anhydride (6 mL) was added and reaction mixture was stirred for 24 h. Ice-water (20 mL) was added at 0°C followed by 25% NH_3(20 mL) and resulting mixture was stirred for 2 h and evaporated under reduced pressure. The residue was extracted with ethyl acetate, washed with water, dried over magnesium sulfate and evaporated to dryness. Semisolid was dissolved in THF (30 mL) and 1N NBu_4F (15 mL) was added dropwise at 0°C to the resulting solution. After 30 min, water (50 mL) was added and THF was removed under reduced pressure and cooled. Solid was filtered off, dried and purified by silica gel column chromatography, using a stepwise gradient of methanol (2–4%) in dichloromethane. Yield 3.1 g (77%).

^{1}H NMR: ($CDCl_3$, 500 MHz, δ): 1.22 (3H, d, $J = 6.8$ Hz, CH_3CH); 1.27 (3H, d, $J = 6.8$ Hz, CH_3CH); 2.79 (1H, dt, $J = 7.1$ Hz, $J = 13.6$ Hz, $(CH_3)_2CH$); 3.59 (1H, dd, $J = 1.8$ Hz, $J = 9.5$ Hz, 4′-H); 3.69 (1H, t, $J = 10.5$, Hz, 6′ax-H), 4.09 (1H, td, $J = 5.1$ Hz, $J = 10.2$ Hz, 5′-H); 4.21 (1H, br d, $J = 13.0$, 1′ax-H); 4.32 (1H, dd, $J = 4.8$ Hz, $J = 10.2$ Hz, 6′eq-H); 4.40–4.50 (3H, m, 1′eq-H, 1′ax-H, 3′-H and 2′-H); 4.79 (1H, br s, 3′-OH), 5.48 (1H, s, PhC*H*); 7.22–7.23 (3H, m, H *arom*); 7.32–7.34 (2H, m, H *arom*); 8.09 (1H, s, 8-H); 10.46 (1H, br s, NH); 12.23 (1H, br s, NH).

^{13}C NMR: ($CDCl_3$, 125 MHz, δ): 18.91 $(CH_3)_2CH$); 19.02 $(CH_3)_2CH$); 36.15 $(CH_3)_2CH$); 55.78 (2′-C); 64.71 (1′-C); 66.03 (3′-C); 66.86 (5′-C); 68.85 (6′-C); 76.75 (4′-C); 101.95 (Ph*C*H); 119.89 (5-C); 125.97–136.97 (4 × C *arom*); 138.11 (8-C); 148.12 (4-C); 149.15 (2-C); 155.91 (6-C); 180.00 (C = O).

HRMS: calcd for $C_{22}H_{26}N_5O_6(M + H)^+$ 456.1882, found 456.1887.

1,5-Anhydro-4,6-*O*-benzylidene-3-*O*-tert-butyldimethylsilyl-2-(N^2-*iso*-butyryl-guanin-9-yl)-2-deoxy-D-*altro*-hexitol (23). A mixture of 1,5-Anhydro-4,6-*O*-benzylidene-2-(N_2-*iso*-butyrylguanin-9-yl)-2-deoxy-D-*altro*-hexitol (2.4 g, 5.2 mmol) and pyridine (150 mL) was evaporated up to 50 mL and imidazole (2.8 g, 40 mmol) and TBDMSCl (3.1 g, 20 mmol) was added under argon at room temperature. The reaction mixture was stirred at 70°C for 8 h. Imidazole (1.4 g 20 mmol) and TBDMSCl (1.5 g, 10 mmol) was added and stirring was continued for 18h. The last operation was repeated and resulting solution was powdered into 5% $NaHCO_3$(250 ml). White semisolid was collected, dissolved in ethyl acetate, washed with 5% $NaHCO_3$, water, dried over magnesium sulfate and solvent was removed under reduced pressure. The title compound was purified by column chromatography on silica gel using 2% MeOH in dichloromethane. Yield 2.7 g (91%).

^{1}H NMR: ($CDCl_3$, 500 MHz, δ): 0.10 (3H, s, CH_3Si); 0.18 (3H, s, CH_3Si); 0.96 (3H, s, $(CH_3)_3Si$); 1.26 (3H, d, $J = 6.2$ Hz, CH_3CH); 1.27 (3H, d, $J = 6.2$ Hz, CH_3CH); 2.69 (1H, dt, $J = 7.1$ Hz, $J = 13.6$ Hz, $(CH_3)_2CH$); 3.57 (1H, dd, $J = 1.8$ Hz, $J = 9.5$ Hz, 4′-H); 3.72 (1H, t, $J = 10.5$, Hz, 6′ax-H), 4.09 (1H, td, $J = 5.1$ Hz, $J = 10.2$ Hz, 5′-H); 4.21 (1H, br d, $J = 13.0$, 1′ax-H); 4.33–4.36 (3H, m, 3′-H, 6′eq-H and 2′-H); 4.45 (1H, dd, $J = 5.1$ Hz, $J = 2.8$ Hz 1′eq-H), 5.46 (1H, s, PhCH); 7.31–7.32 (3H, m, H *arom*); 7.39–7.41 (2H, m, H *arom*); 8.09 (1H, s, 8-H); 8.17 (1H, br s, NH); 11.95 (1H, br s, NH).

^{13}C NMR: ($CDCl_3$, 125 MHz, δ): − 4.91 (CH_3Si); -4.47 (CH_3Si); 18.11 $(CH_3)_3C$); 18.79 $(CH_3)_2CH$); 19.00 $(CH_3)_2CH$); 36.39 $(CH_3)_2CH$); 56.73 (2′-C); 64.65 (1′-C); 66.78 (3′-C); 67.48 (5′-C); 68.95 (6′-C); 76.75 (4′-C); 102.31 (PhCH); 120.84 (5-C); 126.16-137.22 (4 × C *arom*); 137.93 (8-C); 147.37 (4-C); 148.43 (2-C); 155.43 (6-C); 178.10 (C = O).

HRMS: calcd for $C_{28}H_{40}N_5O_6Si$ $(M\text{-}H)^+$ 570.2747, found 570.2733.

1,5-Anhydro-3-*O*-tert-butyldimethylsilyl-2-(N^2-*iso*-butyrylguanin-9-yl)-2-deoxy-6-D-*altro*-hexitol (24). To a solution of 1,5-Anhydro-3-*O*-tert-butyldimethylsilyl-2-(N_2-*iso*-butyrylguanin-9-yl)-2-deoxy-D-*altro*-hexitol (3.2 g, 5.6 mmol) in dichloromethane (60 mL), TFA (15 mL) was added dropwise at 0°C and reaction mixture was stirred for 15 min and co-evaporated with toluene (water bath < 30°C). The crude product was purified by column chromatography on silica gel using using stepwise gradient of methanol (2–4%) in dichloromethane containing triethylamine (0.2%). First fraction contained 1.6 g of recycled starting compound. Second fraction was collected, evaporated to dryness and the crude product was used directly on the next step.

1,5-Anhydro-3-*O*-tert-butyldimethylsilyl-2-(N^2-*iso*-butyrylguanin-9-yl)-2-deoxy-6-*O*-monomethoxytrityl-D-*altro*-hexitol (25). A solution of crude 1,5-anhydro-3-*O*-tert-butyldimethylsilyl-2-(N_2-*iso*-butyrylguanin-9-yl)-2-deoxy-6-*O*-monomethoxytrilyl-D-*altro*-hexitol in pyridine (60 mL) was evaporated up to 20 mL and MMTrCl (1.0g, 3.25 mmol) was added under argon at room temperature. After 6 h methanol (5 mL) and solid sodium hydrocarbonate were added. The volatiles were removed, the residue was coevaporated with toluene (2 × 20 mL) and the title product was purified by column chromatography on silica gel using stepwise gradient of methanol (1–2%) in dichloromethane containing triethylamine (0.2%). Yield 1.8 g (85% on reacted 1,5-anhydro-4,6-*O*-benzylidene-*O*-tert-butyldimethylsilyl-(*iso*-butyrylguanin-9-yl)-2-deoxy-D-*altro*-hexitol).

^{1}H NMR: ($CDCl_3$, 500 MHz, δ): 0.12 (3H, s, CH_3Si); 0.16 (3H, s, CH_3Si); 0.91 (3H, s, $(CH_3)_3Si$); 1.15 (3H, d, $J = 6.9$ Hz, CH_3CH); 1.19 (3H, d, $J = 6.9$ Hz, CH_3CH); 2.07 (1H, br s, 4′-OH); 2.60 (1H, m, $(CH_3)_2CH$); 3.35 (1H, m, 6′ax-H); 3.46 (1H, d, *J* = 10.0, 6′eq-H); 3.67 (2H, m,5′-H and 4′-H); 3.72 (3H, s, C*H*$_3$O); 4.15–4.20 (2H, m, 3′-H, 1′eq-H); 4.21 (1H, br d, *J* = 13.0, 1′ax-H); 4.45 (1H, m, 2′-H); 6.83 (2H, d, *J* = 8.8, H *arom*);7.18–7.48 (12H, m, H *arom*); 8.22 (1H, s, 8-H); 8.74 (1H, br s, NH); 12.04 (1H, br s, NH).

^{13}C NMR: (CDCl$_3$, 125 MHz, δ): – 4.75 (br, CH$_3$Si); 17.92 (*C*H$_3$)$_3$C); 18.76 (*C*H$_3$)$_2$CH); 18.94 (*C*H$_3$)$_2$CH); 36.18 (CH$_3$)$_2$*C*H); 55.16 (*C*H$_3$O); 55.61 (2′-C); 63.89 (1′-C); 64.22 (6′-C); 65.82 (4′-C); 69.86 (3′-C); 76.25 (5′-C); 86.71 (Ph$_3$*C*); 113.16 (C*meta*-PhOCH$_3$); 120.74 (5-C); 126.94-135.32 (5 × C *arom*); 138.70 (8-C); 147.35 (4-C); 144.25 (C *arom*); 144.27 (C *arom*); 148.57 (2-C); 155.62 (6-C); 158.58 (C *arom*); 178.61 (C = O). HRMS: calcd for $C_{41}H_{52}N_5O_7Si$ (M + H) ^{1}754.3635, found 754.3634.

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