DOI: 10.1002/ejoc.200600868

Fmoc-Protected Altritol Phosphoramidite Building Blocks and Their Application in the Synthesis of Altritol Nucleic Acids (ANAs)

Mikhail Abramov,[a] Guy Schepers,[a] Arthur Van Aerschot,[a] and Piet Herdewijn*[a]

Keywords: Altritol nucleoside / Fmoc protecting group / Altritol nucleic acids / Phosphoramidite chemistry

Fmoc-protected altritol nucleoside phosphoramidite building blocks with adenine, guanine, thymine, uracil, cytosine and 5-methylcytosine as bases have been synthesized. These building blocks were used for the synthesis of altritol nucleic acid (ANA) and chimeric ANA-RNA oligonucleotides. Whilst the yields of oligonucleotides were lower than those obtained with 3′-*O*-benzoyl-protected altritol building blocks, the excellent compatibility with Pac-RNA chemistry for the synthesis of chimeric oligonucleotides makes Fmoc a valuable protecting group for the secondary alcohol functions of the sugar moieties of nucleosides for oligonucleotide synthesis.

Introduction

The selection of appropriate protecting groups is a critical issue in successful solid-phase oligonucleotide synthesis. In view of their potential interest as therapeutic or diagnostic agents, we have described the synthesis and physicochemical properties of (4′–6′) altritol nucleic acids (ANAs).[1] However, the synthetic problems associated with the need to protect the additional 3′-hydroxy groups of altritol nucleosides for oligonucleotide synthesis have slowed down the further development of ANA.

The problems in ANA oligonucleotide synthesis have usually been overcome by the use of the benzoyl protecting group for the 3′-hydroxy group, in combination with the phosphoramidite method.[1] However, the problem of 3′→4′ benzoyl migration during the synthesis of the protected building blocks[2] results in difficulties for the large-scale preparation of isomerically pure phosphoramidites, and so we investigated the use of the 3′-*O*-TBDMS protecting group in ANA oligonucleotide synthesis.[3] Although RNA could be produced by this process, the deprotection steps for the preparation of ANA sequences were much more difficult than those for RNA sequences. Steric hindrance in the ANA amidites, such as of the axial TBDMS groups, requires longer coupling times, which increase the formation of side products. Base deprotection with ammonia also needs longer reaction times, which might cause internucleotide cleavage. Desilylation with TBAF is very sensitive to water and could produce salts that must be removed prior to analysis. Triethylamine–tris(hydrogen fluoride) (TEA·3HF) has been used as an alternative to TBAF, but was similarly unsuccessful in cases of long stretches of ANA.

Because of different and not always reproducible deprotection results for the 3′-*O*-TBDMS group in ANA oligonucleotides synthesis (base modification, migration of the phosphate linkage, and degradation have been observed by HRMS analysis), we decided to investigate other protecting strategies for ANA oligonucleotide synthesis.

The decision to use Fmoc as the protecting group was based on the fact that it can be removed from protected bases and sugar moieties by treatment with aliphatic amines such as triethylamine or piperidine,[4] oximate reagent[5] or potassium carbonate in methanol.[6] In addition, Fmoc can be used as a protecting group both for the heterocyclic base and for the 3′-OH group.[7] We decided to use the 2-cyanoethyl *N*,*N*-diisopropylphosphoramidite approach[8] because of its high yield in the internucleotide coupling reaction. The more base-labile 2-cyanoethyl phosphate protecting group should be released more rapidly than the Fmoc protecting group, avoiding migration reactions. Moreover, all protecting groups (except for MMTr) can be removed by β-elimination, which makes a one-step final deprotection procedure possible.[8a]

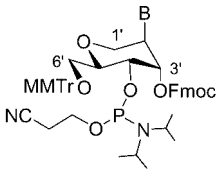
Discussion

Synthesis of ANA Phosphoramidite Building Blocks

Seven phosphoramidites of D-altritol nucleosides incorporating a 3′-*O*-(9-fluorenylmethoxycarbonyl) protecting group – compounds **1a**–**7a** (base moieties are adenine, guanine, uracil, cytosine, thymine and 5-methylcytosine) – were synthesized by a new strategy. The nucleosides were obtained by ring opening of 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol,[3] which was in turn prepared in five steps

[a] Laboratory of Medicinal Chemistry, Rega Institute for Medical Research
Minderbroedersstraat 10, 3000 Leuven, Belgium

from commercially available tetraacetyl α-D-bromoglucose (54% overall yield), basically by the procedure described by Brockway et al. (Figure 1).[9]



1a–7a

B = A^{Fmoc2} (**1a**); G^{Fmoc2} (**2a**); ^{dmf}G (**3a**); T (**4a**); U (**5a**); C^{Fmoc} (**6a**); $^{Me}C^{Fmoc}$ (**7a**)

Figure 1. Structures of amidites **1a**–**7a** containing altritol sugar moieties.

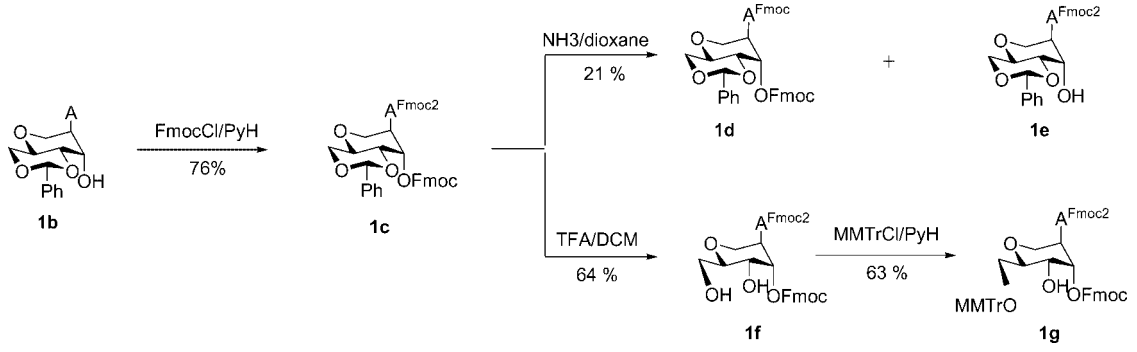
The advantage of this approach is that a D-altritol nucleoside with a free 3′-OH group and protected 4′-OH and 6′-OH groups is obtained, thus avoiding problems with the regioselective introduction of a protecting group in the 3′-position. Different conditions were tested for the nucleophilic opening of the epoxide by the salts of nucleobases. As well as the classical sodium and lithium salts,[1] a softer base (DBU) or a phase-transfer catalyst such as tetrabutylammonium chloride/potassium carbonate could be applied.[3] The preferred reaction conditions proved to be base-dependent.

The fully protected altritol phosphoramidite incorporating an adenine base moiety was obtained in five steps. Treatment of the DBU salt of adenine (3 equiv.) with 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-allitol in DMF at 90 °C for 6 h gave altritol **1b** (Scheme 1) in 70% yield. One-pot Fmoc protection both of the N^6-amino group in the adenine base and of the 3′-OH group in the hexitol moiety was carried out by treatment with Fmoc chloride in pyridine to give only 1,5-anhydro-4,6-*O*-benzylidene-2-[N^6-bis(9-fluorenylmethoxycarbonyl)adenin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-D-*altro*-hexitol (**1c**) in 76% yield. To avoid solubility problems associated with the fully protected building blocks in the solvent used for oligonucleotide synthesis and steric hindrance at the base moiety in the adenine building block, we tried to remove one of the Fmoc protecting groups to obtain 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[N^6-(9-fluorenylmethoxycarbonyl)adenin-9-yl]-D-*altro*-hexitol (**1d**). We tested different conditions for the selective removal of one Fmoc group on the N^6-amino group (pyridine/water, ammonia/dioxane, triethylamine/dioxane), but this resulted either in complete deprotection of hexitol **1c** (to give **1b**) or in partial conversion of **1c** into a mixture of **1d** and the 3′-OH-deprotected compound **1e** in low yield.

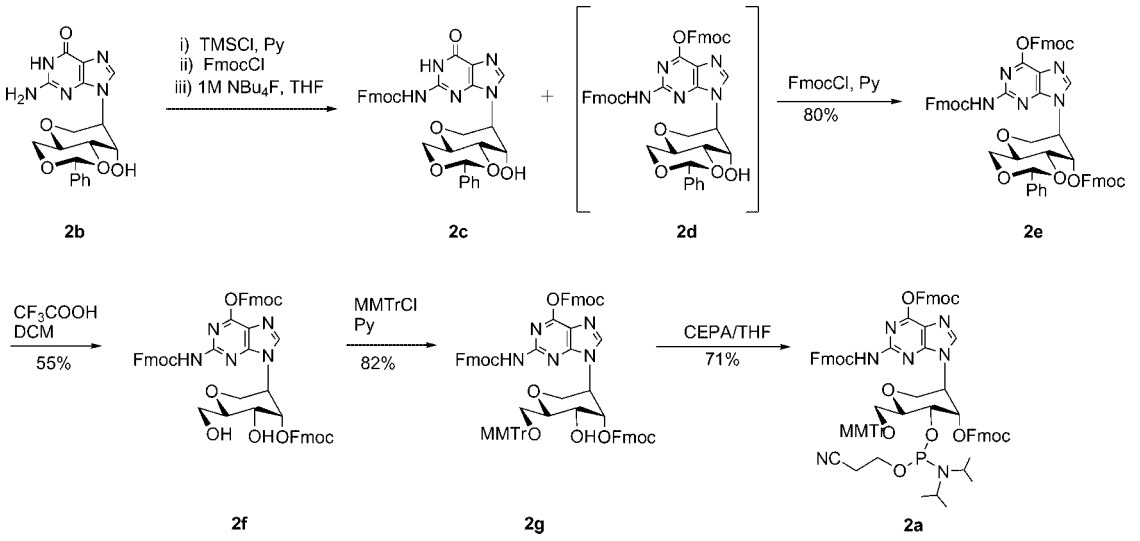
Removal of the benzylidene protecting group of **1c** could be accomplished by treatment with trifluoroacetic acid in dichloromethane without migration of the 3′-*O*-Fmoc protecting group (and without formation of a 3′,4′-cyclic carbonate), giving **1f** in 64% yield. Similarly, the 6′-*O*-monomethoxytrityl group can be introduced under common reaction conditions (pyridine, room temperature). Synthesis of the A(Fmoc)$_3$ phosphoramidite **1a** was accomplished by phosphitylation of the 3′-*O*-Fmoc-6′-*O*-monomethoxytrityl-protected building block **1g** with the aid of 2-cyanoethyl chloro-*N*,*N*-diisopropylposphoramidite (CEPA).[8b]

As might be expected, the guanine congener is a particular case. Previously[1] we had described epoxide opening with the sodium salt of 2-amino-6-chloropurine in DMF in 40% yield. Besides the major product, two side compounds were identified: the N^7-substituted compound and the bis(purinyl) nucleoside. The same reaction with the lithium salt of N^2-acetyl-2-amino-6-[2-(trimethylsilyl)ethoxy]purine afforded the protected guanine nucleoside in 45% yield (after deacetylation), unsatisfactory for large-scale synthesis of the altritol nucleosides, whilst the reaction in the presence of Aliquat 336/K_2CO_3 in DMF gave a 45% yield of the desired compound together with three side compounds. The additional side compound proved to be the N^9-substituted 2-amino-4-(dimethylamino)purine nucleoside. By utilising 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.[3]

Direct Fmoc protection of **2b**[3] (Scheme 2) did not yield the desired N^2,3′-*O*-bis-Fmoc-protected guanine **2e**; only the 3′-*O*-Fmoc-monoprotected compound was formed, in 45% yield. Transient trimethylsilylation of **2b** followed by treatment with Fmoc chloride and deprotection with TBAF, however, yielded a mixture of **2c** and **2d** in a 1:1 ratio, and this was treated with a twofold excess of Fmoc chloride in pyridine to give tris(Fmoc)-protected **2e** exclusively in a 50% yield based on **2b**.



Scheme 1. Synthesis of adenine building block **1g**.



Scheme 2. Synthesis of guanine amidite **2a**.

After removal of the benzylidene group, the primary hydroxy group was protected by treatment with monomethoxytrityl chloride. Finally, the phosphoramidite **2a** was obtained in 71% yield by phosphitylation of the protected building block **2g** with CEPA.[8b] Unfortunately, though, the amidite **2a** has a low solubility in acetonitrile and is not suited for oligonucleotide synthesis in this solvent, and so we synthesized the dimethylformamidine-protected (dmf-protected) G building block.

The ^{dmf}G-protected phosphoramidite **3a** was obtained by starting from 2-amino-6-chloropurine, which was converted into the guanine base **2b**. This was followed by the introduction of the dimethylformamidine protecting group, affording **3b**, and the Fmoc group on 3′-OH, affording **3c** (Scheme 3).

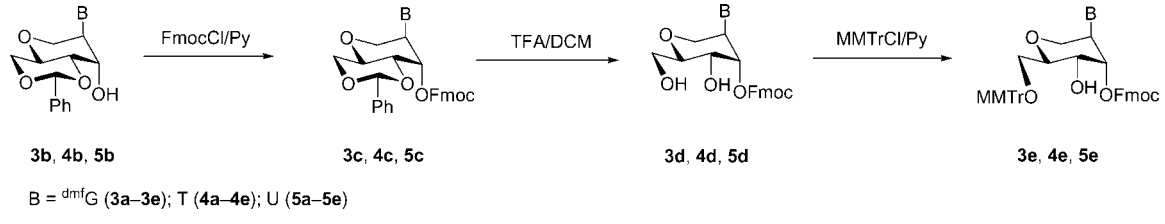
After removal of the benzylidene group, the primary hydroxy group was protected with monomethoxytrityl chloride to yield **3e**. The phosphoramidite **3a** was obtained in 71% yield.[8b]

The fully protected U and T altritol phosphoramidites were each obtained in five steps, from uracil and thymine, respectively. The DBU salts of the bases were each treated with 1,5:2,3-dianhydro-4,6-*O*-benzylidene-D-altritol in DMF, yielding 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-2-(thymin-1-yl)-D-*altro*-hexitol (**4b**, 94%) and 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-2-(uracil-1-yl)-D-*altro*-hexitol (**5b**, 75%).[3] Introduction of the 3′-*O*-Fmoc protecting group was carried out by treatment with Fmoc chloride in pyridine and yielded **4c** and **5c**, respectively (Scheme 3). After removal of the benzylidene protecting groups, the primary hydroxy groups were each protected with a monomethoxytrityl group. Finally, the T(Fmoc) phosphoramidite **4a** and the U(Fmoc) phosphoramidite **5a** were obtained by phosphitylation of the 3′-*O*-Fmoc-6′-*O*-monomethoxytrityl-protected T (**4e**) and U (**5e**) building blocks.[8b]

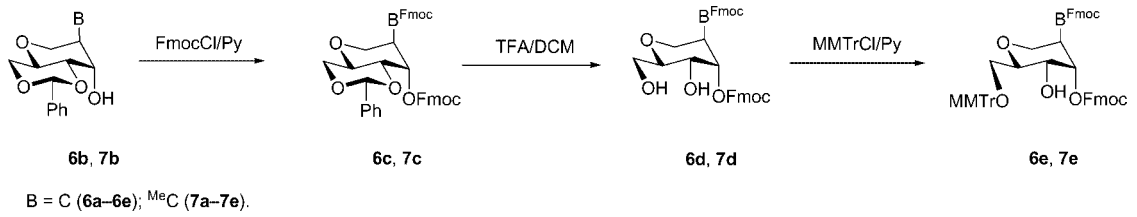
The fully protected C (**6a**) and ^{Me}C (**7a**) phosphoramidites were each obtained in six steps from uracil and thymine, respectively. 1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(uracil-1-yl)-D-*altro*-hexitol (**5b**) and the thymine analogue **4b** were then used as starting materials (Scheme 4) for the synthesis of the protected cytosine (**6b**) and 5-methylcytosine (**7b**) congeners.[1,3] The method used was 1,2,4-triazolyl activation of the 4-positions of the uracil and thymine bases, followed by substitution with ammonia to yield **6b** and **7b**.[10] In all cases investigated, it seems that the conversion of the uracil and thymine bases into the cytosine bases 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 corresponding nucleobases.

The N^4-positions and the 3′-OH groups were each Fmoc-protected in one step, followed by benzylidene removal and 6-*O*-monomethoxytritylation. Finally, the C(Fmoc) and ^{Me}C(Fmoc) phosphoramidites **6a** and **7a** were obtained in 88% yields by phosphitylation of the protected C (**6e**) and ^{Me}C (**7e**) building blocks.

In a preliminary effort to apply the Fmoc protecting group strategy for the synthesis of RNA we started with Fmoc protection of the 2′-OH groups in the ribose moieties



Scheme 3. Synthesis of guanine, thymine and uracil building blocks **3e**–**5e**.



Scheme 4. Synthesis of cytosine and 5-methylcytosine building blocks **6e** and **7e**.

of TIPS-protected rU and ^{dmf}rG. After removal of the TIPS groups with TEA·3HF in pyridine, 2′-*O*-Fmoc-protected rU and ^{dmf}rG were isolated in good yields. During the tritylation of rU and ^{dmf}rG, however, 2′→3′ Fmoc migration was observed and the desired 2′-*O*-Fmoc-protected ribonucleosides were difficult to separate. New reaction conditions for the application of 2′-*O*-Fmoc protection in RNA synthesis are to be investigated.

ANA Oligonucleotide Synthesis

With six new building blocks – **1a** and **3a**–**7a** – to hand, both the coupling and the deprotection conditions needed to be studied. Reactions were first tested and optimized with the thymine analogue **4a**, and decamers containing four altritol blocks in the middle of a series of deoxythymidines (5′-TTT-aT-aT-aT-TTT-3′) were synthesized. In view of the expected steric hindrance, one of the more powerful reported coupling agents, pyridine trifluoroacetate (PTFA),[11] was used with 10 equiv. of the amidite solution and a 14 min coupling time, giving yields of about 70% per coupling. In view of the low coupling yields, in the next series of tests we used 14.5 equiv. of the amidite solution and compared tetrazole (0.45 M), ethylthiotetrazole (0.25 M) and PTFA (0.22 M) as activators. Hardly any difference could be seen in the detritylation graphs, with combined yields over five couplings of 82%, 91% and 90%, respectively. The supports were treated with the AMA reagent[12] at 30 °C for 1.5 h and analysed on a MonoQ ion-exchange column. While detritylation yields were slightly reduced with tetrazole as the activator, no differences in purity could be seen when using the different HPLC profiles as analytical tools. Similar results were obtained with phosphoramidites **1a**, **3a** and **5a**–**7a**.

A series of fully modified and mixed altritol sequences was then prepared on a 1 μmol scale with use of ETT and 14.5 equiv. of amidite (sequences **10**–**14**). To avoid the need for the synthesis of different altritol nucleoside containing supports, a propanediol-modified universal support was used, generating a 3-hydroxypropyl phosphate extension at the 4′-end.[13] Unfortunately, the synthesis turned out to be cumbersome and HPLC profiles after deprotection showed complex mixtures of oligomers, with only small amounts of the desired products being isolated. Overall it seems that coupling might be reasonable, as indicated by good detritylation yields, but that the procedure is not fully reproducible and is sequence-dependent. The reasons for these shortcomings might involve lower solubilities of the fully Fmoc-protected oligomers and steric hindrances due to the bulky protecting groups. Buck et al. had already experimented with Fmoc-protected monomers as transient protecting groups for heterocyclic bases for the synthesis of short oligomers.[4a] For the preparation of longer oligonucleotides, however, they used a protection strategy in solution after having assembled the oligomer on solid support with traditional building blocks (Table 1).

Table 1. Series of fully modified altritol oligomer sequences prepared with amidites **1a**–**7a**.

Oligomer sequences (6′→4′)	Yield (%)[a]
a(TTTGACAAAACC) (**10**)	22[b]
a(GAGACAACGGGT) (**11**)	18[c]
a(CTACTACTTTTC) (**12**)	38[d]
a(GCGCTTTTGCGC) (**13**)	16[e]
a(AAATTTATGTCT) (**14**)	31[f]

[a] Overall coupling yields were calculated from MMTr cation quantitation measurements. [b] AAAA stretch 50%. [c] GGG stretch 25%. [d] First coupling 50%. [e] Last nine couplings 62% overall. [f] Last nine couplings 70% overall.

As well as the suitability of the coupling, deprotection was also investigated, for which a (5′-TTT-aG-aG-aG-TTT-3′) oligomer was prepared.[14] In addition, a thymidine support with the labile Q-linker (based on hydroquinone-*O*,*O*′-diacetic acid)[15] was used to allow rapid cleavage from the support. A large excess of the aG amidite **3a** was used to ensure suitable coupling (22 equiv., in the presence of ETT as the activator), resulting in an almost quantitative overall yield according to detritylation measurements.

The following deprotection conditions were compared by analysis by ion-exchange chromatography under basic conditions (NaOH, pH = 12): (a) ammonia in methanol (2 M) for 4 h at room temp. or (b) at 55 °C, (c) potassium carbonate in methanol (0.05 M) for 4 h at room temp. and (d) at 55 °C, and finally (e) piperidine in anhydrous DMF (10%) for 2 h at room temp. While conditions (a) were not satisfactory, (b) gave a more pronounced peak under the high-salt eluting conditions, but with still many side products. While conditions (c) generated only broad diffuse peaks, heating as in (d) proved detrimental, with mostly shorter fragments being eluted from the HPLC column. Exclusive piperidine treatment for 2 h only afforded a very small, diffuse and broad peak representing the desired oligonucleotide, whilst additional piperidine treatment proved detrimental.

We next tried single or multiple incorporations of adenine (**1a**) and uracil (**5a**) monomers into RNA on a 1 μmol scale, as outlined in Table 2. Phenoxyacetyl-(Pac-)2′-*O*-silyl-

Table 2. Series of mixed altritol oligomer sequences prepared with amidites **1a**–**7a**.

Oligomer sequences (5′→3′)	Yield (%)[a]	OD_{260}	MS calcd.	MS found
GUA UUG ACA GCU AUa**U** CGA AdTdT (**15**)	53	18.3	6665.9	6666.1
GUA UUG ACA GCa**U** AUU CGA AdTdT (**16**)	66	24.2	6665.9	6666.7
GUA Ua**U**G ACA GCU AUU CGA AdTdT (**17**)	68	28.0	6665.9	6666.1
Ga**U**A UUG ACA GCU AUU CGA AdTdT (**18**)	55	25.7	6665.9	6666.1
GUA Ua**U**G ACA GCa**U** AaUa**U** CGA AdTdT (**19**)	55	11.6	6708.0	6708.8
GUa**A** Ua**U**G ACa**A** GCa**U** AUa**U** CGa**A** AdTdT (**20**)	70	8.0	6736.0	6736.1
Ga**U**a**A** a**U**a**U**G a**A**Ca**A** GCa**U** a**A**a**U**a**U** CGa**A** a**A**dTdT (**21**)	34	9.7	6820.1	6820.6
UUC GAA UAG CUG UCA Aa**U**A CdTdT (**22**)	64	32.0	6625.9	6626.1
UUC GAA UAG CUG a**U**CA AUA CdTdT (**23**)	62	22.7	6625.9	6626.3
UUC GAA UAG Ca**U**G UCA AUA CdTdT (**24**)	75	18.0	6625.9	6626.4
UUC GAA a**U**AG CUG UCA AUA CdTdT (**25**)	60	17.8	6625.9	6626.0
Ua**U**C GAA UAG CUG UCA AUA CdTdT (**26**)	65	13.3	6625.9	6626.2

[a] Yields of isolated oligonucleotides. Oligonucleotides were purified by anion-exchange HPLC and the purities and structures of chimeric oligonucleotides were confirmed by anion-exchange HPLC and HRMS. In all cases the purities of the samples were 95% or higher.

(TBDMS-)protected RNA phosphoramidites were applied for chimeric oligonucleotide synthesis. Amidite solution (0.08 M, 18 equiv.) was used for the altritol monomers in the presence of ETT activator (compared to 14 equiv. for the standard silyl-protected RNA monomers), in general ensuring adequate coupling. Final trityl yields, amounts of isolated oligomer (OD_{260}) and mass analyses (M^+) are listed in Table 2. Sequences **15**–**26** were synthesized and deprotected by use of AMA solution at 30 °C for 90 min, followed by a triethylamine–tris(hydrogen fluoride) treatment (1.5 mL NMP + 0.750 mL TEA + 1 mL TEA·3HF) for 3.5 h at 55 °C, allowing removal of the silyl protecting groups. The crude oligonucleotides were purified by anion-exchange HPLC and the purities and structures of the modified oligonucleotides were confirmed by anion-exchange HPLC and HRMS.

Conclusions

The synthesis of six altritol nucleoside phosphoramidite building blocks with Fmoc protection on base and sugar moieties has been accomplished successfully. Coupling yields are generally lower than those obtained with 3′-benzoyl-protected building blocks (protected on the base moieties with the conventional aryl protecting groups). The excellent compatibility with Pac-RNA chemistry for the synthesis of chimeric oligonucleotides points to the value of the Fmoc protecting group for oligonucleotide synthesis, although new research to select the optimal conditions for synthesis and deprotection still needs to be carried out. The full deprotection of the synthesized oligonucleotides is proving to be cumbersome at this moment: this could be due to steric hindrance and/or to low solubilities of the growing oligonucleotides in the solvents used for synthesis and deprotection. In conclusion, the benzoyl group is a better protecting group than Fmoc (for the 3′-OH function) for the synthesis of ANA. Further research will be focussed on the synthesis of 3′-*O*-benzoyl-protected altritol phosphoramidite building blocks, avoiding migration reactions during the synthesis.

Experimental Section

General Procedures: 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. ^1H and ^{13}C NMR spectra were determined with a 200 MHz Varian Gemini apparatus with tetramethylsilane as internal standard for the ^1H 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 – [D_6]DMSO (δ =39.6 ppm) or $CDCl_3$ (δ =76.9 ppm) – for the ^{13}C NMR spectra. For some products a Varian Unity-500 spectrometer (500 MHz for ^1H NMR) was used. Coupling constant values were derived by first-order spectral analysis. Exact mass measurements were performed with a quadrupole/orthogonal acceleration time-of-flight tandem mass spectrometer (qTOF2, Micromass, Manchester, UK) fitted with a standard electrospray ionization interface. Precoated Machery–Nagel Alugram SILG/UV_{254} plates were used for TLC, and the spots were examined under UV light and by use of 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, heated at reflux and distilled. Pyridine was heated at reflux in the presence of potassium hydroxide pellets and distilled. Dimethylformamide was dried with activated molecular sieves (4 Å). HMPA was dried by azeotropic distillation with toluene. Methanolic ammonia was prepared by bubbling NH_3 gas through absolute methanol at 0 °C and stored at 0 °C.

Automated Synthesis of Oligonucleotides with 3′-*O*-Fmoc ANA Building Blocks: The synthesis of oligonucleotides was accomplished by the standard phosphoramidite method on an Exedite synthesiser (Applied Biosystems) with an extended 12 min coupling step. A 15-fold excess of nucleobase acetonitrile solution (200 μL of 0.07 M) and an excess of activator (200 μL) relative to CPG-bound 5′-hydroxy group we used in each coupling cycle. The synthesis scale was 1.0 μmol. Average coupling yield was monitored by online colorimetry by colorimetric quantitation of the trityl fractions. The detritylation solution was 3% TCA in DCM, capping was performed with Ac_2O/THF and 1-methylimidazole/THF/PyH (10%) 1-methylimidazole/THF/PyH, whilst the oxidation solution was I_2 in THF/H_2O/PyH (0.02 M). Tetrazole solution in acetonitrile (0.45 M), *S*-ethylthioterazole solution in MeCN (0.25 M) or pyridinium trifluoroacetate solution in MeCN (0.22 M) was used as an activator.

Deprotection and Purification of Oligonucleotides: Cleavage from the support and base and 3′(4′)-OH deprotection were performed in one step with AMA at 30 °C for 1.5 h. The solution of crude oligonucleotide was desalted on an NAP-25 column and purified by anion-exchange HPLC. The purities and structures of modified oligonucleotides were confirmed by anion-exchange HPLC and HRMS.

Synthesis of 1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol Nucleoside 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidites] 1a–7a: Dry 3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-(monomethoxytrityl)-D-*altro*-hexitol *N*-(9-fluorenylmethoxycarbonyl)-protected nucleoside (1 mmol) was dissolved in dry THF (5 mL). 2,4,6-Collidine (7.5 mmol) was added, followed by *N*-methylimidazole (0.5 mmol). (2-Cyanoethyl)-*N*,*N*-diisopropylphosphonamidic chloride (2.5 mmol) was then added dropwise at room temperature over 5 min. The reaction was complete after 1–2 h as determined by TLC. The reaction mixture was diluted with dichloromethane (50 mL), washed with water and dried with Na_2SO_4. The solvent was removed in vacuo, yielding a viscous oil. Co-evaporation with toluene (2 × 10 mL) afforded the crude phosphoramidites as off-white foams or oils. The phosphoramidites were further purified by silica gel chromatography and precipitated from hexane (150 mL) at –60 °C, yielding white, fine powders in 75–85% yields.

1,5-Anhydro-2-[N^2-bis(9-fluorenylmethoxycarbonyl)adenin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (1a): ^{31}P NMR ($CDCl_3$): δ = 149.75, 152.41 ppm. HRMS: calcd. for $C_{85}H_{79}N_7O_{12}P$ $[MH]^+$ 1420.5524; found 1420.5562.

1,5-Anhydro-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (2a): ^{31}P NMR ($CDCl_3$): δ = 149.46, 151.59 ppm. HRMS: calcd. for $C_{85}H_{79}N_7O_{13}P$ $[MH]^+$ 1436.5474; found 1436.5537.

1,5-Anhydro-2-deoxy-[2-(N^2-dimethylaminomethylene)guanin-9-yl]-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (3a): ^{31}P NMR ($CDCl_3$): δ = 150.9 ppm. HRMS: calcd. for $C_{58}H_{64}N_8O_9P$ $[MH]^+$ 1047.4534; found 1047.4547.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (4a): ^{31}P NMR ($CDCl_3$): δ = 150.02, 151.93 ppm. HRMS: calcd. for $C_{55}H_{60}N_4O_{10}P$ $[MH]^+$ 967.4047; found 967.4099.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-2-(uracil-1-yl)-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (5a): ^{31}P NMR ($CDCl_3$): δ = 150.61, 152.63 ppm. HRMS: calcd. for $C_{55}H_{60}N_4O_{10}P$ $[MH]^+$ 967.4047; found 967.4099.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[N^4-(9-fluorenylmethoxycarbonyl)cytosin-1-yl]-6-*O*-monomethoxytrityl-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (6a): ^{31}P NMR ($CDCl_3$): δ = 149.50, 151.93 ppm. HRMS: calcd. for $C_{70}H_{71}N_5O_{11}P$ $[MH]^+$ 1188.4888; found 1188.4873.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[N^4-(9-fluorenylmethoxycarbonyl)-5-methylcytosin-1-yl]-6-*O*-monomethoxytrityl-D-*altro*-hexitol 4-[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite] (7a): ^{31}P NMR ($CDCl_3$): δ = 150.03, 151.96 ppm. HRMS: calcd. for $C_{70}H_{71}N_5O_{11}P$ $[MH]^+$ 1188.4888; found 1188.4873.

Synthesis of Individual Protected Nucleosides

1,5-Anhydro-2-[N^6,N^6-bis(9-fluorenylmethoxycarbonyl)adenin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol (1g)

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-[N^6,N^6-bis(9-fluorenylmethoxycarbonyl)adenin-9-yl]-3-*O*-(9-fluorenylmethoxycarbonyl)-D-*altro*-hexitol (1c): 9-Fluorenylmethoxycarbonyl chloride (4.2 g, 16.3 mmol) was added under nitrogen in four portions to a solution of **1b**[9] (1.5 g, 4.06 mmol) in dry pyridine (30 mL) and the reaction mixture was stirred at room temperature for 1 h. The reaction was monitored by TLC. MeOH (10 mL) was then added and the stirring was continued for 30 min. The yellow solution was concentrated and co-evaporated to dryness with toluene (2 × 30 mL). The residue was subjected to silica gel flash column chromatography with acetone in dichloromethane (2.5%) as eluent. Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **1c** as a white powder (3.2 g, 76%). ^{1}H NMR ($CDCl_3$): δ = 3.64 (dd, J = 2.6, 9.7 Hz, 1 H, 4′-H), 3.74 (t, J = 10.4 Hz, 1 H, 6′ax-H), 4.10–4.70 [m, 13 H, 1′-H, 5′-H, 6′eq-H, CH_2O and 9-H (Fmoc)], 5.00 (br. s, 1 H, 2′-H), 5.39 (s, 1 H, PhCH), 5.72 (br. s, 1 H, 3′-H), 7.19–7.50 (m, 21 H, H arom), 7.65 (m, 6 H, H arom), 7.80 (d, J = 7.7 Hz, 2 H, H arom), 8.55 (s, 1 H, 8-H), 8.94 (s, 1 H, 2-H) ppm. HRMS: calcd. for $C_{63}H_{50}N_5O_{10}$ $[MH]^+$ 1036.3558; found 1036.3553.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[N^6-(9-fluorenylmethoxycarbonyl)adenin-9-yl]-D-*altro*-hexitol (1d): Compound **1c** (103 mg, 0.1 mmol) was dissolved in dioxane (2 mL) and ammonia (26%, 500 μL) was added at 0 °C. After 5 min, the solution was concentrated and co-evaporated to dryness with toluene (2 × 5 mL). The residue was purified by silica gel flash column chromatography with acetone in dichloromethane (5%) to afford the title compound **1d** as a white solid (17 mg, 21%). ^{1}H NMR ($CDCl_3$): δ = 3.72–3.86 (m, 2 H, 4′-H, 6′ax-H), 4.14–4.78 [m, 11 H, 1′-H, 5′-H, 6′eq-H, CH_2O and 9-H (Fmoc)], 4.98 (br. s, 1 H, 2′-H), 5.50 (s, 1 H, PhCH), 5.66 (br. s, 1 H, 3′-H), 7.20–7.50 (m, 13 H, H arom), 7.65 (m, 4 H, H arom), 7.78 (d, J = 7.7 Hz, 4 H, H arom), 8.60 (s, 1 H, 8-H), 8.81 (br. s, 1 H, 2-NH), 8.90 (s, 1 H, 2-H) ppm. HRMS: calcd. for $C_{63}H_{50}N_5O_{10}$ $[MH]^+$ 814.2877; found 814.2883.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[N^6,N^6-bis(9-fluorenylmethoxycarbonyl)adenin-9-yl]-D-*altro*-hexitol (1f): Compound **1c** (2.9 g, 2.8 mmol) was dissolved in dichloromethane (30 mL) and TFA (4 mL) was added at 0 °C. The reaction was monitored by TLC. After the mixture had been stirred at room temperature for 1 h, ethanol (20 mL) was added and the yellow-brown solution was concentrated and co-evaporated to dryness with toluene (2 × 30 mL). The residue was purified by silica gel flash column chromatography with a stepwise gradient of methanol (2–4%) in dichloromethane to afford the title compound **1f** as a white solid (1.7 g, 64%). ^{1}H NMR ($CDCl_3$): δ = 2.0–2.8 (br. s, 2 H, 4′-OH and 5′-OH), 3.83–3.95 (m, 4 H, 4′-H, 5′-H, 6′-H), 4.10–4.61 [m, 11 H, 1′-H, CH_2O and 9-H (Fmoc)], 5.00 (br. s, 1 H, 2′-H), 5.72 (br. s, 1 H, 3′-H), 7.19–7.50 (m, 21 H, H arom), 7.65 (m, 6 H, H arom), 7.80 (d, J = 7.7 Hz, 2 H, H arom), 8.55 (s, 1 H, 8-H), 8.94 (s, 1 H, 2-H) ppm. HRMS: calcd. for $C_{56}H_{46}N_5O_{10}$ $[MH]^+$ 948.3245; found 948.3253.

1,5-Anhydro-2-[N^6,N^6-bis(9-fluorenylmethoxycarbonyl)adenin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol (1g): Monomethoxytrityl chloride (0.65 g, 2.1 mmol) was added under nitrogen at room temperature to a stirred solution of **1f** (1.6 g, 1.7 mmol) in dry pyridine (15 mL). The reaction was monitored by TLC. After the mixture had been stirred

for 2 h, methanol (3 mL) was added and the solution was concentrated and co-evaporated to dryness with toluene (2 × 15 mL). The residue was purified by silica gel flash column chromatography with acetone in dichloromethane (3%). Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **1g** as a white powder (1.2 g, 63%). ^{1}H NMR ($CDCl_3$): δ = 1.94 (br. s, 1 H, 4′-OH), 3.38 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.56 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.79 (s, 3 H, CH_3), 3.89 (br. s, 2 H, 4′-H, 5′-H), 4.06–4.18 (m, 2 H, 1-H), 4.25–4.69 [m, 9 H, CH_2O and 9-H (Fmoc)], 5.04 (br. s, 1 H, 2′-H), 5.56 (br. s, 1 H, 3′-H), 6.82 (d, J = 8.9 Hz, 2 H, H arom), 7.19–7.50 (m, 24 H, H arom), 7.65 (m, 6 H, H arom), 7.80 (d, J = 7.7 Hz, 2 H, H arom), 8.79 (s, 1 H, 8-H), 8.96 (s, 1 H, 2-H) ppm. HRMS: calcd. for $C_{76}H_{62}N_5O_{11}$ [MH]$^+$ 1220.4446; found 1220.4454.

1,5-Anhydro-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol (2g)

1,5-Anhydro-4,6-*O*-benzylidene-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-D-*altro*-hexitol (2c): 1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(guanin-9-yl)-D-*altro*-hexitol (**2b**,[3] 1.95 g, 5.0 mmol) was co-evaporated with pyridine (2 × 50 mL), and TMSCl (6.4 mL, 50 mmol) was added dropwise at 0 °C under argon to the resulting suspension in pyridine (30 mL). The resulting clear solution was stirred at room temperature for 2 h; Fmoc chloride (5.2 g, 20 mmol) was added in 1 g portions over 3 h and stirring was continued for 1 h. Methanol (10 mL) was added dropwise at 0 °C and the reaction mixture was stirred for 10 min. The resulting mixture was concentrated and co-evaporated with toluene (2 × 30 mL) under reduced pressure. The residue was extracted with ethyl acetate, washed with water, dried with magnesium sulfate and purified by flash silica gel column chromatography with methanol in dichloromethane (1.5%). Yield of 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-2-[N^2-(9-fluorenylmethoxycarbonyl)guanin-9-yl]-3-*O*-trimethylsilyl-D-*altro*-hexitol 3.0 g (66%). ^{1}H NMR ($CDCl_3$): δ = 0.21 (s, 9 H, CH_3Si), 3.54 (dd, J = 1.8, 9.5 Hz, 1 H, 4′-H), 3.72 (t, J = 10.5 Hz, 1 H, 6′ax-H), 4.09–4.70 [m, 9 H, 1′-H, 2′-H, 3′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.44 (s, 1 H, PhCH), 7.20–7.45 (m, 9 H, H arom), 7.55–7.60 [m, 4 H, H arom (Fmoc)], 7.87 (br. s, 1 H, 2-NH), 8.37 (s, 1 H, 8-H), 11.30 (br. s, 1 H, NH) ppm. HRMS: calcd. for $C_{36}H_{38}N_5O_7Si$ [MH]$^+$ 680.2541; found 680.2535. 1,5-Anhydro-4,6-*O*-benzylidene-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-3-*O*-trimethylsilyl-D-*altro*-hexitol (300 mg) was isolated as a minor product. ^{1}H NMR ($CDCl_3$): δ = 0.40 (s, 9 H, CH_3Si), 3.45 (dd, J = 1.8, 9.5 Hz, 1 H, 4′-H), 3.64 (t, J = 10.5, Hz, 1 H, 6′ax-H), 4.09–4.70 [m, 12 H, 1′-H, 2′-H, 3′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.39 (s, 1 H, PhCH), 7.02–7.38 (m, 18 H, 2-NH and H arom), 7.50–7.60 [m, 4 H, H arom (Fmoc)], 8.37 (s, 1 H, 8-H) ppm. HRMS: calcd. for $C_{51}H_{48}N_5O_9Si$ [MH]$^+$ 902.3221; found 902.3228. A solution of 1,5-anhydro-4,6-*O*-benzylidene-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-3-*O*-trimethylsilyl-D-*altro*-hexitol (3.0 g, 3.3 mmol) was dissolved in THF (10 mL) and Bu_4NF (1 N in THF, 6 mL) was added dropwise to the resulting solution at 0 °C. After 30 min, the solution was added slowly (dropwise) to ice-cold water (250 mL) with stirring. The obtained solid was filtered off, dried and purified by flash silica gel column chromatography with methanol in dichloromethane (2.5%). A mixture of two products (2.1 g) was isolated.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-[N^2-(9-fluorenylmethoxycarbonyl)guanin-9-yl]-D-*altro*-hexitol (2c): ^{1}H NMR ([D$_6$]DMSO): δ = 3.64 (dd, J = 1.8, 9.5 Hz, 1 H, 4′-H), 3.79 (t, J = 10.5, Hz, 1 H, 6′ax-H), 4.09–4.70 [m, 9 H, 1′-H, 2′-H, 3′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.58 (s, 1 H, PhCH), 5.73 (br. s, 1 H, 3′-OH), 7.20–7.36 (m, 5 H, H arom), 7.33–7.41 [m, 4 H, H arom (Fmoc)], 7.73–7.80 [m, 4 H, H arom (Fmoc)], 8.08 (s, 1 H, 2-NH), 8.10 (s, 1 H, 8-H), 11.30–11.90 (br. d, 1 H, NH) ppm. HMRS: calcd. for $C_{33}H_{30}N_5O_7$ [MH]$^+$ 608.2145; found 608.2151.

1,5-Anhydro-4,6-*O*-benzylidene-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-D-*altro*-hexitol (2d): ^{1}H NMR ($CDCl_3$): δ = 2.42 (br. s, 1 H, 3′-OH), 3.57 (dd, J = 1.8, 9.5 Hz, 1 H, 4′-H), 3.78 (t, J = 10.5, Hz, 1 H, 6′ax-H), 3.95–4.62 [m, 12 H, 1′-H, 2′-H, 3′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.47 (s, 1 H, PhCH), 7.14–7.48 (m, 18 H, 2-NH and H arom), 7.58–7.65 [m, 4 H, H arom (Fmoc)], 8.32 (s, 1 H, 8-H) ppm. HMRS: calcd. for $C_{48}H_{40}N_5O_9$ [MH]$^+$ 830.2826; found 830.2817.

1,5-Anhydro-4,6-*O*-benzylidene-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)guanin-9-yl]-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-D-*altro*-hexitol (2e): The mixture (2.1 g) of **2c** and **2d** in pyridine (120 mL) was concentrated to 25 mL, Fmoc chloride (4.0 g, 15.4 mmol) was added in 1 g portions over 3 h, and stirring was continued for 1 h. Methanol (10 mL) was added dropwise at 0 °C and the reaction mixture was stirred for a further 10 min. The resulting mixture was concentrated and co-evaporated with toluene (2 × 30 mL) under reduced pressure, and the residue was extracted with ethyl acetate, washed with water, dried with magnesium sulfate and purified by flash silica gel column chromatography with methanol in dichloromethane (1.5%). Yield 2.2 g (42%) based on **2b**. ^{1}H NMR ($CDCl_3$): δ = 3.67 (dd, J = 1.8, 9.5 Hz, 1 H, 4′-H), 3.78 (t, J = 10.5 Hz, 1 H, 6′ax-H), 3.85–4.50 [m, 14 H, 1′-H, 2′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.19 (br. s, 1 H, 3′-OH), 5.30 (s, 1 H, PhCH), 7.00–7.60 (m, 27 H, H arom), 7.70–7.82 [m, 2 H, H arom (Fmoc)], 8.31 (s, 1 H, 8-H) ppm. HRMS: calcd. for $C_{63}H_{50}N_5O_{11}$ [MH]$^+$ 1052.3507; found 1052.3541.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[N^2-(9-fluorenylmethoxycarbonyl)guanin-9-yl]-6-D-*altro*-hexitol (2f): TFA (5 mL) was added dropwise at 0 °C to a solution of **2e** (2.2 g, 2.1 mmol) in dichloromethane (30 mL), and the reaction mixture was stirred for 30 min. Water (100 μL, 5.6 mmol) was added and stirring was continued for 15 min, ethanol (80%, 10 mL) was added, and solvents were removed. The residue was co-evaporated with toluene (2 × 30 mL) and the crude material was subjected to flash silica gel column chromatography with methanol in dichloromethane (4%) to afford the title compound as a white foam (1.0 g, 52%). ^{1}H NMR ($CDCl_3$): δ = 2.0–2.8 (br. s, 2 H, 4-OH and 5-OH), 3.80–4.48 (m, 15 H, 1′-H, 4′-H, 5′-H, 6′-H), [CH_2O and 9-H (Fmoc)], 5.06 (br. s, 1 H, 3′-H), 6.91–7.58 [m, 23 H, 2-NH and H arom (Fmoc)], 7.77–7.80 [m, 2 H, H arom (Fmoc)], 8.85 (s, 1 H, 8-H) ppm. HRMS: calcd. for $C_{56}H_{46}N_5O_{11}$ [MH]$^+$ 964.3194; found 964.3174.

1,5-Anhydro-2-deoxy-2-[N^2,O^6-bis(9-fluorenylmethoxycarbonyl)-guanin-9-yl]-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol (2g): A solution of **2f** (1.0 g, 1 mmol) in pyridine (50 mL) was concentrated to 10 mL and MMTrCl (620 mg, 2 mmol) was added under argon at room temperature. After 3 h, methanol (5 mL) was added and the volatiles were removed. The residue was co-evaporated with toluene (2 × 20 mL) and purified by silica gel flash column chromatography with methanol in dichloromethane (2%). Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **2g** as a white powder (1.0 g, 82%). ^{1}H NMR ($CDCl_3$): δ = 2.04 (br. s, 1 H, 4′-OH), 3.42 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.54 (dd, J = 1.1, 1.1 Hz, 1 H, 6′eq-H), 3.79 (s, 3 H, CH_3), 3.89–4.62 [m, 13 H, 2′-H, 4′-H, 5′-H, CH_2O and 9-H (Fmoc)], 4.96 (br. s, 1 H, 3′-H), 6.82 (d, J = 8.9 Hz, 2 H, H arom), 7.06–7.60 (m, 35 H, H arom and 2-NH), 7.74 [m, 2 H,

H arom (Fmoc)], 8.49 (s, 1 H, 8-H) ppm. HRMS: calcd. for $C_{76}H_{62}N_5O_{12}$ $[MH]^+$ 1236.4395; found 1236.4346.

1,5-Anhydro-2-deoxy-2-[(N^2-dimethylaminomethylene)guanin-9-yl]-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol (3e)

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-[2-(N^2-dimethylaminomethylene)guanin-9-yl]-3-*O*-(9-fluorenylmethoxycarbonyl)-D-*altro*-hexitol (3c): A mixture of **3b** (2.5 g, 5.7 mmol) and pyridine (20 mL) was concentrated to 5 mL, Fmoc chloride (1.75 g, 6.5 mmol) was added in 500 mg portions for 30 min, and stirring was continued for 1 h. Methanol (5 mL) was added dropwise at 0 °C and the reaction mixture was stirred for 10 min. The resulting mixture was concentrated and co-evaporated with toluene (2 × 30 mL) under reduced pressure and the residue was extracted with ethyl acetate, washed with water and purified by flash silica gel column chromatography with methanol in dichloromethane (1.5%). Yield 3.0 g (80%). ^{1}H NMR ($CDCl_3$): δ = 3.09 (s, 6 H, NMe_2), 3.67–3.85 (m, 2 H, 4′-H, 6′ax-H), 4.10–4.60 [m, 7 H, 1′-H, 2′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.51 (s, 1 H, PhC*H*), 5.86 (br. t, 1 H, 3′-H), 7.29–7.44 (m, 9 H, H arom), 7.50–7.68 [m, 2 H, H arom (Fmoc)], 7.77–7.82 [m, 2 H, H arom (Fmoc)], 8.03 (s, 1 H, 8-H), 8.87 (s, 1 H, C*H*), 8.95 (br. s, 1 H, N*H*) ppm. HRMS: calcd. for $C_{36}H_{34}N_6O_8$ $[M]^+$ 662.2489; found 662.2451.

1,5-Anhydro-2-deoxy-2-[N^2-(dimethylaminomethylene)guanin-9-yl]-3-*O*-(9-fluorenylmethoxycarbonyl)-D-*altro*-hexitol (3d): TFA (2 mL) was added dropwise at 0 °C to a solution of hexitol **3c** (2.2 g, 3.3 mmol) in dichloromethane (20 mL) and the reaction mixture was stirred for 30 min. Water (100 μL, 5.6 mmol) was added and stirring was continued for 15 min. The light yellow solution was neutralized with pyridine, washed with saturated NaCl and concentrated to dryness, and the crude material was precipitated from dichloromethane/hexane at 0 °C to afford the title compound **3d** as a white solid in 95% yield. ^{1}H NMR ([D$_6$]DMSO): δ = 3.02 (s, 6 H, NMe), 3.06 (s, 6 H, NMe), 3.60–3.80 (m, 4 H, 4′-H, 6′ax-H, 2 × OH), 4.00–4.60 [m, 7 H, 1′-H, 2′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.43 (br. t, 1 H, 3′-H), 7.29–7.39 (m, 4 H, H arom), 7.55–7.58 [m, 2 H, H arom (Fmoc)], 7.74–7.78 [m, 2 H, H arom (Fmoc)], 8.08 (s, 1 H, 8-H), 8.76 (s, 1 H, C*H*), 11.10 (br. s, 1 H, N*H*) ppm.

1,5-Anhydro-2-deoxy-2-[N^2-(dimethylaminomethylene)guanin-9-yl]-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-D-*altro*-hexitol (3e): A solution of **3d** (1.15 g, 2 mmol) in pyridine (10 mL) was concentrated to 3 mL and MMTrCl (620 mg, 2 mmol) was added under argon at room temperature. After 3 h, methanol (1.5 mL) was added and the solution was washed with water (3 × 50 mL) and dried with magnesium sulfate and the solvents were evaporated to dryness. Precipitation from dichloromethane/hexane at 0 °C afforded the title compound **3e** as a white powder (1.35 g, 80%). ^{1}H NMR ($CDCl_3$): δ = 3.05 (s, 6 H, NMe_2), 3.47 (m, 2 H, 4′-H, 6′ax-H), 3.80 (s, 3 H, CH_3), 3.90 (m, 2 H) and 4.10–4.60 (m, 5 H) [1′-H, 2′-H, 5′-H, 6′eq-H, 9-H and CH_2O (Fmoc)], 5.67 (br. t, 1 H, 3′-H), 6.84–6.88 (m, 2 H, H arom), 7.29–7.62 (m, 16 H, H arom), 7.59–7.66 [m, 2 H, H arom (Fmoc)], 7.77–7.81 [m, 2 H, H arom (Fmoc)], 8.07 (s, 1 H, 8-H), 8.77 (s, 1 H, C*H*), 9.21 (br. s, 1 H, N*H*) ppm. HRMS: calcd. for $C_{49}H_{47}N_6O_8$ $[MH]^+$ 847.3455; found 847.3458.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-D-*altro*-hexitol (4e)

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-(thymin-1-yl)-D-*altro*-hexitol (4c): Fmoc chloride (1.03 g, 4 mmol) was added under nitrogen in four portions to a solution of **4b**[3] (1.08 g, 3 mmol) in dry pyridine (10 mL) and the reaction mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC. MeOH (5 mL) was then added and the stirring was continued for 10 min. The yellow solution was concentrated and co-evaporated to dryness with toluene (2 × 10 mL), and the residue was subjected to silica gel flash column chromatography with methanol in dichloromethane (1.5%) as eluent. Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **4c** as a white powder (1.1 g, 63%). ^{1}H NMR ($CDCl_3$): δ = 2.02 (s, 3 H, 5-Me), 3.76–3.88 [m, 2 H, 4′-H and 9-H (Fmoc)], 4.10–4.60 [m, 7 H, 6′ax-H, 1′ax-H, 5′-H, 6′eq-H, 1′eq-H, CH_2O (Fmoc)], 4.65 (t, J = 2.9 Hz, 1 H, 2′-H), 5.50 (br. s, 1 H, 3′-H), 5.64 (s, 1 H, PhC*H*), 7.23–7.35 (m, 5 H, H arom), 7.35–7.46 (m, 4 H, H arom), 7.62 [d, J = 7.0 Hz, 2 H, H arom (Fmoc)], 7.88 [d, 6-H, J = 7.7 Hz, 2 H, H arom (Fmoc)], 7.88 (d, J = 1.1 Hz, 1 H, 6-H), 8.72 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{34}H_{31}N_2O_8$ $[MH]^+$ 583.2081; found 583.2078.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-(thymin-1-yl)-D-*altro*-hexitol (4d): Compound **4c** (1.75 g, 3 mmol) was dissolved in dichloromethane (30 mL) and TFA (3 mL) was added at 0 °C. The reaction was monitored by TLC. After the mixture had been stirred at room temperature for1 h, ethanol (20 mL) was added and the yellow-brown solution was concentrated and co-evaporated to dryness with toluene (2 × 30 mL). The residue was purified by silica gel flash column chromatography with methanol in dichloromethane (5%) to afford the title compound **4d** as a white solid (1.1 g, 74%). ^{1}H NMR ($CDCl_3$): δ = 1.80 (s, 3 H, CH_3), 3.20 (br. s, 2 H, 6′-OH and 4′-OH), 3.70–3.95 (3 H, m 4′-H, 5′-H, 6′ax-H), 3.96–4.50 [m, 7 H, 6′eq-H, 1′ax-H, 3′-H, 1′eq-H, 9-H and CH_2O (Fmoc)], 5.50 (br. s, 1 H, 3′-H), 7.20–7.40 (m, 4 H, H arom), 7.58 [d, J = 7.0 Hz, 2 H, H arom (Fmoc)], 7.74 [d, J = 7.7 Hz, 1 H, 6-H, H arom (Fmoc)], 7.80 (d, J = 1.1 Hz, 1 H, 6-H), 9.50 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{26}H_{27}N_2O_8$ $[MH]^+$ 495.1768; found 495.1765.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-2-(thymin-1-yl)-D-*altro*-hexitol (4e): MMTrCl (0.95 g, 3 mmol) was added under nitrogen at room temperature to a stirred solution of **4d** (1.0 g, 2 mmol) in dry pyridine (15 mL). The reaction was monitored by TLC. After the mixture had been stirred for 2 h, methanol (3 mL) was added and the solution was concentrated and co-evaporated to dryness with toluene (2 × 15 mL). The residue was purified by silica gel flash column chromatography with methanol in dichloromethane (3%). Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **4e** as a white powder (1.2 g, 52%). ^{1}H NMR ($CDCl_3$): δ = 1.80 (s, 3 H, CH_3), 3.40 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.48 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.78 (s, 3 H, CH_3), 3.72–3.85 (m, 1 H, 4′-H), 4.05–4.30 [m, 4 H, 1′ax-H, 3′-H, 1′eq-H, 5′-H, 9-H (Fmoc)], 4.40–4.50 [m, 2 H, CH_2O (Fmoc)], 4.66 (br. s, 1 H, 2′-H), 5.50 (br. s, 1 H, 3′-H), 6.82 (d, J = 8.9 Hz, 2 H, H arom), 7.22–7.40 (m, 16 H, H arom), 7.58 [d, J = 7.0 Hz, 2 H, H arom (Fmoc)], 7.75 [d, J = 7.7 Hz, 1 H, 6-H, H arom (Fmoc)], 7.80 (d, J = 1.1 Hz, 1 H, 6-H), 9.50 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{46}H_{43}N_2O_9$ $[MNa]^+$ 767.2969; found 767.2977.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-2-(uracyl-1-yl)-D-*altro*-hexitol (5e)

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-(uracil-1-yl)-D-*altro*-hexitol (5c): Fmoc chloride (1.03 g, 4 mmol) was added under nitrogen in four portions to a solution of **5b**[3] (1.04 g, 3 mmol) in dry pyridine (10 mL) and the reaction mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC. MeOH (5 mL) was then added and the stirring

was continued for 10 min. The yellow solution was concentrated and co-evaporated to dryness with toluene (2 × 10 mL), and the residue was subjected to silica gel flash column chromatography with methanol in dichloromethane (1.5%) as eluent. Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **5c** as a white powder (1.1 g, 63%). ^{1}H NMR ($CDCl_3$): δ = 3.76–3.88 [m, 2 H, 4′-H and 9-H (Fmoc)], 4.10–4.60 [m, 7 H, 6′ax-H, 1′ax-H, 5′-H, 6′eq-H, 1′eq-H, CH_2O (Fmoc)], 4.65 (t, J = 2.9 Hz, 1 H, 2′-H), 5.51 (br. s, 1 H, 3′-H), 5.62 (s, 1 H, PhC*H*), 5.82 (dd, J = 1.8, 8.4 Hz, 1 H, 5-H), 7.23–7.35 (m, 5 H, H arom), 7.36–7.46 (m, 4 H, H arom), 7.62 [d, J = 7.3 Hz, 2 H, H arom (Fmoc)], 7.80 [d, J = 7.7 Hz, 2 H, H arom (Fmoc)], 8.05 (d, J = 8.4 Hz, 1 H, 6-H), 9.46 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{32}H_{29}N_2O_8$ [MH]$^+$ 569.1925; found 569.1924.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-(uracil-1-yl)-D-*altro*-hexitol (5d): Compound **5c** (1.70 g, 3 mmol) was dissolved in dichloromethane (30 mL), TFA (3 mL) was added dropwise at 0 °C, and the reaction mixture was stirred for 30 min. Water (100 μL, 5.6 mmol) was added and stirring was continued for 15 min. The light yellow solution was neutralized with pyridine, washed with saturated NaCl and concentrated to dryness, and the crude material was precipitated from dichloromethane/hexane at 0 °C to afford the title compound **5d** as a white solid in 95% yield. ^{1}H NMR ($CDCl_3$): δ = 3.20 (br. s, 2 H, 6′-OH and 4′-OH), 3.70–3.95 (3 H, m, 4′-H, 5′-H, 6′ax-H), 3.96–4.50 [m, 7 H, 6′eq-H, 1′ax-H, 3′-H, 1′eq-H, 9-H and CH_2O (Fmoc)], 5.30 (br. s, 1 H, 3′-H), 5.64 (d, J = 8.1 Hz, 1 H, 5-H), 7.20–7.40 (m, 4 H, H arom), 7.60 [d, J = 7.0 Hz, 2 H, H arom (Fmoc)], 7.71 [d, J = 7.7 Hz, 1 H, 6-H, H arom (Fmoc)], 8.00 (d, J = 8.1 Hz, 1 H, 6-H), 10.0 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{25}H_{25}N_2O_8$ [MH]$^+$ 481.1611; found 481.1611.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-6-*O*-monomethoxytrityl-2-(uracil-1-yl)-D-*altro*-hexitol (5e): Monomethoxytrityl chloride (0.95 g, 3 mmol) was added under nitrogen at room temperature to a stirred solution of **5d** (1.0 g, 2.1 mmol) in dry pyridine (15 mL). The reaction was monitored by TLC. After the mixture had been stirred for 2 h, methanol (3 mL) was added and the solution was concentrated and co-evaporated to dryness with toluene (2 × 15 mL). Precipitation from dichloromethane/hexane at –60 °C afforded the title compound **5e** as a white powder (1.1 g, 72%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.40 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.48 (dd, J = 1.1, 1.1 Hz, 1 H, 6′ax-H), 3.78 (s, 3 H, CH_3), 3.72–3.85 (m, 1 H, 4′-H), 4.05–4.30 [m, 4 H, 1′ax-H, 3′-H, 1′eq-H, 5′-H, 9-H (Fmoc)], 4.40–4.50 [m, 2 H, CH_2O (Fmoc)], 4.66 (br. s, 1 H, 2′-H), 5.50 (br. s, 1 H, 3′-H), 5.88 (d, J = 8.1 Hz, 1 H, 5-H), 6.82 (d, J = 8.9 Hz, 2 H, H arom), 7.22–7.40 (m, 16 H, H arom), 7.58 [d, J = 7.0 Hz, 2 H, H arom (Fmoc)], 7.75 [d, J = 7.7 Hz, 1 H, 6-H, H arom (Fmoc)], 7.80 (d, J = 8.1 Hz, 1 H, 6-H), 8.95 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{45}H_{41}N_2O_9$ [MH]$^+$ 753.2812; found 753.2812.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)cytosin-1-yl]-6-*O*-monomethoxytrityl-D-*altro*-hexitol (6e)

1,5-Anhydro-4,6-*O*-benzylidene-2-(cytosin-1-yl)-2-deoxy-D-*altro*-hexitol (6b): Chlorotrimethylsilane (6.4 mL, 50 mmol) was added under nitrogen to a stirred suspension of **5b**[3] (3.6 g, 10.0 mmol) in dry pyridine (40 mL). After 1 h, the reaction mixture was cooled in an ice bath, 1*H*-1,2,4-triazole (6.9 g, 100 mmol) and phosphorus oxychloride (1.86 mL, 20 mmol) were added, and stirring was continued for 5 h. The volatiles were removed and the residue was co-evaporated with toluene (3 × 20 mL) and partitioned between water and ethyl acetate. The organic layer was washed with water and brine, and the solvents were evaporated to dryness to afford a yellow foam. This crude intermediate was dissolved in dioxane (40 mL), and aqueous ammonia (25%, 15 mL) was added. After the mixture had been stirred for 45 min, the volatiles were evaporated and the solid was co-evaporated with toluene. The residue was suspended in chloroform, co-evaporated with silica gel and subjected to silica gel column chromatography with a stepwise gradient of methanol in dichloromethane (2–10%), to afford the title compound **6b** as a white powder (1.9 g, 55%). ^{1}H NMR ([D$_6$]-DMSO): δ = 3.60 (dd, J = 2.3 and 9.6 Hz, 1 H, 4′-H), 3.64 (t, J = 10.2 Hz, 1 H, 6′-Ha), 3.91 (dd, J = 4.9 and 9.6 Hz, 1 H, 5′-H), 4.00 (m, 1 H, 3′-H), 4.00–4.26 (m, 3 H, 1′-Ha, 1′-He, 6′-He), 4.29 (m, 1 H, 2′-H), 5.65 (s, 1 H, Ph-C*H*), 5.72 (d, J = 4.2 Hz, 1 H, 3′-OH), 5.77 (d, J = 7.5 Hz, 1 H, 5-H), 7.05 and 7.19 (2 × br. s, 2 H, 4-NH$_2$), 7.30–7.45 (m, 5 H, ar-H), 7.94 (d, J = 7.5 Hz, 1 H, 6-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 57.46 (C-2′), 64.00 (C-1′), 64.87 (C-3′), 65.79 (C-5′), 68.28 (C-6′), 76.50 (C-4′), 94.09 (C-5), 101.20 (Ph-*C*H), 126.50 (2 C, ar-C$_o$), 128.10 (2 C, ar-C$_m$), 128.95 (ar-C$_p$), 137.93 (ar-C$_i$), 143.75 (C-6), 154.98 (C-2), 165.19 (C-4) ppm. HRMS (thgly): calcd. for $C_{17}H_{20}N_3O_5$ [MH]$^+$ 346.1403; found 346.1380.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)cytosin-1-yl]-D-*altro*-hexitol (6c): 9-Fluorenylmethoxycarbonyl chloride (5.0 g, 19 mmol) was added under nitrogen in 1 g portions over 1 h to a stirred solution of **6b** (1.5 g, 4.4 mmol) in dry pyridine (20 mL). The reaction mixture was stirred at room temperature for 1 h, the pyridine was removed, the residue was co-evaporated with toluene and suspended in dichloromethane (50 mL), and the organic phase was washed with water. The solvent was removed and the crude material was subjected to flash silica gel column chromatography with a mixture of dichloromethane/ethyl acetate (1:5) as eluent, to afford the title compound **6c** (1.75 g, 51%). ^{1}H NMR ($CDCl_3$): δ = 3.60–3.82 [m, 2 H, 4′-H and 9-H (Fmoc)], 4.05–4.60 [m, 10 H, 6′ax-H, 1′ax-H, 5′-H, 6′eq-H, 1′eq-H, CH_2O (Fmoc)], 4.82 (br. s, 1 H, 2′-H), 5.45 (s, 1 H, PhC*H*), 5.59 (br. s, 1 H, 3′-H), 7.15–7.48 (m, 14 H, 6-H and H arom), 7.50–7.64 [m, 4 H, H arom (Fmoc)], 7.66–7.82 [m, 4 H, H arom (Fmoc)], 7.68 [d, J = 7.7 Hz, 2 H, H arom (Fmoc)], 7.78 [m, 4 H, H arom (Fmoc)], 7.94 (d, J = 7.8 Hz, 1 H, 6-H), 8.95 (br. s, 1 H, NH) ppm.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)cytosin-1-yl]-D-*altro*-hexitol (6d): Compound **6c** (1.2 g, 1.5 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0 °C. TFA (2 mL) was then added and the reaction mixture was stirred at room temperature for 45 min; 80% ethanol (10 mL) was added, solvents were removed in vacuo, and the residue was co-evaporated with toluene. The crude material was subjected to flash silica gel column chromatography with methanol in dichloromethane (2.5%) to afford the title compound **6d** as a white foam (0.75 g, 71%). ^{1}H NMR ($CDCl_3$): δ = 3.05 (br.s, 2 H, 6′-OH and 4′-OH), 3.70–3.95 (3 H, m 4′-H, 5′-H, 6′ax-H), 3.96–4.45 [m, 9 H, 6′eq-H, 1′ax-H, 3′-H, 1′eq-H, 9-H and CH_2O (Fmoc)], 4.63 (m, 1 H, 3′-H), 5.49 (br. s, 1 H, 3′-H), 6.81 (d, J = 7.7 Hz, 1 H), 7.26–7.48 (m, 8 H, H arom), 7.55 [m, 4 H, H arom (Fmoc)], 7.65 [m, 4 H, H arom (Fmoc)], 8.04 (d, J = 7.7 Hz, 1 H) ppm. HMRS: calcd. for $C_{40}H_{36}N_3O_9$ [MH]$^+$ 702.2450; found 702.2480.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)cytosin-1-yl]-6-*O*-monomethoxytrityl-D-*altro*-hexitol (6e): Monomethoxytrityl chloride (0.46 g, 1.42 mmol) was added at room temperature under nitrogen to a solution of **6d** (0.65 g, 0.9 mmol) in dry pyridine (6 mL). After 4 h, methanol

(1 mL) was added, the volatiles were removed, and the residue was co-evaporated with toluene. The residue was subjected to flash silica gel column chromatography with acetone (2%) in dichloromethane, to afford the title compound **6e** as a white solid (0.55 g, 60%). ^{1}H NMR ($CDCl_3$): δ = 3.38 (dd, J = 1.1, 10.5 Hz, 1 H, 6′ax-H), 3.48 (dd, J = 1.1, 10.5 Hz, 1 H, 6′ax-H), 3.78 (s, 3 H, CH_3), 3.72–3.85 (m, 1 H, 4′-H), 4.05–4.30 [m, 4 H, 1′ax-H, 1′eq-H, 5′-H, 9-H (Fmoc)], 4.40–4.50 [m, 4 H, CH_2O (Fmoc)], 4.66 (br. s, 1 H, 2′-H), 5.50 (br. s, 1 H, 3′-H), 5.78 (dd, J = 1.8, 8.1 Hz, 1 H, 6-H), 6.82 (d, J = 8.9 Hz, 2 H, H arom), 7.22–7.40 (m, 20 H, H arom), 7.58 [m, 2 H, H arom (Fmoc)], 7.68 [m, 2 H, H arom (Fmoc)], 7.75 [m, 4 H, H arom (Fmoc)], 8.25 (d, J = 8.1 Hz, 1 H, 6-H), 8.93 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{60}H_{52}N_3O_{10}$ $[MH]^+$ 974.3653; found 974.3633.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)-5-methylcytosin-1-yl]-6-*O*-monomethoxytrityl-D-*altro*-hexitol (7e)

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-2-(5-methylcytosin-1-yl)-D-*altro*-hexitol (7b): Chlorotrimethylsilane (6.4 mL, 50 mmol) was added under nitrogen to a stirred suspension of **4b**[3] (3.6 g, 10.0 mmol) in dry pyridine (40 mL). After 1 h, the reaction mixture was cooled in an ice bath, 1*H*-1,2,4-triazole (6.9 g, 100 mmol) and phosphorus oxychloride (1.86 mL, 20 mmol) were added, and stirring was continued for 5 h. The volatiles were removed and the residue was co-evaporated with toluene (3 × 25 mL) and partitioned between water and ethyl acetate. The organic layer was washed with water and brine, and the solvents were evaporated to dryness to afford a yellow foam. This crude intermediate was dissolved in dioxane (40 mL), and aqueous ammonia (25%, 15 mL) was added. After the mixture had been stirred for 45 min, the volatiles were evaporated and the solid was co-evaporated with toluene. The residue was suspended in chloroform, adsorbed on silica gel and subjected to silica gel column chromatography with a stepwise gradient of methanol in dichloromethane (2–10%), to afford the title compound **7b** as a white powder (2.0 g, 55%). ^{1}H NMR ([D$_6$]-DMSO): δ = 1.97 (s, 3 H, CH_3), 3.53 (dd, J = 2.4, 9.5 Hz, 1 H, 4′-H), 3.69 (t, J = 10.4 Hz, 1 H, 6′ax-H), 3.85–4.15 (m, 7 H, 1′ax-H, 5′-H, 6′eq-H, 3′-H, 1′ax-H, 2′-H and 3′-OH), 5.61 (s, 1 H, PhC*H*), 6.90 (br. s, 2 H, NH_2), 7.29–7.32 (m, 3 H, H arom), 7.39–7.45 (m, 2 H, H arom), 7.75 (s, 1 H, 6-H) ppm. HMRS: calcd. for $C_{18}H_{21}N_3O_5$ $[MH]^+$ 360.1559; found 360.1554.

1,5-Anhydro-4,6-*O*-benzylidene-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)-5-methylcytosin-1-yl]-D-*altro*-hexitol (7c): 9-Fluorenylmethoxycarbonyl chloride (5.0 g, 19 mmol) was added under nitrogen over 1 h in 1 g portions to a stirred solution of **7b** (1.5 g, 4.2 mmol) in dry pyridine (20 mL). The reaction mixture was stirred at room temperature for 1 h and the pyridine was removed. The residue was co-evaporated with toluene and suspended in dichloromethane (50 mL), and the organic phase was washed with water. The solvent was removed and the crude material was subjected to flash silica gel column chromatography with a mixture of dichloromethane/ethyl acetate (1:5) as eluent, to afford the title compound **7c** (1.45 g, 43%). ^{1}H NMR ($CDCl_3$): δ = 2.13 (s, 3 H, 5-Me), 3.77–3.83 [m, 2 H, 4′-H and 9-H (Fmoc)], 4.20–4.55 [m, 10 H, 6′ax-H, 1′ax-H, 5′-H, 6′eq-H, 1′eq-H, CH_2O (Fmoc)], 4.65 (br. s, 1 H, 2′-H), 5.49 (br. s, 1 H, 3′-H), 5.61 (s, 1 H, PhC*H*), 7.23–7.35 (m, 5 H, H arom), 7.35–7.46 (m, 8 H, H arom), 7.58 [d, J = 7.0 Hz, 2 H, H arom (Fmoc)], 7.68 [d, J = 7.7 Hz, 2 H, H arom (Fmoc)], 7.78 [m, 4 H, H arom (Fmoc)], 7.94 (d, J = 1.1 Hz, 1 H, 6-H), 12.42 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{48}H_{42}N_3O_9$ $[MH]^+$ 804.2921; found 804.2911.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)-5-methylcytosin-1-yl]-D-*altro*-hexitol (7d): Compound **7c** (1.2 g, 1.5 mmol) was dissolved in dichloromethane (15 mL) and cooled to 0 °C. TFA (2 mL) was then added, and the reaction mixture was stirred at room temperature for 45 min. Ethanol (80%, 10 mL) was added, solvents were removed, and the residue was co-evaporated with toluene. The crude material was subjected to flash silica gel column chromatography with methanol in dichloromethane (2.5%), to afford the title compound **7d** as a white foam (0.70 g, 65%). ^{1}H NMR ($CDCl_3$): δ = 2.05 (s, 3 H, CH_3), 3.70–3.95 (3 H, m 4′-H, 5′-H, 6′ax-H), 3.96–4.50 [m, 9 H, 6′eq-H, 1′ax-H, 3′-H, 1′eq-H, 9-H and CH_2O (Fmoc)], 4.63 (br. s, 1 H, 3′-H), 5.35 (br. s, 1 H, 3′-H), 6.20 (br. s, 2 H, 6′-OH and 4′-OH), 7.20–7.40 (m, 8 H, H arom), 7.58 [m, 4 H, H arom (Fmoc)], 7.74 [m, 4 H, H arom (Fmoc)], 8.40 (d, J = 1.1 Hz, 1 H, 6-H), 9.50 (br. s, 1 H, NH) ppm. HMRS: calcd. for $C_{41}H_{38}N_3O_9$ $[MH]^+$ 716.2608; found 716.2605.

1,5-Anhydro-2-deoxy-3-*O*-(9-fluorenylmethoxycarbonyl)-2-[*N*^{4}-(9-fluorenylmethoxycarbonyl)-5-methylcytosin-1-yl]-6-*O*-monomethoxytrityl-D-*altro*-hexitol (7e): Monomethoxytrityl chloride (0.46 g, 1.42 mmol) was added at room temperature under nitrogen to a solution of **7d** (0.65 g, 0.9 mmol) in dry pyridine (6 mL). After 4 h, methanol (1 mL) was added, the volatiles were removed, and the residue was co-evaporated with toluene. The residue was subjected to flash silica gel column chromatography with acetone (2%) in dichloromethane, to afford the title compound **7e** as a white solid (0.55 g, 60%). ^{1}H NMR ($CDCl_3$): δ = 1.96 (s, 3 H, CH_3), 3.38 (dd, J = 1.1, 10.5 Hz, 1 H, 6′ax-H), 3.48 (dd, J = 1.1, 10.5 Hz, 1 H, 6′ax-H), 3.78 (s, 3 H, CH_3), 3.72–3.85 (m, 1 H, 4′-H), 4.05–4.30 [m, 4 H, 1′ax-H, 1′eq-H, 5′-H, 9-H (Fmoc)], 4.40–4.50 [m, 4 H, CH_2O (Fmoc)], 4.66 (br. s, 1 H, 2′-H), 5.50 (br. s, 1 H, 3′-H), 6.82 (d, J = 8.9 Hz, 2 H, H arom), 7.22–7.40 (m, 20 H, H arom), 7.58 [m, 2 H, H arom (Fmoc)], 7.68 [m, 2 H, H arom (Fmoc)], 7.75 [m, 4 H, H arom (Fmoc)], 8.09 (d, J = 1.1 Hz, 1 H, 6-H) ppm. HMRS: calcd. for $C_{61}H_{54}N_3O_{10}$ $[MH]^+$ 988.3765; found 988.3796.

Acknowledgments

The authors thank the DWTC for financial support.

[1] B. Allart, K. Khan, H. Rosemeyer, G. Schepers, C. Hendrix, K. Rothenbacher, F. Seela, A. Van Aerschot, P. Herdewijn, *Chem. Eur. J.* **1999**, *5*, 2424–2431.

[2] B. Allart, R. Busson, J. Rozenski, A. Van Aerschot, P. Herdewijn, *Tetrahedron* **1999**, *55*, 6527–6546.

[3] M. Abramov, A. Marchand, P. Herdewijn, *Nucleosides, Nucleotides Nucleic Acids* **2004**, *23*, 439–455.

[4] a) L. H. Koole, H. M. Moody, N. L. H. L. Broeders, P. J. L. M. Quaedflieg, W. H. A. Kuijpers, M. H. P. van Genderen, A. J. J. M. Coenen, S. van der Wal, H. M. Buck, *J. Org. Chem.* **1989**, *54*, 1657–1664; b) Y. Ma, E. Sonveaux, *Biopolymers.* **1989**, *28*, 965–973; c) C. Gioeli, J. Chattopadhyaya, *J. Chem. Soc., Chem. Commun.* **1982**, 672–674.

[5] M. D. Hagen, C. Scalfi-Happ, E. Happ, S. Chladek, *J. Org. Chem.* **1988**, *53*, 5040–5045; Y. Zhou, S. Chladek, L. Romano, *J. Org. Chem.* **1994**, *59*, 556–563; M. D. Hagen, S. Chladek, *J. Org. Chem.* **1989**, *54*, 3189–3195.

[6] P. J. L. M. Quaedflieg, A. P. van der Heiden, L. H. Koole, A. J. J. M. Coenen, S. van der Wal, E. M. Meijer, *J. Org. Chem.* **1991**, *56*, 5846–5859.

[7] J. Heikkila, J. Chattopadhyaya, *Acta Chem. Scand. Ser. B* **1983**, *37*, 263–265; C. Scalfi-Happ, E. Happ, S. Chladek, *Nucleosides Nucleotides* **1987**, *6*, 345–348; A. B. Sierzchala, D. J. Dellinger, J. R. Betley, T. K. Wyrzykiewicz, C. M. Yamada, *J. Am. Chem. Soc.* **2003**, *125*, 13427–13441; J. S. Oliver, A. Oyelere, *J. Org.*

Chem. **1996**, *61*, 4168–4171; P. A. Sacca, A. Fontana, J. M. Montserrat, A. M. Iribarren, *Chem. Biodiversity* **2004**, *1*, 595–600; M. Ushioda, M. Kadokura, T. Moriguchi, A. Kobori, M. Aoyagi, K. Seio, M. Sekine, *Helv. Chim. Acta* **2002**, *85*, 2930–2945; J. Amato, A. Galeone, G. Oliviero, L. Mayol, G. Piccialli, M. Varra, *Eur. J. Org. Chem.* **2004**, 2331–2336.

[8] a) F. Himmelbach, R. Charubala, W. Pfleiderer, *Tetrahedron* **1984**, *40*, 54–72; b) S. A. Scaringe, C. Francklyn, N. Usman, *Nucleic Acid Res.* **1990**, *18*, 5433–5441.

[9] C. Brockway, P. Kocienski, C. J. Pant, *J. Chem. Soc., Perkin Trans. 1* **1984**, 875–878.

[10] A. Miah, C. B. Reese, Q. Song, *Nucleosides, Nucleotides Nucleic Acids* **1997**, *16*, 53–65.

[11] A. Eleuteri, D. C. Capaldi, A. H. Krotz, D. L. Cole, V. T. Ravikumar, *Org. Process Res. Dev.* **2000**, *4*, 182–189.

[12] M. P. Reddy, N. B. Hanna, F. Farooqui, *Tetrahedron Lett.* **1994**, *35*, 4311–4314.

[13] A. Van Aerschot, T. Saison-Behmoaras, J. Rozenski, C. Hendrix, G. Scherpers, G. Verhoeven, P. Herdewijn, *Bull. Soc. Chim. Belg.* **1995**, *104*, 717–720.

[14] H. M. Moody, M. H. P. Vangenderen, L. H. Koole, H. J. M. Kocken, E. M. Meijer, H. M. Buck, *Nucleic Acids Res.* **1989**, *17*, 4769–4782.

[15] R. T. Pon, S. Y. Yu, *Tetrahedron Lett.* **1997**, *38*, 3327–3330.

Received: October 3, 2006
Published Online: January 25, 2007

