# Improved Synthesis of Anhydrohexitol Building Blocks for Oligonucleotide Synthesis☆

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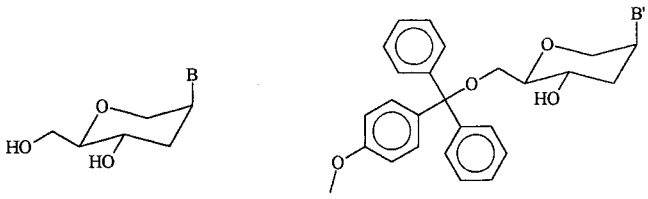
The synthesis of the four building blocks used for the preparation of hexitol nucleic acids were optimized. The nucleoside analogues with a purine base moiety are best prepared by a nucleophilic substitution reaction, whereas the pyrimidine nucleosides can best be obtained using Mitsunobu-type conditions.

Oligonucleotides with a conformationally rigid backbone may have an advantage over natural DNA or RNA, insofar as they hybridize more strongly with the targeted mRNA due to the lower change in entropy during duplexation[1]. At present, four types of backbone-modified oligonucleotides, with strong hybridizing capabilities, are candidates for further development as antisense constructs. These are the peptide nucleic acids[2], N3′ → P5′ phoshoramidates[3], 2′-methoxyethyl RNA[4], and hexitol nucleic acids[5]. Further study of these oligomers is dependent on the availability of large amounts of the protected, modified nucleosides for oligonucleotide synthesis. Previous synthesis of the hexitol nucleosides **1**–**4** (hA, hT, hC and hG; Figure 1; h stands for hexitol and A, T, C, G for the nucleobases) was time-consuming and with low yield[6]. The conversion of D-glucose diacetonide to 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (**9**), which is used as common starting material for the four building blocks, was recently optimized[7]. The synthesis may be performed on large scale without the use of chromatographic purification procedures. We have now optimized the remaining steps in the procedure for the synthesis of the four protected hexitol nucleoside building blocks **5**–**8** (Figure 1). This optimization has been achieved from the point of view of regioselectivity, mild reaction conditions and high yields. Principally, the alkylation of the bases, or the alcohol-base condensation reaction, was studied; which was carried out by nucleophilic displacement reaction or under Mitsunobu-type reaction conditions. Because product purification after Mitsunobu reaction is a tedious problem[8], especially in large-scale preparations, priority was first given to the nucleophilic displacement reaction. With the exception of the alkylation of the cytosine base, all synthetic steps leading to the protected hexitol building blocks were optimized to over 80% yield.

Figure 1. Structure of anhydrohexitol nucleosides and protected analogues for oligonucleotide synthesis




**1-4**

**1** *h*G, B = guanin-9-yl
**2** *h*A, B = adenin-1-yl
**3** *h*C, B = cytosin-1-yl
**4** *h*T, B = thymin-9-yl

**5** B' = $N^2$-isobutyryl-guanin-9-yl
**6** B' = $N^6$-benzoyl-adenin-9-yl
**7** B' = $N^4$-benzoyl-cytosin-1-yl
**8** B' = thymin-1-yl

## Results and Discussion

Previously[6], we reported that reaction of 2-amino-6-chloropurine with hexitol tosylate **10a** in DMF at 120°C for 5 h, in the presence of potassium carbonate and 18-crown-6, afforded the coupled product **11a** in a 19% yield after chromatographic purification (Scheme 1). Using the readily solube tetrabutylammonium salt **12** of 2-amino-6-iodopurine[9], alkylation of the anhydrohexitol triflate **10b** was straightforward in $CH_2Cl_2$ at room temperature, affording, after chromatographic purification, the desired N-9 isomer **11b** in a 70% overall yield for the two steps, without formation of the N-7 regioisomer. About 10% of the elimination product **13** was formed.

The protected nucleoside **11b** could be converted into hG (**1**) in 83% yield (Scheme 2) by treatment with aqueous HCl (10%) at 100°C, resulting in deprotection of the benzylidene moiety and concomitant hydrolysis of the iodo group.

Silylation of the guanosine analogue **1**, in order to introduce the isobutyryl group at the 2-amino group by transient protection, was carried out in boiling pyridine. Upon addition of isobutyric anhydide at room temperature a full

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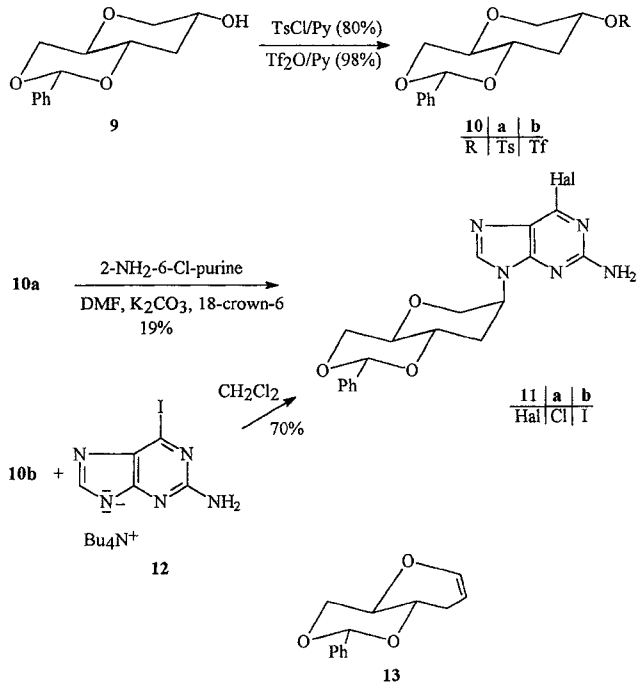
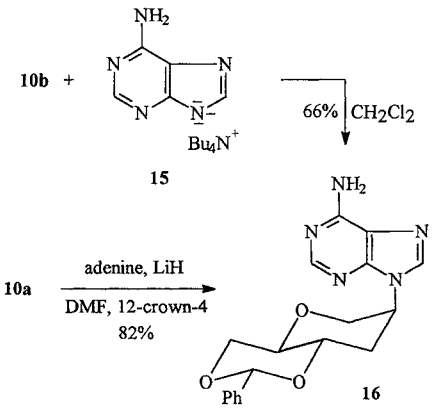
Scheme 1




Scheme 3

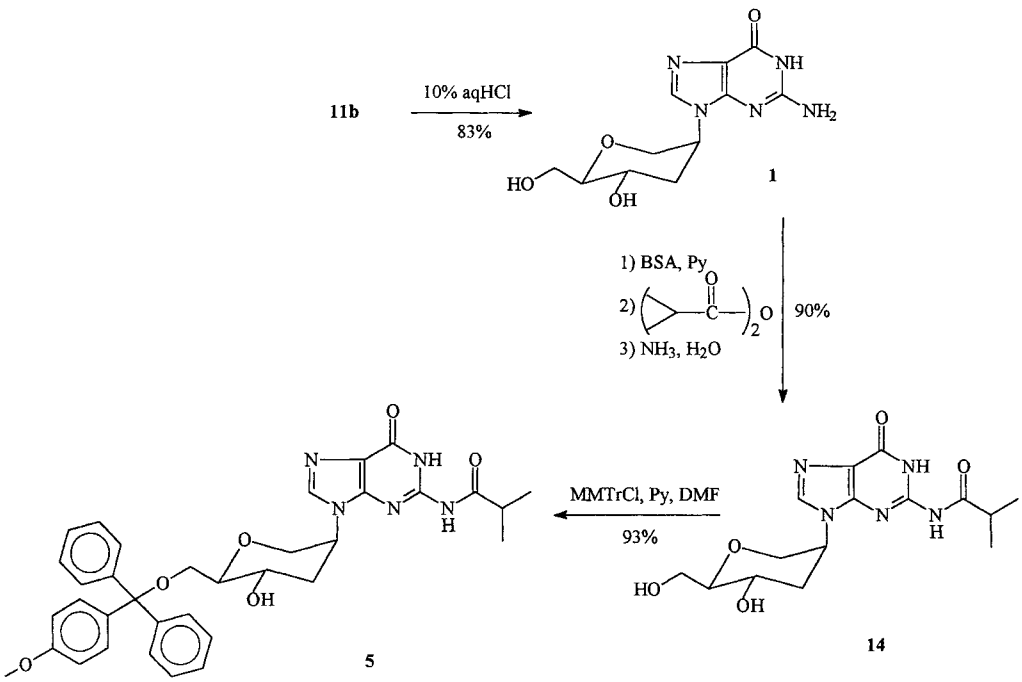



conversion to **14** was obtained (90% yield). Introduction of the isobutyryl group to the iodo compound **11b** was unsuccessful. Compound **14** was monomethoxytritylated to **5** in a mixture of pyridine/DMF (1:1) using 1.3 equivalents of monomethoxytrityl chloride (MMTrCl).

For the synthesis of the adenine analogue the same coupling strategy was at first applied (Scheme 3). Formation of the tetrabutylammonium salt of adenine **15** went smoothly and the salt was easily isolated, in a 80% yield, after precipitation from $CH_2Cl_2$/diethyl ether. The coupling via the triflate gave a maximum yield of 66%. During scaling-up (10 g) more problems were encountered, mainly due to the thermal instability of the triflate. Due to the high cost of triflic anhydride, and the fact that we obtained quite good yields in the past[6] with the tosylate strategy (56%), we decided to optimize the latter approach. The triflate route is now recommended and used exclusively for the guanine compound.

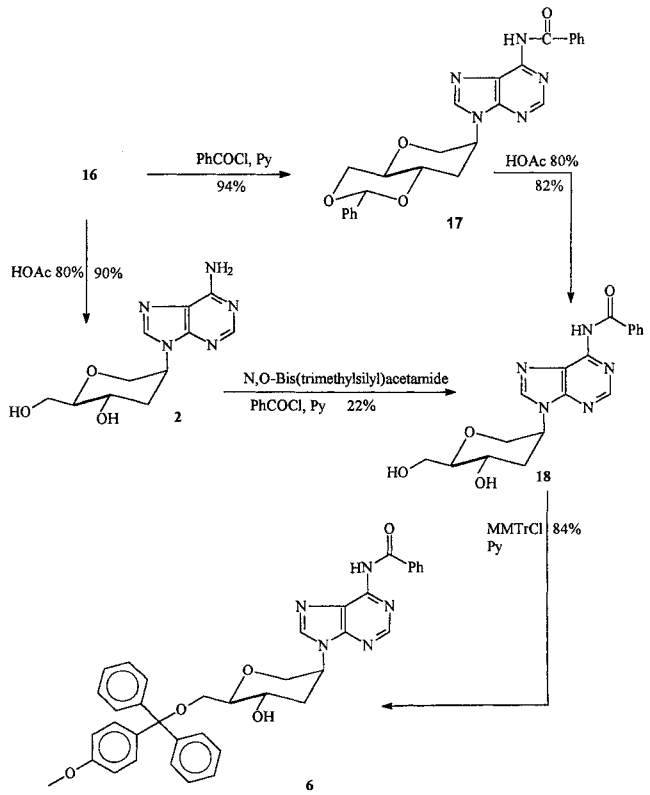
Nucleophilic displacement of the tosylate[6,10] of hexitol **10a** with the lithium salt of adenine, formed in situ with lithium hydride in the presence of 12-crown-4, afforded the coupled product **16** in 82% yield. The use of 18-crown-6 ether did not improve the reaction and led to a much lower yield (61%). The overall yield of this route compared very well with that of the triflate route (Scheme 3), but here the yields are more reproducible. Also, in this reaction, no N-7 regioisomer was noticed.

Scheme 2



By using 5 equivalents of benzoyl chloride, amine **16** was converted to **17** in high yield (94%, Scheme 4). During hydrolysis of the benzylidene moiety of **17**, to yield **18**, temperature control is crucial. Hydrolysis at 90°C led to concomitant hydrolysis of the amide function with formation of **2** (±30%). This base deprotection is avoided by lowering the reaction temperature to 60°C. This product was then transformed to **6** by tritylation in 84% yield. Hereto, 1.7 equivalents of monomethoxytrityl chloride was used and the reaction was conducted at room temperature for 72 h. The alternative route via hexitol nucleoside **2** was less attractive, probably due to incomplete transient protection of **2**. Although complete dissolution was obtained and silylation was carried out at an elevated temperature (110°C), invariably, substantial amounts of starting material **2** were present and purification became problematic.

Scheme 4




Alkylation of pyrimidines (cytosine and thymine) is generally more troublesome than that of purines[4–9]. When attempting the triflate strategy for the coupling, problems were encountered with the difficult purification of the tetrabutylammonium salts of cytosine (**19**) and thymine (Scheme 5). These products could not be uniformly characterized and were isolated as sticky oils or foams, which invariably contained some TBAOH. In addition, these salts are poorly soluble in dichloromethane. Hence, the coupling reaction of **19** with the triflate **10b** did not lead to the desired results.

Therefore we turned to the classical tosylate displacement reaction. The temperature of the reaction was adjusted to 110–120°C. Lower temperatures gave rise to incomplete reactions, and higher temperatures to more elimination reactions and other side compounds. Dimethylformamide proved to be the best solvent. Other dipolar aprotic solvents, such as DMA and NMP, did not improve the yield, but gave more purification problems. A work-up by extraction with brine/EtOAc was the most convenient. Other factors which were investigated were the leaving group, the nature of the counterion, and the use of a protecting group in the base (such as monomethoxytrityl) in order to increase solbility.

Table 1. Reaction of the tosylate **10a** with monomethoxytritylated cytosine

| Base MH | Yield (%) $N^1$ isomer **20a** | Yield (%) $O^2$ isomer **20b** | Temp. [°C] | Ratio $N^1/O^2$ isomer | Reaction time [h] |
|---|---|---|---|---|---|
| LiH | 27 | 35 | 120 | 1:1.3 | 16 |
| NaH | 20 | 40 | 120 | 1:2 | 16 |
| KH | 20 | 40 | 120 | 1:2 | 3 |
| $Cs_2CO_3$ | 14 | 43 | 120 | 1:3 | 16 |

Scheme 5

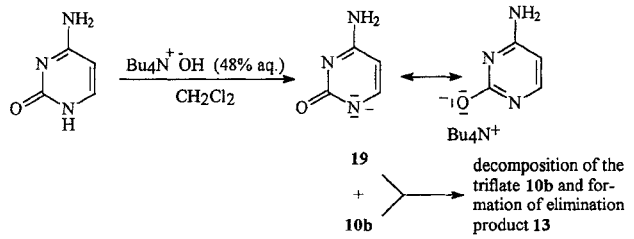
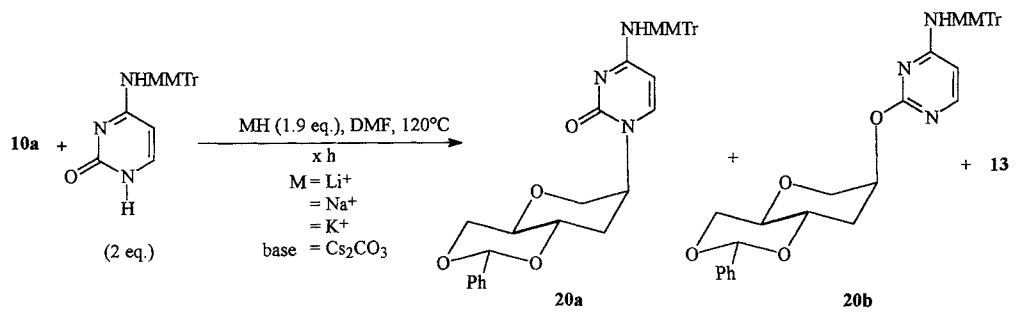



Table 1 summarizes the data obtained after condensation of the tosylate **10a** with monomethoxytritylated cytosine in the presence of different bases (Scheme 6). The following

Scheme 6



conclusions could be made; whatever base is used, a mixture of $N^1$- and $O^2$-alkylated compound (**20a** and **20b**) was obtained; the smaller the cationic species the more of the desired $N^1$ isomer **20a** was obtained, and invariably 15–20% of the elimination product **13** was formed. Therefore, in our further investigations, lithium hydride was used as base.

In a second set of experiments the influence of the leaving group and the base protection was tested using the lithium salt of either cytosine or monomethoxytritylated cytosine (Scheme 7 and Table 2). As expected, the reaction with the better leaving group, chlorobenzenesulfonate, was much faster. However, more of the $O^2$ isomer was formed in both cases (cytosine and monomethoxytritylcytosine) but since in the latter case somewhat more of the undesired $O^2$ isomer was formed, cytosine was preferred over monomethoxytritylcytosine. The reactivity of the mesylate and the tosylate are comparable.

Table 2. Reaction of either cytosine or monomethoxytrityl cytosine with the chlorobenzenesulfonate **10c** and with the tosylate **10a**

| Leaving group | R group | Yield (%) $N^1$ isomer | Yield (%) $O^2$ isomer | Ratio $N^1/O^2$ isomer | Reaction time [h] |
|---|---|---|---|---|---|
| *p*-ClSO$_3$ | H | 25 | 50 | 1:2.5 | 4 |
| | MMTr | 24 | 38 | 1:1.6 | 4 |
| *p*-CH$_3$SO$_3$ | H | 30 | 33 | 1:1.1 | 16 |
| | MMTr | 27 | 35 | 1:1.3 | 16 |

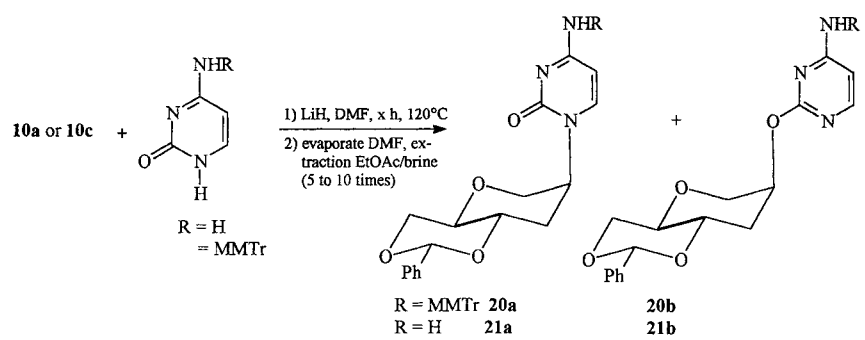
The combination ROTs/LiH/cytosine was further investigated in the presence of 12-crown-4 ether. However, more of the undesired $O^2$ isomer was formed ($N^1/O^2$ = 1:1.4 versus $N^1/O^2$ = 1:1.1). Based on the problems encountered for protecting the base in compound **2**, we decided to introduce the benzoyl function on **21a** (Scheme 8) prior to removing the benzylidene function. A good yield of **22** was obtained using not less than ten equivalents of benzoyl chloride (85%).

Because of the reather low overall yield (max 20%) of compound **22** obtained via the 3-step procedure (Scheme 8), we reinvestigated the one-step procedure[6] from the hexitol **9** and benzoylcytosine under Mitsunobu conditions[11]. The reaction was carried out by adding very slowly DEAD in THF to a THF solution of $Ph_3P$, alcohol **9** and the acid component, benzoylcytosine. After purification on silica gel using a combination of ether and hexane/EtOAc mixtures (in which triphenylphosphane oxide was separated) the product **22** could be isolated in pure form in 34% yield. Attempts to increase the yield using different order or protocols for mixing the reagents failed. Addition of the solid base to the reagents or addition of the alcohol **9** and $Ph_3P$ in THF to a THF solution of the base and DEAD, gave a 15% (together with the $O^2$ isomer) and a 27% yield, respectively. The poor solubility of benzoylcytosine and the formation of the salt **23**, when formed preferentially as side product, are the major reasons for this low yield. However, this yield is still better, and the route is shorter than the classical nucleophilic displacement strategy. Also the problems of product purification normally encountered with Mitsunobu reaction mixtures were solved. The generally lower yield of the condensation reaction with the cytosine base (compared with thymine and uracil) is due to formation of a larger amount of *O*-alkylated compound. Hydrolysis of amide **22** to diol **24** gave good results at moderate temperature (94% yield). Compound **24** was tritylated to afford **7** using monomethoxytrityl chloride in pyridine.

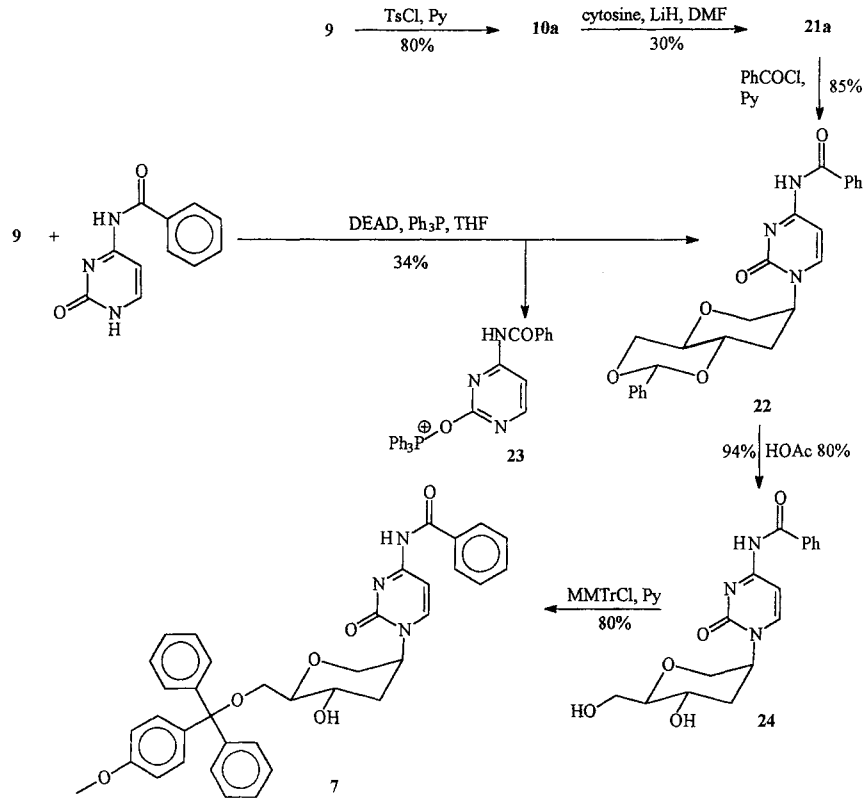
Finally, the nucleophilic displacement strategy was investigated for the thymine analogue. As for the cytosine compound, the combination LiH/tosylate of the sugar gave the best results. On a scale of 5 mmol of the tosylate, the reaction gave 48% of the $N^1$ isomer **25** (Scheme 9, Table 3), 16% of the $O^2$ isomer and 15% of the elimination product **13**. On scaling up (15 mmol), however, the yield of the desired $N^1$ isomer **25** was considerably lower (24%). This is due to the fact that at 110°C the lithium salt of thymine in DMF formed a gel-like suspension, with a quite high viscosity, resulting in a much lower reactivity. By increasing the temperature to 120°C the reaction was still not complete (even after 48 h), but more side products were formed. Analogously to the cytosine condensation, use of the crown ether 12-crown-4 increased the ratio in favour of the unwanted $O^2$ isomer.

Instead of exploring further the direct nucleophilic displacement reaction we then went on to investigate the Mitsunobu strategy. Instead of using a suspension in dioxane[6], the reaction with $N^3$-benzoylthymine was carried out using tetrahydrofuran as solvent; as for the cytosine analogue and as described for the synthesis of the uracil congener[14]. In this way a clear solution was obtained. After chromatographic purification, **26** was isolated in analytically pure quality in 80% yield. After hydrolysis of the benzoyl group

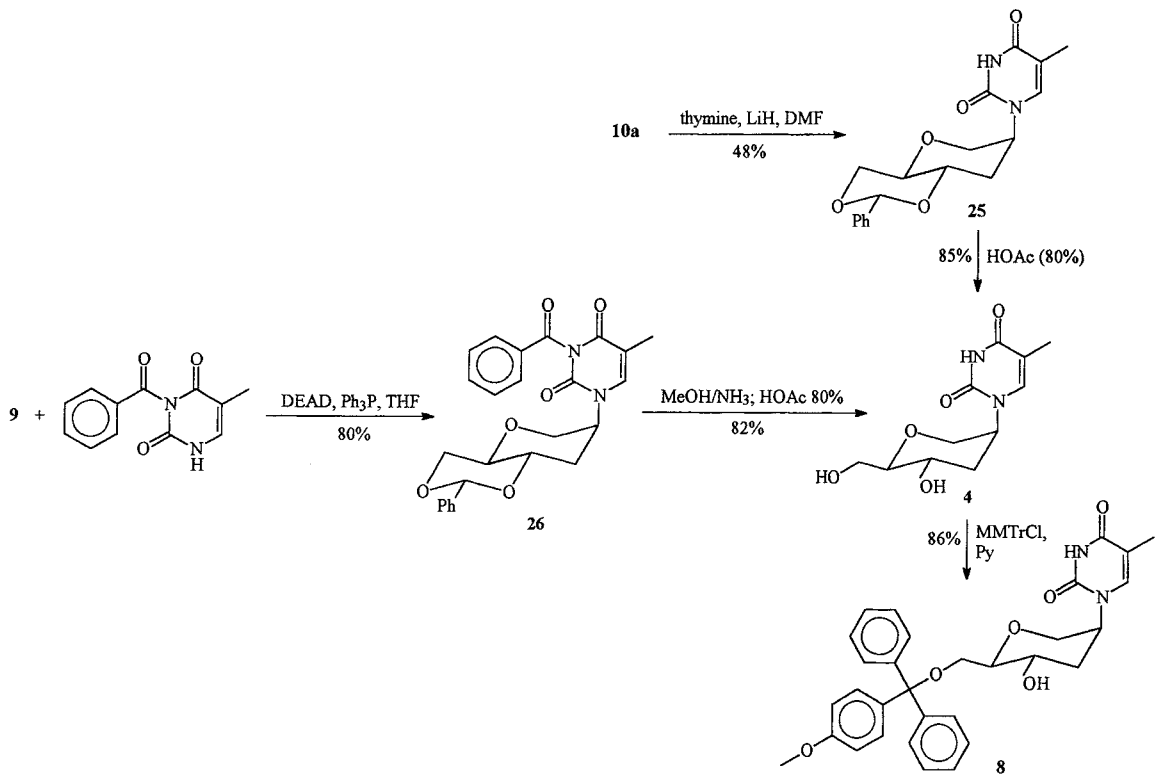
Scheme 7



Scheme 8




Scheme 9




with ammonia in methanol, **25** was immediately deprotected yielding the diol **4** in 82% yield after chromatography. By using the Mitsunobu strategy, the total yield for the displacement strategy was improved to 65% yield. The tritylation to **8** was carried out with 1.5 equivalents of MMTrCl at room temperature (86%).

Table 3. Reaction of thymine and **10a** in the presence of LiH

| Scale [mmol] | Temp. [°C] | 12-crown-4 | Reaction time [h] | Yield (%) $N^1$ isomer **25** | Yield (%) $O^2$ isomer | Ratio $N^1/O^2$ isomer |
|---|---|---|---|---|---|---|
| 5 | 110 | – | 24 | 48 | 16 | 3:1 |
| 15 | 120 | – | > 48 | 24 | 13 | 2:1 |
| 15 | 120 | + | > 48 | 24 | 17 | 1.5:1 |

In conclusion, during this optimization we improved the alklyation procedures for the coupling of purine bases with the anhydrohexitol ring using sulfonate-activating groups on the anhydrohexitol ring. For the synthesis of the pyrimidine analogues the Mitsunobu reaction is the method of choice.

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## Experimental Section

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. The $^1$H-NMR and $^{13}$C-NMR spectra were determined using a 200-MHz Varian Gemini apparatus with tetramethylsilane as an internal standard for the $^1$H-NMR spectra, and [$D_6$]DMSO ($\delta$ = 39.6) or $CDCl_3$ ($\delta$ = 76.9) for the $^{13}$C-NMR spectra. Liquid secondary-ion mass spectra (LSIMS) were obtained using a KRATOS concept 1H mass spectrometer. Precoated Macherey-Nagel Alugram SIL G/$UV_{254}$ plates were used for TLC, and the spots were examined with UV light and using a sulfuric acid/anisaldehyde spray. Column chromatography was performed on ACROS silica gel (0.060–0.200 nm). Anhydrous solvents were obtained as follows: DMF was dried over molecular sieves; dichloromethane was stored on phosphorus pentoxide, refluxed and distilled; tetrahydrofuran was stored on sodium/benzophenone, refluxed and distilled; pyridine was stored on calcium hydride, refluxed and distilled. Elemental analyses were performed at the University of Konstanz, Germany.

*1,5-Anhydro-4,6-O-benzylidene-3-deoxy-2-O-(p-tolylsulfonyl)-D-ribo-hexitol* (**10a**): Tosyl chloride (11.44 g, 60 mmol) was added to a solution of 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol[7] (11.8 g, 50 mmol) in 100 ml of dry pyridine and stirred overnight at 50°C. The mixture was diluted with dichloromethane (300 ml) and washed with saturated $NaHCO_3$ solution. The organic phase was dried with $MgSO_4$, evaporated and co-evaporated 3 times with toluene. The yellow-orange solid product (17 g) was purified by crystallization from 350 ml of ethanol. After the solid was dissolved in the boiling ethanol, the solution was filtered hot and the filtrate was then cooled in the refrigerator (4°C) overnight. The crystals obtained were washed with cold ethanol yielding 15.5 g of **10a** (39.74 mmol, 80%). – LSIMS (thgly); *m/z*: 391 [$MH^+$]. – $^1$H NMR ($CDCl_3$): $\delta$ = 1.8 (q, 1H, 3ax-H, *J* = 11.5 Hz), 2.39 (m, 1H, 3eq-H), 2.46 (s, 3H, $CH_3$), 3.25 (dt, 1H, 5-H, *J* = 10 Hz, *J* = 4.8 Hz), 3.35 (t, 1H, 1ax-H, *J* = 10.8 Hz), 3.48 (dt, 1H, 4-H, *J* = 8.9 Hz, *J* = 4.1 Hz), 3.62 (t, 1H, 6ax-H, *J* = 10.4 Hz), 4.00 (dd, 1H, 1eq-H, *J* = 10.9 Hz, *J* = 5.4 Hz), 4.28 (dd, 1H, 6eq-H, *J* = 10.4 Hz, *J* = 4.8 Hz), 4.55 (tt, 1H, 2-H, *J* = 10.4 Hz, *J* = 5.2 Hz), 5.46 (s, 1H, PhCH), 7.30–7.45 (m, 7H, aromatic H), 7.75–7.85 (m, 2H, aromatic H). – $^{13}$C NMR ($CDCl_3$): $\delta$ = 21.64 ($CH_3$), 35.45 (C-3), 68.88 (C-6), 69.24 (C-1), 73.03 (C-4, C-2), 75.70 (C-5), 101.70 (PhCH), 126.06 (C-*o*/Ph, 2 C), 127.73 (C-*m*/Ts, 2 C), 128.32 (C-*m*/Ph, 2 C), 129.18 (C-*p*/Ph), 130.01 (C-*o*/Ts, 2 C), 133.40 (C-*p*/Ts), 136.53 (C-*i*/Ph), 145.21 (C-*i*/Ts).

*2'-(2-Amino-6-iodopurin-9-yl)-1',5'-anhydro-4',6'-O-benzylidene-2',3'-dideoxy-D-arabino-hexitol* (**11b**): The yield of **11b** was gradually improved to 70%, mainly due to optimization of the following parameters.

1) *The Quality of the Tetrabutylammonium Salt of 6-Iodo-9H-purin-2-amine* (**12**): According to the literature prescription[9], this product was formed by adding aqueous tetrabutylammonium hydroxide to a slurry of powdered 6-iodo-9*H*-purin-2-amine, followed by concentration of the residue from toluene and crystallization from ethyl acetate. Crystallization, however, proved to be very difficult. The salt **12** which is contaminated with tetrabutylammonium hydroxide (TBAOH), could be purified by ultrasonic treatment (15 g in 500 ml of EtOAc) lasting 30 min, followed by filtration. A white product was obtained in 80% yield, which was then used as such. Purification attempts by recrystallization from $CH_2Cl_2$/diethyl ether failed.

2) *Formation of the Triflate:* In order to obtain a quantitative conversion of the alcohol **9** into the triflate **10b**, the quantity of pyridine and triflic anhydride was slightly increased (20%) with respect to the literature values[8] and the temperature was lowered from 0°C to –5°C, in view of the instability of the triflate. During the work-up of the reaction below 20°C, the 5% $NaHSO_4$ solution (pH = 1), used to extract most of the pyridine from the organic layer, was replaced by a 1 M $KH_2PO_4$ solution (pH = 5) since the former solution causes some acidic hydrolysis of the benzylidine moiety of **10b**.

3) *Work-up Procedure after Coupling with the Base:* According to ref.[8] the solvent ($CH_2Cl_2$) is evaporated, and the organic phase (toluene an EtOAc) is washed twice with 30% aqueous $H_3PO_4$ and six times with water, until the tetrabutylammonium ion was absent. In our case, this procedure did not lead to a pure material and therefore we filtered off the precipitate formed and purified the residue **11b** using column chromatography with gradient elution, leading to the following modus operandus.

Triflic anhydride (9.24 ml, 36.6 mmol; a solution of 10 ml of triflic anhydride in 15 ml of anhydrous dichloromethane was used) was added dropwise, over 5 min, to a stirred solution of 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (6 g, 25.42 mmol) and pyridine (3.7 ml, 45.76 mmol) in 36 ml of anhydrous $CH_2Cl_2$ at –5°C. After 10 min of additional stirring (TLC: hexane/EtOAc, 8:2, $R_f$ = 0.77), the reaction mixture was worked up below 20°C. The mixture was quenched with ice and diluted to 200 ml with $CH_2Cl_2$. The organic layer was washed with ice-cold water (2×), cold 1 M $KH_2PO_4$ (200 ml) and ice-cold water. The aqueous washings were extracted with $CH_2Cl_2$. The combined organic layers were dried with $MgSO_4$, filtered and concentrated at low temperature to yield the triflate **10b** as a white-yellow solid product. The last traces of pyridine were removed under vacuum using an oil pump. This yielded 9.13 g of **10b** (24.8 mmol, 98%).

A solution of the triflate **10b** (9.13 g, 24.8 mmol) in anhydrous $CH_2Cl_2$ (24 ml) was added to a stirred, ice-cold solution of the tetrabutylammonium salt **12**[9] (15.33 g, 30.5 mmol) in anhydrous $CH_2Cl_2$ (36 ml). After 30 min, the ice-bath was removed and the reaction mixture stirred overnight at room temperature. A precipitate was filtered off, washed with $CH_2Cl_2$, and the filtrate was absorbed on silica gel. Purification by column chromatography with gradient elution (hexane/EtOAc, 55:45, 50:50, 45:55) afforded 8.49 g (17.67 mmol, 70% based on **9**) of the coupled product **11b**. – M.p. 204°C. – LSIMS (thgly); *m/z*: 480 [$MH^+$]. – $^1$H NMR ($CDCl_3$): $\delta$ = 2.14 (dt, 1H, 3'ax-H, *J* = 11.6 Hz, *J* = 4.5 Hz), 2.61 (br. d, 1H, 3'eq-H, *J* = 13.5 Hz), 3.6 (m, 2H, 5'-H, 4'-H), 3.77 (t,

1H, 6'ax-H, $J$ = 9.7 Hz), 4.10 (dd, 1H, 1'ax-H, $J$ = 13.2 Hz, $J$ = 2.8 Hz), 4.39 (m, 2H, 1'eq-H, 6'eq-H), 4.8 (br. s, 1H, 2'-H), 5.15 (s, 2H, $NH_2$), 5.49 (s, 1H, PhCH), 7.35–7.45 (m, 5H, aromatic H), 8.29 (s, 1H, 8-H). – $^{13}C$ NMR ($CDCl_3$): δ = 32.79 (C-3'), 50.79 (C-2'), 68.83 (C-6'), 69.21 (C-1'), 73.78 (C-4'), 74.49 (C-5'), 101.7 (PhCH), 122.8 (C-5), 125.94 (C-*o*-/Ph, 2 C), 128.23 (C-*m*, 2 C), 129.08 (C-*p*/Ph), 131.9 (C-*i*/Ph), 136.96 (C-6), 140.56 (C-8), 149.6 (C-4), 158.74 (C-2). – $C_{18}H_{18}N_5O_3I$ (479.28): calcd. C 45.11, H 3.79, N 14.61; found C 45.26, H 4.01, N 14.37.

*1',5'-Anhydro-2',3'-dideoxy-2'-(guanin-9-yl)-*D*-arabino-hexitol* (**1**): A suspension of **11b** (8.18 g, 17.06 mmol) in 160 ml of 10% aqueous HCl was heated at 100°C for 2 h. After cooling to room temperature, the yellow-brown solution was washed with 60 ml of $CH_2Cl_2$ to remove benzaldehyde. The acidic yellow water phase was neutralized with 120 ml of NaOH (4 N), using phenolphthaleine as indicator. At pH = 7 the product started to precipitate. This suspension was concentrated and the white product was dissolved in boiling water (745 ml) and filtered while hot. The solution, after standing for 1 h, was cooled in the refrigerator (4°C) overnight. The crystals obtained were filtered off and washed with cold water yielding 3.98 g of **1** (14.16 mmol, 83%). – M.p. > 300°C. – UV ($H_2O$): $\lambda_{max}$ (ε) = 253 nm (9100). – LSIMS (thgly); *m/z*: 282 [$MH^+$]. – $^1H$ NMR ([$D_6$]DMSO): δ = 1.8 (m, 1H, 3'ax-H), 2.17 (br., 1H, 3'eq-H), 3.2–3.7 (m, 3H, 5'-H, 4'-H, 6A-H), 3.79 (dd, 1H, 1'ax-H, $J$ = 12.5 Hz, $J$ = 2.2 Hz), 4.05–4.15 (m, 2H, 1'eq-H, 6B-H, 4.52 (br. s, 1H, 2'-H), 4.63 (t, 1H, 6'-OH, $J$ = 6 Hz), 4.91 (d, 1H, 4'-OH, $J$ = 5.3 Hz), 6.46 (br. s, 2H, $NH_2$), 7.87 (s, 1H, 8-H). – $^{13}C$ NMR ([$D_6$]DMSO): δ = 36.3 (C-3'), 50.2 (C-2'), 61.0 (C-6'), 61.2 (C-4'), 68.4 (C-1'), 83.2 (C-5'), 116.3 (C-5), 136.9 (C-8), 151.5 (C-4), 154.1 (C-2), 157.9 (C-6). – $C_{11}H_{15}N_5O_4$ (281.27): calcd. C 46.97, H 5.38, N 24.90; found C 46.68, H 5.46, H 24.62.

*1,5-Anhydro-2,3-dideoxy-2-(N²-isobutyrylguanin-9-yl)-*D*-arabino-hexitol* (**14**): A suspension of the guanine nucleoside **1** (3.58 g, 12.74 mmol) in 160 ml of dry pyridine was treated with bis(trimethylsilyl)acetamide (16.57 m, 63.7 mmol) and refluxed for 8 h. The clear dark-red solution was stirred overnight at room temperature (16 h). This solution was treated with isobutyric anhydride (1.89 ml, 63.7 mmol) and stirred overnight (24 h). The mixture was cooled to 0°C and 20 ml of water was added. After 15 min, the solution was treated with 20 ml of aqueous $NH_3$ (25%) and stirred for 2 h at room temperature. The volatiles were removed in vacuo and the remaining brown suspension was treated with 200 ml of water, stirred and filtered. The precipitate was successively washed three times with 50 ml of water and three times with 100 ml of EtOAc/diethyl ether (1:1 mixture). This yielded 3.74 g of pure **14** (10.65 mmol, 90%). – M.p. 258°C. – LSIMS (thgly); *m/z*: 352 [$MH^+$]. – $^1H$ NMR ([$D_6$]DMSO): δ = 1.13 [d, 6H, HC($CH_3$)$_2$, $J$ = 7 Hz], 1.87 (dt, 1H, 3'ax-H, $J$ = 12.3 Hz, $J$ = 4.2 Hz), 2.23 (br. d, 1H, 3'eq-H, $J$ = 12.8 Hz), 2.78 [sept, 1H, *H*C($CH_3$)$_2$, $J$ = 7 Hz], 3.18 (m, 2H, 5'-H, 4'-H), 3.45–3.75 (m, 2H, 6'-H), 3.85 (dd, 1H, 1'ax-H, $J$ = 12.5 Hz, $J$ = 2.7 Hz), 4.17 (d, 1H, 1'eq-H, $J$ = 12.4 Hz), 4.65 (m, 2H, 2'-H, 6'-OH), 5.01 (br. s, 1H, 4'-OH), 8.15 (s, 1H, 8-H), 10.2 (br. s, 1H, NH). – $^{13}C$ NMR ([$D_6$]DMSO): δ = 19.03 (2 × Me), 34.84 (C-3'), 36.19 [H*C*(Me)$_2$], 50.43 (C-2'), 60.56 (C-6', C-4'), 68.05 (C-1'), 83.08 (C-5'), 119.68 (C-5), 138.62 (C-8), 148.01 (C-2), 148.66 (C-4), 155.12 (C-6), 180.25 (NHC=O). – $C_{15}H_{21}N_5O_5$ (351.36): calcd. C 51.28, H 6.02, N 19.93; found: C 51.18, H 6.29, N 19.87.

*1',5'-Anhydro-2',3'-dideoxy-2'-(N²-isobutyrylguanin-9-yl)-6'-O-monomethoxytrityl-*D*-arabino-hexitol* (**5**): A suspension of **14** (4.03 g; 11.481 mmol) in 60 ml of anhydrous DMF and 60 ml of anhydrous pyridine was heated to 120°C. After the diol was dissolved, the red-brown solution was cooled to room temperature. This solution was treated with monomethoxytrityl chloride (4.6 g, 14.92 mmol, 1.3 equiv.) and stirred overnight at room temperature (TLC: $CH_2Cl_2$/MeOH, 94:6; starting material: $R_f$ = 0.33). The use of 2 equivalents of monomethoxytrityl chloride leads to the formation of 15% bistritylated compound. The mixture was quenched with 200 ml of a saturated $NaHCO_3$ solution and extracted four times with 100 ml of $CH_2Cl_2$. The organic phase was dried with $MgSO_4$, filtered, evaporated and co-evaporated with toluene (3×). Purification by column chromatography with gradient elution (100% $CH_2Cl_2$, $CH_2Cl_2$/MeOH, 99:1, 98:2, 97:3) afforded 6.65 g (10.67 mmol, 93%) of pure product **5**. – M.p. 165°C. – LSIMS (thgly/NaOAc); *m/z*: 646 [$MNa^+$]. – $^1H$ NMR ($CDCl_3$): δ = 1.19 [d, 6H, HC($CH_3$)$_2$, $J$ = 6.8 Hz], 1.80 (t, 1H, 3'ax-H, $J$ = 12.6 Hz), 2.20 (s, 1H, 4'-OH), 2.32 (br. d, 1H, 3'eq-H, $J$ = 12.1 Hz), 2.70 [sept, 1H, *H*C($CH_3$)$_2$, $J$ = 6.9 Hz], 3.35–3.50 (m, 4H, 5'-H, 4'-H, 6A-H, 6B-H), 3.75 (m, 4H, 1'ax-H, $OCH_3$), 4.20 (d, 1H, 1'eq-H, $J$ = 13.4 Hz), 4.52 (br. s, 1H, 2'-H), 6.8 (d, 2H, aromatic H), 7.10–7.50 (m, 12H, aromatic H), 8.1 (s, 1H, 8-H), 9.66 (br. s, 1H, NH). – $^{13}C$ NMR ($CDCl_3$): δ = 18.82 (Me), 18.90 (Me), 35.82 (C-3'), 36.02 [H*C*(Me)$_2$], 50.71 (C-2'), 55.11 ($OCH_3$), 63.33 (C-4'), 63.88 (C-6'), 68.50 (C-1'), 81.13 (C-5'), 86.70 (C$^{Tr}$O), 113.13 (C-*m'*, 2 C), 120.05 (C-5), 126.93 (C-*p*, 2 C), 127.84 (C-*o*, 4 C), 128.28 (C-*m*, 4 C), 130.24 (C-*o'*, 2 C), 135.19 (C-*i'*), 138.56 (C-8), 144.06 (C-*i*, 2 C), 147.85 (C-2), 148.82 (C-4), 156.03 (C-6), 158.52 (C-*p'*), 180.00 (NHC=O). – $C_{35}H_{37}N_5O_6$ (623.71): calcd. C 67.48, H 5.98, N 11.23; found: C 67.78, H 6.06, N 10.99.

*2'-(Adenin-9-yl)-1',5'-anhydro-4',6'-O-benzylidene-2',3'-dideoxy-*D*-arabino-hexitol* (**16**): A mixture of 1.35 g (10 mmol) of adenine, 76 mg of lithium hydride (9.5 mmol) and 0.32 ml of 12-crown-4 (2 mmol) in 60 ml of dry DMF was stirred at 110°C for 1 h. After a solution of 1.95 g (5 mmol) of the tosylate **10a**, in 13 ml of anhydrous DMF, was added, stirring was continued for 8 h at 110°C (TLC: hexane/EtOAc 8:2). The reaction mixture was cooled, quenched with water (0.09 ml, 5 mmol) and concentrated. The residue was dissolved in dichloromethane (100 ml) and the organic phase was washed with saturated $NaHCO_3$ solution (200 ml) and water (2 × 100 ml). The combined water phases were extracted with $CH_2Cl_2$. After drying with $MgSO_4$ and filtering, the organic phase was concentrated and the resulting residue was purified by column chromatography on silica gel with gradient elution ($CH_2Cl_2$/MeOH, 99:1, 98:2, 97:3, 96:4), yielding 1.45 g (4.1 mmol, 82% yield) of **16**. – M.p. 227°C. – For spectral data, see ref.[6], p. 2038, compound **13**. – $C_{18}H_{19}N_5O_3$ (353.38): calcd. C 61.18, H 5.42, N 19.82; found: C 61.25, H 5.79, N 19.56.

*1',5'-Anhydro-2'-(N⁶-benzoyladenin-9-yl)-4',6'-O-benzylidene-2',3'-dideoxy-*D*-arabino-hexitol* (**17**): A pre-evaporated (3× in dry pyridine) yellow solution of the amine **16** (5.9 g; 16.71 mmol) in 160 ml of dry pyridine was treated at 0°C with benzoyl chloride (9.7 ml, 83.57 mmol) and stirred overnight. The orange-brown solution was cooled in an ice-bath and 18 ml of water was added. After 5 min, 35 ml of an aqueous solution of $NH_3$ (25%) was added and the mixture stirred for 2 h at room temperature. The volatiles were removed in vacuo and co-evaporated three times with toluene to remove all pyridine. The resulting solid was diluted with 250 ml of $CH_2Cl_2$ and washed with 200 ml of a saturated $NaHCO_3$ solution. The organic phase was dried, filtered, concentrated and purified on silica gel with gradient elution (hexane/EtOAc, 80:20, 90:10, 100% EtOAc). This procedure yielded 7.2 g (15.75 mmol, 94%) of **17**. – M.p. 150°C. – LSIMS (thgly); *m/z*: 458 [$MH^+$]. – $^1H$ NMR ($CDCl_3$): δ = 2.25 (m, 1H, 3'ax-H), 2.66 (br. d, 1H, 3'eq-H, $J$ = 12 Hz), 3.65 (m, 2H, 5'-H, 4'-H), 3.78 (t, 1H, 6'ax-H, $J$ = 9.8 Hz), 4.16 (dd, 1H, 1'ax-H, $J$ = 13.5 Hz, $J$ = 2.5 Hz), 4.38 (dd,

1H, 6'eq-H, $J$ = 10.2 Hz, $J$ = 3.6 Hz), 4.48 (br. d, 1H, 1'eq-H, $J$ = 13.3 Hz), 5.07 (br. s, 1H, 2'-H), 5.49 (s, 1H, PhCH), 7.5 (m, 8H, aromatic H), 8.04 (d, 2H, aromatic H, $J$ = 6.7 Hz), 8.55 (s, 1H, 8-H), 8.77 (s, 1H, 2-H), 9.30 (s, 1H, NH). – $^{13}$C NMR ($CDCl_3$): 33.08 (C-3'), 51.01 (C-2'), 68.83 (C-6'), 69.42 (C-1'), 73.80 (C-4'), 74.55 (C-5'), 101.99 (PhCH), 122.8 (C-5), 125.94 (C-*o*/Ph, 2 C), 127.88 (C-*m*/Ph, 2 C), 128.25 (C-*m*/Bz, 2 C), 128.76 (C-*o*/Bz, 2 C), 129.1 (C-*p*/Ph), 132.73 (C-*p*/Bz), 133.49 (C-*i*/Bz), 136.89 (C-*i*/Ph), 142.04 (C-8), 149.58 (C-4), 152.00 (C-2), 152.51 (C-6), 164.72 (HN-C=O). – $C_{25}H_{23}N_5O_4$ (457.49): calcd. C 65.64, H 5.07, N 15.31; found C 65.72, H 5.09, N 15.44.

*1',5'-Anhydro-2'-($N^6$-benzoyladenin-9-yl)-2',3'-dideoxy-D-arabino-hexitol* (**18**): The benzylidene compound **17** (8.06 g, 17.63 mmol) was taken up in 450 ml of 80% HOAc and heated at 60°C for 6 h. After evaporation and co-evaporation with toluene, the residue was dissolved in a minimum of a $CH_2Cl_2$/MeOH (1:1) mixture and ether (500 ml) was added slowly while stirring. The precipitate was filtered off and washed with ether. After drying, 5.35 g (14.5 mmol, 82%) of **18** was obtained. – M.p. 220°C. – LSIMS (thgly); *m/z*: 370 [$MH^+$]. – $^1$H NMR ([$D_6$]DMSO): δ = 1.97 (dt, 1H, 3'ax-H, $J$ = 13 Hz, $J$ = 3.9 Hz), 2.36 (br. d, 1H, 3'eq-H, $J$ = 13.2 Hz), 3.23 (m, 1H, 5'-H), 3.5–3.8 (m, 3H, 6'-H, 4'-H), 3.93 (dd, 1H, 1'ax-H, $J$ = 12.7 Hz, $J$ = 2.1 Hz), 4.30 (br. d, 1H, 1'eq-H, $J$ = 12.5 Hz), 4.7 (t, 1H, 6'-OH, $J$ = 6.22 Hz), 4.98 (br. s, 1H, 2'-H), 5.00 (d, 1H, 4'-OH, $J$ = 5.5 Hz), 7.58 (m, 3H, aromatic H), 8.05 (d, 2H, aromatic H, $J$ = 6.9 Hz), 8.62 (s, 1H, 8-H), 8.75 (s, 1, 2-H), 11.18 (s, 1H, NH). – $^{13}$C NMR ([$D_6$]DMSO): δ = 35.84 (C-3'), 50.75 (C-2'), 60.50 (C-6', C-4'), 67.90 (C-1'), 83.11 (C-5'), 125.08 (C-5), 128.55 (C-*m*, C-*o*, 4 C), 132.51 (C-*p*), 133.50 (C-*i*), 143.48 (C-8), 150.26 (C-4), 151.49 (C-2), 152.37 (C-6), 165.67 (HN-C=O). – $C_{18}H_{19}N_5O_4$ (369.38): calcd. C 58.53, H 5.18, N 18.96; found C 58.45, H 5.39, N 18.92.

*1',5'-Anhydro-6'-O-monomethoxytrityl-2',3'-dideoxy-2'-($N^6$-benzoyladenin-9-yl)-D-arabino-hexitol* (**6**): A pre-evaporated (3× in dry pyridine) solution of the diol **18** (5.35 g, 14.49 mmol) in 340 ml of dry pyridine was treated at room temperature with monomethoxytrityl chloride (7.85 g, 24.64 mmol) and stirred for 2 d (TLC: $CH_2Cl_2$/MeOH, 94:6; starting compound: $R_f$ = 0.50). Smaller quantities of monomethoxytrityl chloride gave uncomplete reaction and higher temperatures gave rise to bistritylated product. The mixture was quenched with 300 ml of saturated $NaHCO_3$ solution and extracted with 400 ml of $CH_2Cl_2$ (2×). The organic phase was dried with $MgSO_4$, filtered, evaporated and co-evaporated with toluene (3×). Purification by column chromatography on silica gel with gradient elution (100% $CH_2Cl_2$, $CH_2Cl_2$/MeOH, 99:1, $CH_2Cl_2$/MeOH, 98:2, $CH_2Cl_2$/MeOH, 97:3) afforded 7.79 g (12.15 mmol, 84%) of pure **6**. – M.p. 125°C. – LSIMS (thgly/NaOAc); *m/z*: 664 [$MNa^+$]. – $^1$H NMR ($CDCl_3$): δ = 1.98 (dt, 1H, 3'ax-H, $J$ = 11.6 Hz, $J$ = 4.4 Hz), 2.54 (br. d, 1H, 3'eq-H, $J$ = 12.6 Hz), 2.88 (br. s, 1H, 4'-OH), 3.47 (m, 3H, 5'-H, 4'-H, 6A-H), 3.77 (br. s, 4H, 6B-H, $OCH_3$), 3.96 (dd, 1H, 1'ax-H, $J$ = 12.9 Hz, $J$ = 2.5 Hz), 4.34 (br. d, 1H, 1'eq-H, $J$ = 12.8 Hz), 4.98 (br. s, 1H, 2'-H), 6.84 (d, 2H, aromatic H, $J$ = 8.9 Hz), 7.38 (m, 15H, aromatic H), 8.02 (d, 2H, aromatic H, $J$ = 8.3 Hz), 8.54 (s, 1H, 8-H), 8.77 (s, 1H, 2-H), 9.3 (br. s, 1H, NH). – $^{13}$C NMR ($CDCl_3$): δ = 35.68 (C-3'), 50.64 (C-2'), 55.19 ($OCH_3$), 64.33 (C-4', C-6'), 69.03 (C-1'), 80.65 (C-5'), 87.16 ($C^{Tr}O$), 113.13 (C-*m'*, 2 C), 120.00 (C-5), 127.15 (C-p, 2 C), 127.86 (C-*m*/Bz, 2 C), 128.02 (C-*o*, 4 C), 128.19 (C-*m*, 4 C), 128.78 (C-*o*/Bz, 2 C), 130.20 (C-*o'*, 2 C), 132.67 (C-*p*/Bz), 133.68 (C-*i*/Bz), 134.90 (C-*i'*), 142.39 (C-8), 143.76 (C-*i*, 2 C), 149.46 (C-4), 152.48 (C-2, C-6), 158.68 (C-*p'*), 164.67 (NHC=O). – $C_{38}H_{35}N_5O_5$ (641.73): calcd. C 71.12, H 5.50, N 10.91; found C 71.02, H 5.56, N 10.84.

*1',5'-Anhydro-2'-($N^4$-benzoylcytosin-1-yl)-4',6'-O-benzylidene-2',3'-dideoxy-D-arabino-hexitol* (**22**): To a suspension of 1.5 g (7 mmol) of $N^4$-benzoylcytosine[12], 0.87 g (3.68 mmol) of the alcohol **9** and 2.29 g (8.75 mmol) of triphenylphosphane in 100 ml of anhydrous THF, was added over 200 min 1.38 ml (8.75 mmol) of diethyl azodicarboxylate in 10 ml of anhydrous THF. The mixture was stirred overnight at room temperature. The precipitate, which contained almost exclusively product **23**, was filtered off and the volatiles of the filtrate were removed in vacuo. The crude product (ca. 7 g) was adsorbed on silica gel (15 g) and purified by column chromatography with gradient elution (100% diethyl ether, hexane/EtOAc, 20:80, 10:90, 100% EtOAc) yielding 0.548 g (1.26 mmol, 34%) of the amide **22**. – M.p. 226°C. LSIMS (thgly); *m/z*: 434 [$MH^+$]. – $^1$H NMR ($CDCl_3$): δ = 2.15 (dt, 1H, 3'ax-H, $J$ = 11.9 Hz, $J$ = 4.8 Hz), 2.58 (br. d, 1H, 3'eq-H, $J$ = 12.9 Hz), 3.45–3.75 (m, 2H, 5'-H, 4'-H), 3.79 (t, 1H, 6'ax-H, $J$ = 10.2 Hz), 4.06 (dd, 1H, 1'ax-H, $J$ = 13.7 Hz, $J$ = 3 Hz), 4.30 (d, 1H, 1'eq-H, $J$ = 13.8 Hz), 4.36 (dd, 1H, 6'eq-H, $J$ = 10 Hz, $J$ = 4.7 Hz), 4.95 (br. s, 1H, 2'-H), 5.55 (s, 1H, PhCH), 7.30–7.65 (m, 9H, aromatic H + 5-H), 7.92 (d, 2H, aromatic H, $J$ = 7.8 Hz), 8.48 (d, 1H, 6-H, $J$ = 7.4 Hz), 9.00 (br. s, 1H, NH). – $^{13}$C NMR ($CDCl_3$): δ = 31.98 (C-3'), 52.83 (C-2'), 68.74 (C-6'), 68.93 (C-1'), 73.51 (C-4'), 74.22 (C-5'), 96.6 (C-5), 101.94 (PhCH), 125.97 (C-*o*/Ph, 2 C), 127.53 (C-*m*/Bz, 2 C), 128.26 (C-*o*/Bz, 2 C), 128.97 (C-*m*/Ph, 2 C), 129.08 (C-*p*/Ph), 132.88 (C-*i*/Bz), 133.16 (C-*p*/Bz), 137 (C-*i*/Ph), 147.05 (C-6), 155.14 (C-2), 161.97 (C-4), 166.63 (HN-C=O). – $C_{24}H_{23}N_3O_5$ (433.46): calcd. C 66.50, H 5.35, N 9.69; found C 66.54, H 5.56, N 9.47.

*1',5'-Anhydro-2'-($N^4$-benzoylcytosin-1-yl)-2',3'-dideoxy-D-arabino-hexitol* (**24**): The benzylidene compound **22** (3.51 g, 8.1 mmol) was taken up in 200 ml of 80% HOAc and heated at 60°C for 3 h. After evaporation and co-evaporation with toluene, the residue was dissolved in a minimum of $CH_2Cl_2$/MeOH (1:1), and diethyl ether (150 ml) was added slowly while stirring. Ultrasonic treatment was necessary to obtain a good precipitate. This precipitate was filtered off and washed with ether. After drying, this procedure yielded 1.92 g (5.56 mmol, 69%) of **24**. The filtrate was concentrated and purified on silica gel with gradient elution ($CH_2Cl_2$/MeOH, 98:2, 96:4, 93:7), affording another 0.7 g (2.02 mmol, 25%) of **24**, raising the total yield to 2.62 g (7.6 mmol, 94%). – M.p. 130°C. – LSIMS (thgly); *m/z*: 346 [$MH^+$]. – $^1$H NMR ([$D_6$]DMSO): δ = 1.79 (dt, 1H, 3'ax-H, $J$ = 13.6 Hz, $J$ = 4.4 Hz), 2.2 (br. d, 1H, 3'eq-H, $J$ = 13.7 Hz), 3.14 (m, 1H, 5'-H), 3.5–3.7 (m, 3H, 6'-H, 4'-H), 3.81 (dd, 1H, 1'ax-H, $J$ = 12.9 Hz, $J$ = 2.9 Hz), 4.14 (br. d, 1H, 1'eq-H, $^2J$ = 13.2 Hz), 4.60 (m, 2H, 6'-OH + 2'-H), 4.95 (d, 1H, 4'-H), $J$ = 5.1 Hz), 7.31 (d, 1H, 5-H, $J$ = 7.3 Hz), 7.57 (m, 3H, aormatic H), 8.01 (d, 2H, aromatic H, $J$ = 7 Hz), 8.50 (d, 1H, 6-H, $J$ = 7.4 Hz), 11.19 (s, 1H, OCNH). – $^{13}$C NMR ([$D_6$])DMSO): δ = 34.78 (C-3'), 52.30 (C-2'), 60.53 (C-6', C-4'), 67.23 (C-1'), 82.84 (C-5'), 95.87 (C-5), 128.51 (C-*m*, C-*o*, 4 C), 132.76 (C-*p*, C-*i*, 2 C), 148.09 (C-6), 155.01 (C-2), 162.56 (C-4), 167.48 (HNC=O). – $C_{17}H_{19}N_3O_5 \cdot 2\ H_2O$ (381.39): calcd. C 53.54, H 6.08, N 11.02; fond C 53.47, H 6.02, N 10.94.

*1',5'-Anhydro-2'-($N^4$-benzoylcytosin-1-yl)-2',3'-dideoxy-6'-O-monomethoxytrityl-D-arabino-hexitol* (**7**): The tritylation to afford **7** was done with 1.7 equivalents of monomethoxytrityl chloride at room temperature or by using 1.2 equivalents of monomethoxytrityl chloride at 50°C for 1 h. A pre-evaporated (3× in dry pyridine) solution of the diol **24** (2.72 g, 7.88 mmol), in 100 ml of dry pyridine, was treated at room temperature with monomethoxytrityl chloride (4.26 g, 13.4 mmol) and stirred overnight (TLC: $CH_2Cl_2$/MeOH, 95:5; starting material: $R_f$ = 0.34). – The mixture was quenched with 200 ml of saturated $NaHCO_3$ solution and extracted with 200 ml of $CH_2Cl_2$ (2×). The organic phase was dried with

$MgSO_4$, filtered, evaporated and co-evaporated with toluene (3×). Purification by column chromatography with gradient elution ($CH_2Cl_2$/MeOH, 99:1, 98:2, 97:3, 96:4) afforded 3.88 g (6.28 mmol, 80%) of pure **7**. – M.p. 140°C. – LSIMS (thgly/NaOAc); *m/z*: 640 [$MNa^+$]. – $^1H$ NMR ($CDCl_3$): δ = 1.9 (m, 1H, 3'ax-H), 2.55 (br. d, 1H, 3'eq-H, *J* = 12.2 Hz), 3.08 (br. s, 1H, 5'-H), 3.42 (m, 3H, 4'-H, 6'-H), 3.79 (s, 3H, $OCH_3$), 3.89 (dd, 1H, 1'ax-H, *J* = 13.5 Hz, *J* = 2.9 Hz), 4.01 (br. s, 1H, 4'-OH), 4.25 (br. d, 1H, 1'eq-H, *J* = 13.5 Hz), 4.83 (br. s, 1H, 2'-H), 6.85 (d, 2H, aromatic H, *J* = 8.8 Hz), 7.2–7.7 (m, 18H, aromatic H + 5-H), 8.73 (br. d, 2H, NH + 6-H, *J* = 7.6 Hz). – $^{13}C$ NMR ($CDCl_3$): δ = 35.02 (C-3'), 52.72 (C-2'), 55.15 ($OCH_3$), 62.08 (C-4'), 62.73 (C-6'), 68.38 (C-1'), 80.81 (C-5'), 86.61 ($C^{Tr}O$), 96.43 (C-5), 113.21 (C-*m'*, 2 C), 127.11 (C-*p*, 2 C), 127.86 (C-*m*/Bz, 2 C), 127.97 (C-*o*, 4 C), 128.37 (C-*m*, 4 C), 128.85 (C-*o*/Bz, 2 C), 129.96 (C-*o'*, 2 C), 132.72 (C-*p*/Bz), 132.88 (C-*i*/Bz), 135.6 (C-*i'*), 143.93 (C-*i*), 147.98 (C-6), 155.54 (C-2), 158.56 (C-*p'*), 161.92 (C-4), 166.26 (NHC=O). – $C_{37}H_{35}N_3O_6$ (617.70): calcd. C 71.95, H 5.71, N 6.80; found C 71.95, H 5.87, N 6.87.

*1',5'-Anhydro-2'-(N³-benzoylthymin-1-yl)-4',6'-O-benzylidene-2',3'-dideoxy-D-arabino-hexitol* (**26**): To a solution of 2.4 g (10.46 mmol) of $N^3$-benzoylthymine[13], 1.23 g (5.23 mmol) of the alcohol **9** and 3.43 g (13.08 mmol) of triphenylphosphane in 100 ml of anhydrous THF was added 2.06 ml (13.08 mmol) of diethyl azodicarboxylate in 15 ml of anhydrous THF via a dropping funnel over a period of 60 min. The mixture was stirred overnight at room temperature. The volatiles were removed in vacuo. The crude foam (ca. 10 g) was adsorbed on silica gel (20 g) and purified by gradient elution (hexane/EtoAc, 80:20, 70:30, 60:40). This procedure yielded 1.88 g (4.19 mmol, 80%) of white crystals of **26**. – M.p. 200°C. – LSIMS (thgly); *m/z*: 449 [$MH^+$]. – $^1H$ NMR ($CDCl_3$): δ = 2.02 (s, 3H, $CH_3$), 2.07 (m, 1H, 3'ax-H), 2.47 (br. d, 1H, 3'eq-H, *J* = 13.9 Hz), 3.53 (dt, 1H, 5'-H, *J* = 9.6 Hz, *J* = 4.7 Hz), 3.8 (m, 1H, 4'-H), 3.8 (t, 1H, 6'ax-H, *J* = 10.2 Hz), 4.02 (dd, 1H, 1'ax-H, *J* = 13.7 Hz, *J* = 3.5 Hz), 4.28 (br. d, 1H, 1'eq-H, *J* = 13.9 Hz), 4.37 (dd, 1H, 6'eq-H, *J* = 10.5 Hz, *J* = 4.7 Hz), 4.73 (br. s, 1H, 2'-H), 5.64 (s, 1H, PhCH), 7.3–7.9 (m, 8H, aromatic H), 7.95 (s + d, 3H, 6H + aromatic 2H, 6-H). – $^{13}C$ NMR ($CDCl_3$): δ = 32.91 (C-3'), 51.67 (C-2'), 68.80 (C-6', C-1'), 73.64 (C-4'), 74.24 (C-5'), 101.99 (PhCH), 110.72 (C-5), 126.01 (C-*o*/Ph, 2 C), 128.35 (C-*p*/Ph), 129.12 (C-*m*/Bz + C-*o*/Bz, 4 C), 130.41 (C-*m*/Ph, 2 C), 131.54 (C-*i*/Bz), 135.02 (C-*p*/Bz), 136.97 (C-*i*/Ph), 137.71 (C-6), 149.74 (C-2), 162.61 (C-4), 168.91 (HNC=O). – $C_{25}H_{24}N_2O_6 \cdot 0.5\,H_2O$ (507.48): calcd. C 65.64, H 5.51, N 6.12; found C 65.74, H 5.69, N 6.10.

*1',5'-Anhydro-2',3'-dideoxy-2-(thymin-1-yl)-D-arabino-hexitol* (**4**): Product **26** (1.83 g, 4.085 mmol) was taken up in 100 ml of methanol saturated with ammonia and stirred at room temperature. During this reaction a precipitate is formed. Dichloromethane (50 ml) was added and the suspension dissolved. The solution was stirred for another 90 min at room temperature (TLC: hexane/EtOAc, 50:50; $R_f$ = 0.12). Evaporation and co-evporation with toluene (3×) left crude **25** (1.8 g) as a white solid which was taken up in 75 ml of 80% acetic acid and heated at 60°C for 3 h. After evaporation and co-evaporation with toluene, the residue was purified on silica gel with gradient elution ($CH_2Cl_2$/MeOH, 95:5, 93:7), affording 0.86 g (3.36 mmol, 82%) of the diol **4**. – M.p. 170°C. – LSIMS (thgly); *m/z*: 357 [$MH^+$]. – $^1H$ NMR ([$D_6$]DMSO): δ = 1.76 (s, 3H, $CH_3$), 1.75 (m, 1H, 3'ax-H), 2.08 (br. d, 1H, 3'eq-H, *J* = 13.8 Hz), 3.13 (m, 1H, 5'-H), 3.35 (m, 1H, 4'-H), 3.6 (m, 2H, 6'-H), 3.73 (dd, 1H, 1'ax-H, *J* = 12.9 Hz, *J* = 3.4 Hz), 3.99 (br. d, 1H, 1'eq-H, *J* = 12.8 Hz), 4.5 (br. s, 1H, 2'-H), 4.65 (br. s, 1H, 6'-OH), 4.91 (br. s, 1H, 4'-OH), 7.88 (s, 1H, 6-H), 11.25 (s, 1H, 3-H). – $^{13}C$ NMR ([$D_6$]DMSO): δ = 12.45 ($CH_3$), 35.25 (C-3'), 50.13 (C-2'), 60.30 (C-6'), 60.75 (C-4'), 67 (C-1'), 82.42 (C-5'), 108.37 (C-5), 139.02 (C-6), 151.05 (C-2), 163.9 (C-4). Additional spectral data are published in ref.[4], p. 2039, product **4d**. – $C_{11}H_{16}N_2O_5 \cdot 2\,H_2O$ (276.29): calcd. C 45.20, H 6.90, N 9.58; found C 45.33, H 6.88, N 9.38.

*1',5'-Anhydro-2',3'-dideoxy-6'-O-monomethoxytrityl-2'-(thymin-1-yl)-D-arabino-hexitol* (**8**): A pre-evaporated (3× in dry pyridine) solution of the diol **4** (2.77 g, 10.82 mmol) in 100 ml of dry pyridine was treated at room temperature with monomethoxytrityl chloride (5.85 g, 18.39 mmol) and stirred overnight (TLC: $CH_2Cl_2$/MeOH, 90:10; starting material $R_f$ = 0.66). – The mixture was quenched with 200 ml of saturated $NaHCO_3$ solution and washed with 200 ml of $CH_2Cl_2$ (2×). The organic phase was dried with $MgSO_4$, filtered, evaporated and co-evaporated three times with toluene. Purification by column chromatography with gradient elution (100% $CH_2Cl_2$, $CHCl_2$/MeOH, 99:1, 98:2, 95:5) afforded 4.9 g (9.28 mmol, 86%) of **8**. – M.p. 125°C. – LSIMS (thgly/NaOAc); *m/z*: 551 [$MNa^+$]. – $^1H$ NMR ($CDCl_3$): δ = 1.86 (s, 3H, $CH_3$), 1.85 (m, 1H, 3'ax-H), 2.28 (d, 1H, 4'-OH, *J* = 3.8 Hz), 2.38 (br. d, 1H, 3'eq-H, *J* = 14 Hz), 3.31 (m, 1H, 5'-H), 3.45 (m, 2H, 4'-H, + 6'ax-H), 3.78 (s, 3H, $OCH_3$), 3.81 (dd, 1H, 1'ax-H, *J* = 13 Hz, *J* = 3.4 Hz), 3.98 (m, 1H, 6'eq-H), 4.18 (br. d, 1H, 1'eq-H, *J* = 13.2 Hz), 4.67 (br. s, 1H, 2'-H), 6.83 (d, 2H, aromatic H, *J* = 8.7 Hz), 7.2–7.5 (m, 12H, aromatic H), 8.00 (s, 1H, 6-H), 9.2 (br. s, 1H, NH). – $^{13}C$ NMR ($CDCl_3$): δ = 12.80 ($CH_3$), 35.47 (C-3'), 50.98 (C-2'), 55.21 ($OCH_3$), 63.13 (C-4'), 63.30 (C-6'), 68.47 (C-1'), 80.90 (C-5'), 86.80 ($C^{Tr}O$), 110.41 (C-5), 113.26 (C-*m'*, 2 C), 127.11 (C-*p*, 2 C), 127.97 (C-*o*, 4 C), 128.17 (C-*m*, 4 C), 130.22 (C-*o'*, 2 C), 135.03 (C-*i'*), 138.57 (C-6), 143.91 (C-*i*), 151.03 (C-2), 158.69 (C-*p'*), 163.82 (C-4). – $C_{31}H_{32}N_2O_6$ (528.60): calcd. C 70.44, H 6.10, N 5.30; found C 70.28, H 6.09, N 5.49.

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☆ Dedicated to Professor *Wolfgang Pfleiderer* on the occasion of his 70th birthday.


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