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Azido Functionalized Nucleosides Linked to Controlled Pore Glass as Suitable Starting Materials for Oligonucleotide Synthesis by the Phosphoramidite Approach

Frederik Müggenburg,^[a] Alexander Biallas,^[a] Mégane Debiais,^[b] Michael Smietana,^[b] and Sabine Müller^{*[a]}

In memory of Klaus Hafner

It has long been debated whether easily reducible azide groups can withstand the conditions of oligonucleotide synthesis by phosphoramidite chemistry. We have synthesized various 2'- and 3'-azido modified nucleosides and immobilized them on controlled pore glass (CPG) to be used as starting material for the synthesis of oligonucleotides (ONs) with 3'-terminal azide (attached to C2' or C3'). In a model study, immobilized 3'-azidoadenosine was used as a starting block for the synthesis of a series of oligodeoxynucleotides (ODNs) of increasing length. Upon synthesis, the ODNs were enzymatically digested into

monomers and analyzed by RP-HPLC. A peak corresponding to 3'-azidoadenosine was clearly identified in all samples. Quantitative analysis showed that 3'-azidoadenosine was present in nearly the expected ratio to deoxycytidine, which was used as an internal standard. Most importantly, the ratio remained the same for all three ODNs regardless of their length, demonstrating that a higher number of coupling cycles does not lead to higher degradation of the azide. Thus, 2'- or 3'-azido nucleosides attached to a solid support are excellent starting materials for the synthesis of oligonucleotides with 3'-terminal azide.

Introduction

Over the past decade, techniques for covalent labeling of nucleic acids have tremendously developed and nowadays a number of orthogonal methods are offered for attaching a specific dye or other probe to the nucleic acid of interest.^[1] Among those, the copper catalyzed azide alkyne cycloaddition (CuAAC) or the copper free strain-promoted azide alkyne cycloaddition (SPAAC) are often utilized to form DNA- or RNA-bioconjugates.^[2–4] Both methods require one reactant carrying an azido group and the other offering an alkyne moiety. Interestingly, when it comes to the necessary functionalization of RNA or DNA, one observes that usually the group to be conjugated carries the azido moiety, while the oligonucleotide

(ON) is functionalized with the alkyne.^[5–7] Presumably, this is caused by the widely held view that azido groups cannot withstand the conditions of the standard phosphoramidite procedure for chemical ON synthesis.^[8–10] In fact, the azido group is easily reduced, while phosphoramidites are easily oxidized, which makes unfavorable interactions between these two groups seem likely. As a matter of fact, azido modified phosphoramidites have been reported to be inherently unstable as they degrade quickly when dissolved.^[11] Although, some specially designed phosphoramidite azides have been successfully used for the chemical synthesis of 5'-azido-modified ONs on solid phase.^[12] Furthermore, the reactivity of azides towards phosphoramidites has been used intentionally to produce cyclic phosphoramidates and phosphorazides.^[13] Owing to this reactivity, azido modifications are often inserted into oligonucleotides enzymatically,^[14,15] or chemically after solid phase ON synthesis has been concluded.^[16–18] H-phosphonate or phosphotriester chemistry was also used instead of the phosphoramidite approach for the synthesis of azido-modified oligonucleotides,^[19–21] or synthesis of 3'-terminal azido modified ODNs was conducted in reverse orientation from 5' to 3'-end.^[10] Yet, there have been reports on the chemical synthesis of azido modified ONs by the standard phosphoramidite strategy.^[22–24] It seems that the azido group can generally withstand the conditions of ON synthesis on solid phase as long as it is not part of a phosphoramidite building block, but is used instead as a polymer-bound 3'-terminal nucleoside from which the synthesis starts. Although the chemical synthesis of ONs with an azido group at the 3'-end of the sequence seems to be generally possible, to our knowledge there have been no

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attempts to quantitatively investigate the stability of the azide as a function of the length of the ON constructed from the 3'-azido modified starting material. The 3'-terminal azide can be introduced by linking a 2'- or 3'-azido modified nucleoside to solid support, which is then used as starting material in ON synthesis. This requires the preparation of the modified nucleoside and its immobilization by an appropriate chemistry. Here, we report on the synthesis of three azido functionalized nucleosides, their conjugation to CPG, and the evaluation of azide stability over multiple phosphoramidite coupling cycles.

Results and Discussion

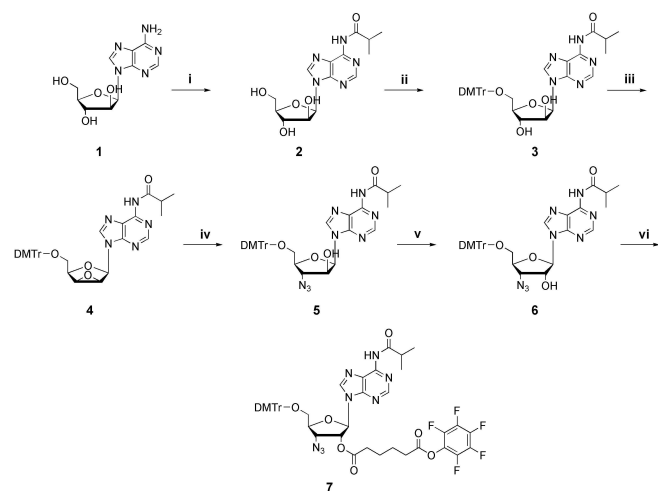
With the aim to prepare 3'-azido functionalized oligonucleotides for our projects involving postsynthetic conjugation of fluorescence dyes to ONs of interest by CuAAC, or Click ligation of RNA molecules,^[22] we have prepared several 2'- and 3'-azido modified monomers, namely 2'-azidoadenosine, 3'-azidoadenosine and 3'-azidouridine, appropriately protected to be linked to solid support and as such to be used as starting material in DNA or RNA synthesis. The preparation of the 3'-azido modified adenosine derivative **7** described here was based on a strategy previously used in our laboratory,^[22] but with some variation in the order of reaction steps to prevent unwanted decomposition of the azide (Scheme 1).

The synthesis was started from the arabinose derivative of adenosine **1**, which is commercially available. First, the exocyclic amino function was protected using isobutyryl chloride in tandem with transient protection of the hydroxyl groups with trimethylsilyl chloride (TMS-Cl),^[25] which yielded the *N*-protected nucleoside **2**. Next step was the selective masking of the

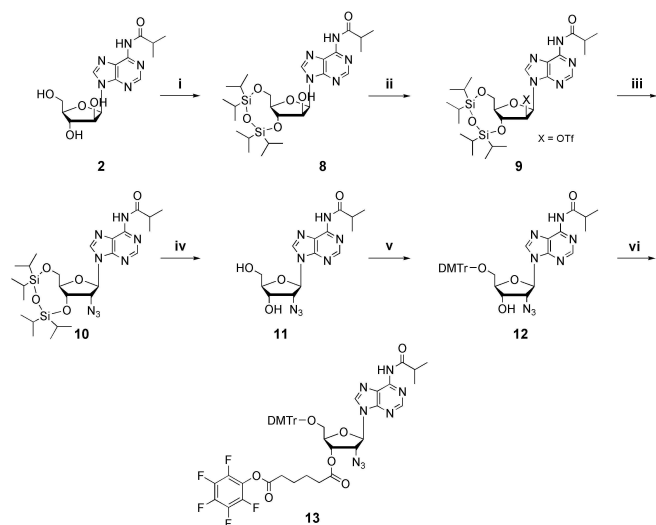
5'-hydroxyl group using 4,4'-dimethoxytrityl chloride (DMTr-Cl). The tritylated compound **3** was easily converted into the epoxide **4** by reaction with triphenylphosphine and diisopropyl azodicarboxylate (DIAD).^[26] The latter was used as a substitute for the potentially explosive diethyl azodicarboxylate (DEAD). Due to the steric influence of the nucleobase, the 3'-hydroxyl group appears to be the preferred leaving group yielding the epoxide **4**. Indeed, we have observed the clear formation of a single product, which by NMR analysis and further downstream reactions was confirmed to be the expected epoxide **4**. The epoxide was treated with lithium azide to obtain the 3'-azido functionalized nucleoside **5**, whose identity was clearly confirmed by NMR. The generation of the desired 3'-azido derivative is generally favored over the respective 2'-azido isomer,^[27] although in our case the preference for the 3'-isomer was somewhat diminished, presumably due to the influence of the DMTr-group. This influence of the C5' substituent has been discussed previously by Goodman et al.,^[27] who showed that the absence of an electronegative moiety at the C5' position increases the likelihood of a nucleophilic attack at the C2'. The correct placement of the azido group at C3' was confirmed by 2D COSY NMR characterization (Figure S1). The introduction of the azido function left the nucleoside again in the arabinose configuration. Epimerization of **5** was carried out following a procedure by Micura et al.,^[28] converting the nucleoside into the desired ribose configuration **6**. The last step was the esterification of the 2'-hydroxyl group with a dipentafluorophenyl (Pfp) ester of adipic acid.^[23] The final compound **7** was then used for immobilization onto amino functionalized CPG support.

For our future studies regarding the influence of the position of the azide (2'- or 3'-) on its functionality, the 2'-azido isomer **13** was synthesized, also starting from the arabinose derivative of adenosine **1** (Scheme 2). The overall strategy was adapted from a procedure of the Micura group for preparation of 2'-azido guanosine.^[20] The first step of the synthesis was identical to the one for the synthesis of the 3'-azido derivative. However, now the resulting *N*-protected nucleoside **2** was treated with tetraisopropylsiloxane dichloride (TIPDS-Cl) for selective protection of the 3'- and 5'-hydroxyl group. The resulting diprotected nucleoside **8** was treated with trifluoromethanesulfonyl chloride in order to activate the 2'-hydroxyl function as leaving group in intermediate **9**. Next step was an S_N2 -reaction with lithium azide, which furnished the azido modified nucleoside **10** in excellent yields. The removal of the TIPDS group was easily performed using a solution of tetrabutylammonium fluoride and acetic acid. The deprotected nucleoside **11** was tritylated with DMTr-Cl, and in the last step the 3'-hydroxyl group of derivative **12** was reacted with the Pfp ester of adipic acid as described above for the 3'-azido derivative **6** (Scheme 1). Accordingly, the obtained final product **13** was then used for immobilization onto amino-functionalized CPG.

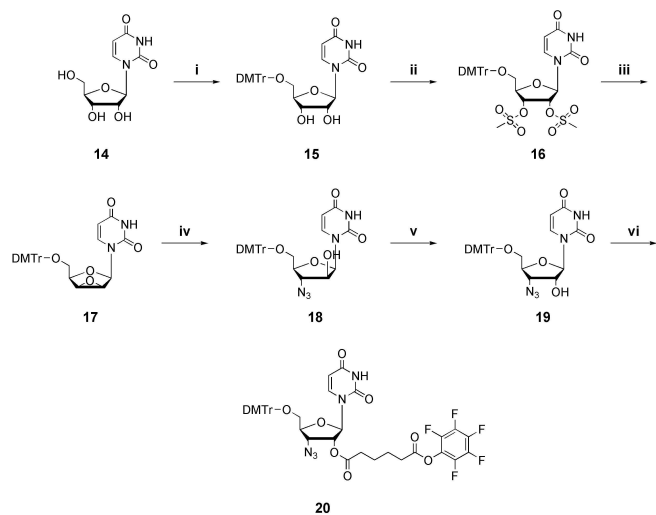
In order to extend the set of azido modified nucleosides to a pyrimidine derivative, 3'-azido uridine was synthesized and converted into compound **20** (Scheme 3), following a previous strategy by Amin et. al. for preparation of a 3'-azido modified



Scheme 1. Synthesis of functionalized 3'-azidoadenosine **7**. Reaction conditions: (i a) 5 eq. TMS-Cl in pyridine, 1 h, RT; (i b) 2 eq. isobutyryl chloride in pyridine, overnight, RT; (i c) $\text{NH}_3/\text{H}_2\text{O}$, in pyridine, 30 min, RT, 74%; (ii) 1.3 eq. DMTr-Cl, in pyridine, overnight, RT, 53%; (iii) 1.85 eq. PPh_3 , 1.85 eq. DIAD, in DMF, 1 h, 55 °C, 85%; (iv) 5 eq. LiN_3 in DMF, 3 h, 55 °C, 40%; (v a) 3 eq. DMAP, 1.5 eq. trifluoromethanesulfonyl chloride in CH_2Cl_2 , 30 min, 0 °C; (v b) 5 eq. $\text{CF}_3\text{COO}^- \text{K}^+$, 2 eq. 18-crown-6, 1.5 eq. DIPEA in toluene, 2 h, 60 °C, 64%; (vi) 1 eq. DMAP, 4.9 eq. Pfp adipic acid ester in pyridine/DMF (1 : 1), 2 h, RT, 58%.



Scheme 2. Synthesis of functionalized 2'-azido adenosine **13**. Reaction conditions: (i) 1.2 eq. 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane, in pyridine overnight, RT, 67%; (ii) 1.5 eq. DMAP, 2.5 eq. Et₃N, 1.5 eq. trifluoromethanesulfonyl chloride, in CH₂Cl₂, 30 min, 0 °C; (iii) 5 eq. LiN₃ in DMF, overnight, RT, 90%; (iv) 1 M tetrabutylammonium fluoride, 1.9 eq. acetic acid, in THF, 30 min, RT, 71%; (v) 1.3 eq. DMTr-Cl, in pyridine, 4.5 h, RT, 57%; (vi) 1 eq. DMAP, 4.9 eq. Pfp adipic acid ester in pyridine/DMF (1 : 1), 2 h, RT, 60%.



Scheme 3. Synthesis of 3'-azido modified uridine derivative **20**. Reaction conditions: (i) 1.3 eq. DMTr-Cl, in pyridine, overnight, RT, 67%; (ii) 15 eq. methanesulfonyl chloride, in pyridine, 1.5 h, 0–25 °C, 98%; (iii) 1 M NaOH, in ethanol, 1 h, 60 °C, 89%; (iv) 5 eq. LiN₃ in DMF, overnight, 55 °C, 44%; (v a) 3 eq. DMAP, 1.5 eq. trifluoromethanesulfonyl chloride in CH₂Cl₂, 30 min, 0 °C; (v b) 5 eq. CF₃COO⁻ K⁺, 2 eq. 18-crown-6, 1.5 eq. DIPEA in toluene, 2 h, 60 °C, 73%; (vi) 1 eq. DMAP, 4.9 eq. Pfp adipic acid ester in pyridine/DMF (1 : 1), 2 h, RT, 54%.

arabino nucleoside.^[29] First, uridine was 5'-tritylated with DMTr-Cl. The 2'- and 3'-hydroxyl groups were then activated with methanesulfonyl chloride to obtain compound **16** in excellent yields. The conversion of **16** into the epoxide **17** was carried out by treatment with a 1 M NaOH solution in ethanol. Reaction with lithium azide produced the desired 3'-azido

isomer **18**. Analogous to compound **5**, the correct placement of the azido group was confirmed by 2D COSY NMR characterization (Figure S2). Again, epimerization was necessary to convert compound **18** into the desired ribonucleoside **19**. Treatment with the adipic acid ester as described above delivered the activated ester **20**.

All three azidonucleoside derivatives (**7**, **13** and **20**) were linked to amino functionalized CPG. The subsequently determined loading densities ranged from 44 to 74 μmol/g (see SI for more detailed information).

All three immobilized azido modified nucleosides were used as starting material for oligonucleotide synthesis by the phosphoramidite approach to produce ONs functionalized with an azide at the 3'-terminus (attached to either the 2'- or 3'-position of the 3'-terminal nucleoside, Figures S13 to S18). Actually, we have performed such syntheses already in the past, however without exact examination of the stability of the azido group over multiple synthesis cycles.^[22] In order to closer investigate the stability of the azido group under the conditions of ON synthesis, the performance of one of the synthesized azido modified derivatives, 3'-azido adenosine linked to CPG, was systematically investigated in a representative study. Three different ODNs of increasing length (11-, 16- and 21-mer) were synthesized (Table 1). For the sake of simplicity, we decided for DNA synthesis, assuming that the results would be transferable also to the synthesis of 3'-azido modified RNA.

Upon chain assembly, oligonucleotides were cleaved from the solid support, deprotected and purified by ion exchange HPLC. Purity and correct sequence length were confirmed by gel electrophoresis and MALDI-TOF analysis (Figure S3).

To further investigate whether the azido group stayed intact during the iterative treatments with phosphoramidite solution over multiple coupling cycles, oligonucleotides were digested into monomers by phosphodiesterase (PDE) and alkaline shrimp phosphatase (ASP). Monomers were then separated via reversed phase-HPLC (Figure 1A). For each ODN the enzymatic digestion and subsequent HPLC analysis were carried out in triplets.

Nucleoside standards were used for identification of peaks (Figures S4–S8). Additionally, the RP HPLC fraction containing 3'-azido adenosine was analysed by LC-MS to confirm that the azido group indeed stayed intact (Figure S9). For internal standardization, the area of the 3'-azido adenosine peak was set in relation to the peak area corresponding to deoxycytidine, and the determined ratio (3'-azido A to dC) in comparison to the theoretically expected ratio was taken as a measure of the stability of 3'-azido adenosine in the individual samples (Table 2).

Table 1. Synthesized DNA sequences.		
	Sequence ^[a]	Length (nt)
ODN 1	5'-GTCC GTCC A*-3'	11
ODN 2	5'-GTCC GTCC GTCC A*-3'	16
ODN 3	5'-GTCC GTCC GTCC GTCC A*-3'	21

[a] A* = 3'-azido modified adenosine.

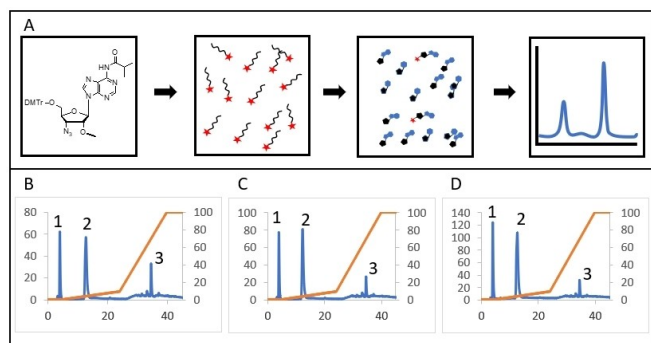


Figure 1. A: Workflow for the stability assay: First step was the synthesis of the azido modified starting block, which was used in chemical ODN synthesis. The isolated DNA sequences were then enzymatically digested into monomers and analysed with RP-HPLC. B: RP-HPLC analysis of enzymatically digested ODN 1; C: RP-HPLC analysis of enzymatically digested ODN 2; D: RP-HPLC analysis of enzymatically digested ODN 3; blue line: absorption at 260 nm; orange line: percentage of buffer B; peak 1: dC; peak 2: dG + T; peak 3: 3'-azido A; horizontal axis: buffer volume (ml); primary vertical axis: absorption (mAU); secondary vertical axis: percentage of buffer B. Enlarged versions of the individual chromatograms can be found in the SI (Figures S10–S12).

	Expected Ratio of A* to dC ^[a]	Detected Ratio of A* to dC ^[a]	Normalized Ratio of A* to dC ^[a]
ODN 1	1 to 4	1 to 4.4	0.91 to 1
ODN 2	1 to 6	1 to 6.4	0.96 to 1
ODN 3	1 to 8	1 to 8.7	0.92 to 1

[a] A* = 3'-azido modified adenosine.

If the azido group is inherently unstable during the chemical ODN synthesis, the detected ratio should clearly deviate from the expected one, and the discrepancy should become more pronounced with increasing sequence length.

Peak areas corresponding to 3'-azido A and dC (Figure 1B–D) were integrated and divided by the extinction coefficients of the respective nucleosides. In the absence of an exact extinction coefficient for 3'-azidoadenosine the coefficient of adenosine was used as an approximation.^[30] Furthermore, for the sake of comparability, the calculated values for the dC peaks were normalized to the amount of dC in the respective ODN. Thus, ideally, the normalized ratio between deoxycytidine and 3'-azidoadenosine peaks should be 1 to 1. A detailed description of the analysis and all the performed calculations is provided in the SI (Tables S1–S3).

As shown in Table 2, the detected ratio of the two peaks for 3'-azidoadenosine (A*) and dC nearly matches the expected one, although the data shows a slightly smaller amount of 3'-azidoadenosine than expected. However, this appears to be a general phenomenon in all three samples, possibly resulting from the approximate extinction coefficient for 3'-azidoadenosine used for normalization of peak areas (see above). Most importantly, there is no trend that would indicate increased decomposition with increasing lengths of the oligonucleotide. On the contrary, the best result with a 3'-azido:dC ratio of

0.96:1 was obtained for the 16mer sequence (ODN2), while for the 11mer and the 21mer a slightly stronger deviation from the 1:1 ratio is observed (Table 1 and 2). In summary, this indicates, that there is no significant reduction of the 3'-terminal azide by the iterative treatment, in the individual coupling steps, of the growing oligonucleotide chain with phosphoramidite solution. Thus, the 3'-terminal azido function is largely stable under the conditions of ON synthesis by the standard phosphoramidite approach.

Conclusion

The suitability of solid supports loaded with 2'- or 3'-azidonucleosides for oligonucleotide synthesis by the phosphoramidite method has long been controversial. It has been reported that phosphoramidites of azido-modified nucleosides are inherently unstable, and this was considered to be a problem that would apply to any azidonucleoside that iteratively interacts with phosphoramidites during ON synthesis. There are very few reports on the synthesis of 3'-azido modified oligonucleotides by the phosphoramidite approach.^[22–24] However, there is a lack of information on the stability of the azide and the extent of possible decomposition by reduction. Here we have shown that a 3'-azido nucleoside linked to CPG is an excellent starting material for oligonucleotide synthesis by the phosphoramidite method, and we concluded that this applies also to 2'-azido nucleosides. The azido function remains largely stable during chain extension, regardless of the number of coupling cycles and thus the number of repeated contacts with phosphoramidites, which could act as reducing agents. Thus, we have provided evidence that oligonucleotides with 3'-terminal azide can be synthesized by the standard phosphoramidite approach without the need for special protocols such as enzymatic or postsynthetic conjugation of an azide functionality or ON synthesis in the reverse direction. Furthermore, we have provided protocols for the synthesis of three different sugar azido modified nucleosides and their immobilization onto CPG. These materials can be used for the synthesis of DNA or RNA fragments with 3'-terminal C2'- or C3'-azide. Such modified nucleic acids are important synthons, well suited for click ligation to an alkyne-modified nucleic acid^[22] or click conjugation of other functionalities, such as for example dyes or spin probes.^[1]

Experimental Section

General

Unless otherwise stated, all reactions were carried out under argon atmosphere and all reagents were bought from commercial suppliers. Dry solvents for reactions were obtained commercially (DMF, CH₂Cl₂, pyridine) and used without further purification. Toluene was dried over CaH₂ and distilled onto 3 Å molecular sieve. ¹H, ¹³C and ¹⁹F NMR spectra were recorded on a Bruker Avance III 400 or 500 MHz instrument or a Bruker Avance Neo 600 MHz spectrometer. The chemical shifts were referenced to the residual

proton signal of the deuterated solvents relative to TMS: DMSO- d_6 (2.50 ppm) for ^1H NMR spectra; DMSO- d_6 (39.52 ppm) for ^{13}C NMR spectra. ^{19}F NMR spectra were indirectly referenced to CCl_3F .^[31] ^1H and ^{13}C assignments were based on COSY, HSQC and HMBC experiments. MS measurements were performed on a Bruker microflex MALDI-ToF MS with 3-hydroxypyridine-2-carboxylic acid (HPA) being used as a matrix.

Synthesis of 6-*N*-isobutyryl-9- β -D-arabinofuranosyladenine (2): 9- β -D-arabinofuranosyladenine **1** (1.6 g, 6 mmol) was co-evaporated three times with 12 ml dry pyridine and suspended in 42 ml dry pyridine. The solution was cooled to 0 °C and TMS-Cl (3.8 ml, 30 mmol, 5 eq.) was added. The solution was stirred for 1 h at room temperature under argon. The reaction mixture was again cooled to 0 °C and isobutyryl chloride (1.26 ml, 12 mmol, 2 eq.) was added and the mixture was stirred overnight at room temperature, under argon atmosphere. The following day the mixture was cooled to 0 °C for a final time and H_2O and concentrated ammonia solution (9 ml respectively) were added dropwise to the solution. The mixture was stirred for 30 min at room temperature after which the solvents were removed. The oily residue was co-evaporated three times with 10 ml toluene and purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5→90:10). The product **2** (1.49 g 4.42 mmol, 74%) was isolated as white foam. $R_f=0.20$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); ^1H NMR (600 MHz, DMSO- d_6) δ 10.65 (s, 1H, H-N(6)), 8.58 (s, 1H, H-C(2)), 8.57 (s, 1H, H-C(8)), 7.35 (m, 2H, DMT H-C(ar)), 7.22 (m, 7H, DMTr H-C(ar)), 6.82 (m, 4H, DMTr H-C(ar)), 6.03 (d, $J=4.6$ Hz, 1H, H-C(1')), 5.60 (d, $J=5.6$ Hz, 1H, HO-C(2')), 5.26 (d, $J=5.8$ Hz, 1H, HO-C(3')), 4.76 (q, $J=5.1$ Hz, 1H, H-C(2')), 4.33 (q, $J=5.4$ Hz, 1H, H-C(3')), 4.10 (q, $J=4.7$ Hz, 1H, H-C(4')), 3.72 (2 s, 6H, O- CH_3), 3.23 (m, 2H, H₂-C(5')), 2.96 (sept, $J=6.8$ Hz, 1H, -CH-(CH_3)₂), 1.13 (dd, $J_1=0.9$ Hz, $J_2=6.8$ Hz, 6H, -CH-(CH_3)₂); ^{13}C NMR (150 MHz, DMSO- d_6): δ 175.31 (C=O), 158.03 (C(ar)-DMTr), 158.01 (C(ar)-DMTr), 151.69 (C(6)), 151.65 (C(2)), 149.87 (C(4)), 144.82 (C(ar)-DMTr), 142.92 (C(8)), 135.54, 135.49, 129.68, 129.64, 127.76, 127.65, 126.63 (C(ar)-DMTr), 124.13 (C(5)), 113.12, 113.11 (C(ar)-DMTr), 88.18 (C(1')), 85.46 (tert. C-DMTr), 83.20 (C(4')), 72.91 (C(2')), 70.30 (C(3')), 63.66 (C(5')), 55.00, 54.99 (OCH₃), 34.34 (CH-(CH_3)₂), 19.24, 19.22 (CH-(CH_3)₂); m/z calcd for $\text{C}_{14}\text{H}_{19}\text{N}_5\text{O}_5 + \text{H}^+$: 338.15 [$M + \text{H}$]⁺; found: 338.61.

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-6-*N*-isobutyryl-9- β -D-arabino-furanosyl-adenine (3): Compound **2** (683 mg, 2 mmol) was co-evaporated three times with dry pyridine and suspended in 15 ml dry pyridine. 4,4'-dimethoxytrityl chloride (900 mg, 2.65 mmol, 1.3 eq.) was added in two portions over 1 h. The reaction was carried out overnight. The next day the reaction was quenched with 12 ml methanol and the solvents were removed (not to complete dryness). The residue was diluted with CH_2Cl_2 and added to 40 ml saturated NaHCO_3 solution. The aqueous phase was extracted three times with 30 ml CH_2Cl_2 . The organic phases were pooled, dried over Na_2SO_4 and evaporated. The residue was co-evaporated twice with 12 ml toluene and the crude product was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 98:2→95:5). The product (694 mg, 1.08 mmol, 53%) was isolated as white foam. $R_f=0.35$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); ^1H NMR (600 MHz, DMSO- d_6) δ 10.65 (s, 1H, H-N(6)), 8.58 (s, 1H, H-C(2)), 8.57 (s, 1H, H-C(8)), 7.35 (m, 2H, DMTr H-C(ar)), 7.22 (m, 7H, DMTr H-C(ar)), 6.82 (m, 4H, DMTr H-C(ar)), 6.03 (d, $J=4.6$ Hz, 1H, H-C(1')), 5.60 (d, $J=5.6$ Hz, 1H, HO-C(2')), 5.26 (d, $J=5.8$ Hz, 1H, HO-C(3')), 4.76 (q, $J=5.1$ Hz, 1H, H-C(2')), 4.33 (q, $J=5.4$ Hz, 1H, H-C(3')), 4.10 (q, $J=4.7$ Hz, 1H, H-C(4')), 3.72 (2 s, 6H, O- CH_3), 3.23 (m, 2H, H₂-C(5')), 2.96 (sept, $J=6.8$ Hz, 1H, -CH-(CH_3)₂), 1.13 (dd, $J_1=0.9$ Hz, $J_2=6.8$ Hz, 6H, -CH-(CH_3)₂), 6H, -CH-(CH_3)₂); ^{13}C NMR (150 MHz, DMSO- d_6): δ 175.31 (C=O), 158.03 (C(ar)-DMTr), 158.01 (C(ar)-DMTr), 151.69 (C(6)), 151.65 (C(2)), 149.87 (C(4)), 144.82 (C(ar)-DMTr), 142.92 (C(8)), 135.54, 135.49, 129.68, 129.64, 127.76, 127.65, 126.63 (C(ar)-DMTr), 124.13 (C(5)), 113.12, 113.11 (C(ar)-DMTr), 88.18 (C(1')), 85.46 (tert.

C-DMTr), 83.20 (C(4')), 72.91 (C(2')), 70.30 (C(3')), 63.66 (C(5')), 55.00, 54.99 (OCH₃), 34.34 (CH-(CH_3)₂), 19.24, 19.22 (CH-(CH_3)₂); m/z calcd for $\text{C}_{35}\text{H}_{37}\text{N}_5\text{O}_7 + \text{Na}^+$: 662.68 [$M + \text{Na}$]⁺; found: 662.95.

Synthesis of 2',3'-anhydro-5'-O-(4,4'-dimethoxytrityl)-6-*N*-isobutyryl-9- β -D-lyxofuranosyl-adenine (4): Compound **3** (694 mg, 1.08 mmol) and triphenylphosphine (425 mg, 1.62 mmol, 1.85 eq.) were co-evaporated two times with 12 ml dry CH_2Cl_2 and dissolved in 15 ml dry DMF. The solution was heated to 55 °C and DIAD (320 μl , 1.62 mmol, 1.85 eq.) was added dropwise. The reaction proceeded for 1 h, during which the colour of the solution turned from orange to yellow. After the reaction was concluded, the solvent was removed under vacuum. The oily residue was dissolved in 30 ml CH_2Cl_2 and added to 40 ml saturated NaHCO_3 solution. The aqueous phase was extracted three times with 30 ml CH_2Cl_2 . The organic phases were pooled, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1→98:2). The product (567 mg, 0.91 mmol, 85%) was isolated as white foam. $R_f=0.50$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); ^1H NMR (600 MHz, DMSO- d_6) δ 10.71 (s, 1H, H-N(6)), 8.69 (s, 1H, H-C(2)), 8.31 (s, 1H, H-C(8)), 7.41 (m, 2H, DMTr H-C(ar)), 7.28 (m, 7H, DMTr H-C(ar)), 6.86 (m, DMTr H-C(ar)), 6.45 (d, $J=0.8$ Hz, 1H, H-C(1')), 4.36 (m, 2H H-C(2')/H-C(4')), 4.27 (m, 1H, H-C(3')), 3.73 (2 s, $J=1.3$ Hz, 6H, O- CH_3), 3.25 (m, 2H, H₂-C(5')), 2.96 (sept, $J=6.9$ Hz, 1H, -CH-(CH_3)₂), 1.13 ($J_1=0.9$ Hz, $J_2=6.9$ Hz, 6H, -CH-(CH_3)₂); ^{13}C NMR (150 MHz, DMSO- d_6) δ 175.30 (C=O), 158.12, 158.11 (C(ar)-DMTr), 152.10 (C(6)), 151.68 (C(2)), 149.90 (C(4)), 144.73 (C(ar)-DMTr), 141.60 (C(8)), 135.38, 135.33, 131.51, 131.45, 129.71, 129.69, 127.84, 127.65, 126.73 (C(ar)-DMTr), 123.13 (C(5)), 113.20 (C(ar)-DMTr), 85.70 (tert. C-DMTr), 80.74 (C(1')), 76.65 (C(4')), 62.39 (C(5')), 56.94 (C(3')), 56.27 (C(2')), 55.02 (OCH₃), 34.35 (CH-(CH_3)₂), 19.24, 19.21 (CH-(CH_3)₂); m/z calcd for $\text{C}_{35}\text{H}_{35}\text{N}_5\text{O}_6 + \text{H}^+$: 622.27 [$M + \text{H}$]⁺; found: 622.09.

Synthesis of 3'-azido-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-6-*N*-isobutyryl-9- β -D-arabinofuranosyl-adenine (5): Compound **4** (567 mg, 0.91) was co-evaporated twice with 12 ml dry CH_2Cl_2 and dissolved in 12 ml dry DMF. The solution was heated to 55 °C and LiN_3 (223 mg, 4.56 mmol, 5 eq.) was added. After 3 h the solvent was removed under vacuum. The oily residue was dissolved in 30 ml CH_2Cl_2 and added to 20 ml saturated NaHCO_3 solution. The aqueous phase was extracted three times with 30 ml CH_2Cl_2 . The organic phases were pooled, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 99:1→98:2). The product (224 mg, 0.37 mmol, 40%) was isolated as white foam. $R_f=0.40$ ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5); ^1H NMR (600 MHz, DMSO- d_6) δ 10.64 (s, 1H, H-N(6)), 8.59 (s, 1H, H-C(2)), 8.46 (s, 1H, H-C(8)), 7.37 (m, 2H, DMTr H-C(ar)), 7.24 (m, 7H, DMTr H-C(ar)), 6.83 (m, 4H, DMTr H-C(ar)), 6.41 (d, $J=6.5$ Hz, 1H, H-C(1')), 6.19 (d, $J=5.5$ Hz, 1H, HO-C(2')), 4.63 (m, 1H, H-C(2')), 4.53 (t, $J=8.2$ Hz, 1H, H-C(3')), 3.96 (m, 1H, H-C(4')), 3.72 (2 s, 6H, OCH₃), 3.37 (m, 1H, H₃-C(5')), 3.28 (m, 1H, H₂-C(5')), 2.97 (sept, $J=6.8$ Hz, 1H, -CH-(CH_3)₂), 1.13 (dd, $J_1=1.4$ Hz, $J_2=6.9$ Hz, 6H, -CH-(CH_3)₂); ^{13}C NMR (150 MHz, DMSO- d_6) δ 175.31 (C=O), 158.09, 158.07 (C(ar)-DMTr), 151.99 (C(6)), 151.59 (C(2)), 149.68 (C(4)), 144.61 (C(ar)-DMTr), 143.42 (C(8)), 135.33, 135.30, 129.67, 129.64, 127.81, 127.65, 126.73 (C(ar)-DMTr), 123.41 (C(5)), 113.17, 113.15 (C(ar)-DMTr), 85.70 (tert. C-DMTr), 82.52 (C(1')), 78.28 (C(4')), 74.14 (C(2')), 64.85 (C(3')), 63.30 (C(5')), 55.00 (OCH₃), 34.33 (CH-(CH_3)₂), 19.26, 19.23 (CH-(CH_3)₂); m/z calcd for $\text{C}_{35}\text{H}_{35}\text{N}_5\text{O}_6 + \text{H}^+$: 665.28 [$M + \text{H}$]⁺; found: 665.56.

Synthesis of 3'-azido-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-6-*N*-isobutyryl-adenine (6): Compound **5** (224 mg, 0.37 mmol) and 4-dimethylaminopyridine (DMAP) (136 mg, 1.11 mol, 3 eq.) were co-evaporated two times with dry CH_2Cl_2 and dissolved in 20 ml dry CH_2Cl_2 . Trifluoromethanesulfonyl chloride (53 μl , 0.56 mmol, 1.5 eq.) was added dropwise at 0 °C. The reaction proceeded for 30 min at

0°C after which the solution was diluted with 30 ml CH₂Cl₂ and treated with 20 ml saturated NaHCO₃ solution. The aqueous phase was extracted three times with 30 ml CH₂Cl₂. The organic phases were pooled, dried and evaporated. The residue was dissolved in 20 ml dry toluene and potassium trifluoroacetate (281 mg, 1.85 mmol, 5 eq.) as well as 18-crown-6 (196 mg, 0.74 mmol, 2 eq.) and DIPEA (96 μl, 0.56 mmol, 1.5 eq.) were added. The solution was heated to 60°C and the reaction proceeded for 2 h. The solvent was removed under vacuum. The residue was dissolved in 20 ml CH₂Cl₂ and added to 20 ml saturated NaHCO₃ solution. The aqueous phase was extracted three times with 20 ml CH₂Cl₂. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (CH₂Cl₂/MeOH, 99:1→98:2). The product (158 mg, 0.24 mmol, 64%) was isolated as white foam. *R*_f=0.40 (CH₂Cl₂/MeOH 95:5); ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.67 (s, 1H, H-N(6)), 8.59 (s, 1H, H-C(2)), 8.58 (s, 1H, H-C(8)), 7.33 (m, 2H, DMTr H-C(ar)), 7.22 (m, 7H, DMTr H-C(ar)), 6.82 (m, 4H, DMTr H-C(ar)), 6.39 (d, *J*=5.4 Hz, 1H, HO-C(2')), 6.05 (d, *J*=4.3 Hz, 1H, H-C(1')), 5.18 (q, *J*=5.1 Hz, 1H, H-C(2')), 4.47 (t, *J*=5.7 Hz, 1H, H-C(3')), 4.15 (m, 1H, H-C(4')), 3.72 (2 s, 6H, OCH₃), 3.27 (m, 2H, H₂-C(5')), 2.96 (sept, *J*=6.9 Hz, 1H CH-(CH₃)₂), 1.13 (dd, *J*₁=0.6 Hz, *J*₂=6.9 Hz, 6H, CH-(CH₃)₂); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 175.31 ((C=O)), 158.08, 158.06 (C(ar)-DMTr), 151.72 (C(6)), 151.61 (C(2)), 149.95 (C(4)), 144.66 (C(ar)-DMTr), 142.97 (C(8)), 135.34, 129.66, 129.63, 127.80, 127.61, 126.70 (C(ar)-DMTr), 124.12 (C(5)), 113.16 (C(ar)-DMTr), 88.35 (C(1')), 85.68 (tert. C-DMTr), 80.29 (C(4')), 73.52 (C(2')), 63.16 (C(5')), 61.02 (C(3')), 55.00 (OCH₃), 34.34 (CH-(CH₃)₂), 19.23, 19.21 (CH-(CH₃)₂); *m/z* calcd for C₃₃H₃₆N₆O₆ + H⁺: 665.28 [M + H]⁺; found: 665.10.

Synthesis of 3'-azido-3'-deoxy-2'-O-[(1,6-dioxo-6-(pentafluorophenyl-oxy)hexyl]-5'-O-(4,4'-dimethoxytrityl)-6-N-isobutyryl-adenosine (7): Compound 6 (158 mg, 0.24 mmol) and DMAP (29 mg, 0.24, 1 eq.) were co-evaporated twice with 6 ml dry CH₂Cl₂ and dissolved in 4 ml dry DMF/pyridine (1:1). The dipentafluorophenyl adipic acid ester (561 mg, 1.17 mmol, 4.9 eq.) was added. The solution was stirred for 2 h, after which the solvent was removed under vacuum. The oily residue was co-evaporated twice with dry CH₂Cl₂ and then immediately purified via column chromatography (CH₂Cl₂/acetone, 96:4). The product (154 mg, 0.14 mmol, 58%) was isolated as white crystals. *R*_f=0.20 (CH₂Cl₂/acetone 96:4); ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.70 (s, 1H, H-N(6)), 8.63 (s, 1H, H-C(2)), 8.62 (s, 1H, H-C(8)), 7.30 (m, 2H, DMTr-H-C(ar)), 7.20 (m, 7H, DMTr-H-C(ar)), 6.80 (m, 4H, DMTr-H-C(ar)), 6.30 (d, *J*=3.1 Hz, 1H, H-C(1')), 6.18 (dd, *J*₁=3.1 Hz, *J*₂=5.7 Hz, 1H, H-C(2')), 5.03 (dd, *J*₁=5.7 Hz, *J*₂=7.6 Hz, 1H, H-C(3')), 4.15 (m, 1H, H-C(4')), 3.71 (2 s, 6H, O-CH₃), 3.26 (m, 2H, H₂-C(5')), 2.95 (sept, *J*=6.8 Hz, 1H, CH-(CH₃)₂), 2.81 (t, *J*=6.9 Hz, 2H, CH₂), 1.69 (m, 4H, CH₂), 1.12 (d, *J*=6.8 Hz, 6H, CH-(CH₃)₂); ¹³C NMR (150 MHz, DMSO-*d*₆) δ 175.28, 171.73, 162.30 (C=O), 158.08 (C(ar)-DMTr), 151.89 (C(2)), 151.35 (C(4)), 150.03 (C(6)), 144.53 (C(ar)-DMTr), 142.93 (C(8)), 135.25 (C(ar)-DMTr), 129.61, 127.78, 127.56, 126.70 (C(ar)-DMTr), 123.95 (C(5)), 113.14 (C(ar)-DMTr), 86.44 (C(1')), 85.68 (tert. C-DMTr), 80.56 (C(4')), 74.48 (C(2')), 62.07 (C(5')), 59.57 (C(3')), 54.99 (OCH₃), 34.35 (CH-(CH₃)₂), 32.62 (CH₂), 32.08 (CH₂), 23.46 (CH₂), 23.32 (CH₂), 19.20 (CH-(CH₃)₂); ¹⁹F NMR (565 MHz, DMSO-*d*₆) δ -153.58 (F-C(ar)), -158.07 (F-C(ar)), -162.62 (F-C(ar)); *m/z* calcd for C₄₇H₄₃F₅N₆O₉ + H⁺: 959.31 [M + H]⁺; found: 959.49.

Synthesis of 3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-6-N-isobutyryl-9-β-D-arabinofuranosyl-adenine (8): Compound 2 (1.48 g 4.4 mmol) was co-evaporated three times with 12 ml dry pyridine and dissolved in 36 ml dry pyridine. 1,3-dichloro-1,1,3,3-tetraisopropyl-disiloxane (1.69 ml, 5.28 mmol, 1.2 eq.) was added and the solution was stirred overnight. After the reaction was completed the solvent was removed and the oily residue was co-evaporated twice with 10 ml toluene. The oily crude product was

diluted with CH₂Cl₂ and added to 40 ml of saturated NaHCO₃-Solution. The aqueous phase was extracted 3 times with 30 ml CH₂Cl₂. The organic phases were pooled, washed with 40 ml H₂O and dried over Na₂SO₄. The solvent was removed and the crude product was purified by column chromatography (CH₂Cl₂/MeOH 98:2). The product (1.7 g, 2.93 mmol 67%) was isolated as white foam. *R*_f=0.35 (CH₂Cl₂/MeOH 95:5); ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.64 (s, 1H, H-N(6)), 8.61 (s, 1H, H-C(2)), 8.31 (s, 1H, H-C(8)), 6.35 (d, *J*=6.3 Hz, 1H, H-C(1')), 5.82 (d, *J*=5.7 Hz, 1H, HO-C(2')), 4.58 (m, 2H, H-C(2')/H-C(3')), 4.14 (m, 1H, H₃-C(5')), 3.95 (m, 1H, H₅-C(5')), 3.84 (m, 1H, H-C(4')), 2.97 (sept, *J*=6.8 Hz, 1H, (CH)-(CH₃)₂), 1.17-0.94 (m, 34H, CH-(CH₃)₂/(CH)₄(CH₃)₈OSi₂); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 175.30 (C=O), 151.95 (C(4)), 151.45 (C(2)), 149.70 (C(6)), 142.81 (C(8)), 123.51 (C(5)), 82.13 (C(1')), 79.65 (C(4')), 75.32 (C(2')), 74.91 (C(3')), 61.52 (C(5')), 34.34 (CH-(CH₃)₂), 19.26, 19.24 (CH-(CH₃)₂), 17.37, 17.25, 17.24, 17.21, 16.94, 16.90, 16.84, 16.79 ((CH)₄(CH₃)₈OSi₂), 12.78, 12.45, 12.27, 11.98 ((CH)₄(CH₃)₈OSi₂); *m/z* calcd for C₂₆H₄₅N₅O₆Si₂ + H⁺: 580.30 [M + H]⁺; found: 580.47.

Synthesis of 3',5'-O-(1,1,3,3-tetraisopropylidisiloxane-1,3-diyl)-2'-O-[(trifluoromethyl)sulfonyl]-6-N-isobutyryl-9-β-D-arabinofuranosyl-adenine (9): Compound 8 (1.39 g, 2.4 mmol) and 440 mg DMAP (3.6 mmol, 1.5 eq.) were co-evaporated three times with 16 ml dry CH₂Cl₂ and dissolved in 100 ml dry CH₂Cl₂. The solution was cooled to 0°C and triethylamine (840 μl, 6 mmol, 2.5 eq.) was added. The reaction was started by the addition of trifluoromethanesulfonyl chloride (385 μl, 3.6 mmol, 1.5 eq.). The reaction mixture was stirred for 30 min at 0°C under argon. The solution was diluted with CH₂Cl₂, washed with 40 ml saturated NaHCO₃-Solution dried over Na₂SO₄ and evaporated. The crude product was used in the next step without further purification.

Synthesis of 2'-azido-2'-deoxy-3',5'-O-(1,1,3,3-tetraisopropyl-disiloxane-1,3-diyl)-6-N-isobutyryl-adenosine (10): The intermediate 9 (1.07 g, 1.5 mmol) was dissolved in dry DMF and treated with LiN₃ (367 mg, 7.5 mmol, 5 eq.). The solution was stirred for 16 h at room temperature. The solvent was removed under high vacuum. The oily residue was co-evaporated twice with toluene, dissolved in 40 ml ethyl acetate and added to 40 ml of saturated NaHCO₃-solution. The aqueous phase was extracted three times with 40 ml of ethyl acetate. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (hexane/ethyl acetate 1:1). The product (817 mg, 1.35 mmol, 90%) was isolated as a white foam. *R*_f=0.45 (CH₂Cl₂/MeOH 95:5); ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.69 (s, 1H, H-N(6)), 8.56 (s, 1H, H-C(2)), 8.52 (s, 1H, H-C(8)), 5.93 (d, *J*=1.4 Hz, 1H, H-C(1')), 5.41 (dd, *J*₁=5.9 Hz, *J*₂=8.35 Hz, 1H, H-C(2')), 5.09 (dd, *J*₁=1.4 Hz, *J*₂=5.9 Hz, 1H), 4.06-3.95 (m, 3H, H-C(4')/H₂-C(5')), 2.95 (sept, *J*=6.8 Hz, 1H, CH(CH₃)₂), 1.17-1.01 (m, 34H, CH-(CH₃)₂/(CH)₄(CH₃)₈OSi₂); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 175.30 (C=O), 151.45 (C(2)), 150.99 (C(4)), 150.03 (C(6)), 143.18 (C(8)), 124.36 (C(5)), 86.37 (C(1')), 81.05 (C(4')), 71.66 (C(2')), 64.42 (C(3')), 60.55 (C(5')), 34.35 (CH-(CH₃)₂), 19.24, 19.21 (CH-(CH₃)₂), 17.28 17.16, 17.14, 17.13, 17.07, 16.90, 16.81, 16.76 ((CH)₄(CH₃)₈OSi₂), 12.73, 12.36, 12.18, 12.15 ((CH)₄(CH₃)₈OSi₂); *m/z* calcd for C₂₆H₄₄N₈O₅Si₂ + H⁺: 605.31 [M + H]⁺; found: 605.14.

Synthesis of 2'-azido-2'-deoxy-6-N-isobutyryl-adenosine (11): Compound 10 (817 mg, 1.35 mmol) was dissolved in 5.3 ml 1 M tetrabutylammonium fluoride in THF and acetic acid (153 μl, 2.57 mmol 1.9 eq.) was immediately added to the solution. The mixture was stirred for 30 min at room temperature under argon. The solvent was evaporated and the residue was co-evaporated twice with 15 ml CH₂Cl₂. The crude product was purified by column chromatography (CH₂Cl₂/MeOH 97:3). The product (345 mg, 0.96 mmol, 71%) was isolated as a white foam. *R*_f=0.30 (CH₂Cl₂/MeOH 95:5); ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.68 (s, 1H, H-N(6)), 8.69 (s, 1H, H-C(8)), 8.67 (s, 1H, H-C(2)), 6.14 (d, *J*=5.6 Hz, 1H,

H-C(1')), 6.07 (d, $J=5.4$ Hz, 1H, HO-C(3')), 5.15 (t, $J=5.5$ Hz, 1H, HO-C(5')), 4.71 (t, $J=5.4$ Hz, 1H, H-C(2')), 4.59 (q, $J=5.0$ Hz, 1H, H-C(3')), 4.01 (q, $J=4.0$ Hz, 1H, H-C(4')), 3.70 (m, 1H, H_a-C(5')), 3.59 (m, 1H, H_b-C(5')), 2.96 (m, $J=6.8$ Hz, 1H, CH(CH₃)₂), 1.13 (d, $J=6.8$ Hz, 6H, CH(CH₃)₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 175.34 (C=O), 151.87 (C(2)), 151.58 (C(4)), 149.96 (C(6)), 142.42 (C(8)), 124.08 (C(5)), 85.88 (C(4')), 85.30 (C(1')), 70.86 (C(3')), 64.48 (C(2')), 60.72 (C(5)), 34.35 (CH(CH₃)₂), 19.24, 19.22 (CH(CH₃)₂); *m/z* calcd for C₁₄H₁₈N₈O₄ + H⁺: 363.15 [M + H]⁺; found: 363.53;

Synthesis of 2'-azido-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-6-N-isobutyryl-adenosine (12): Compound 11 (380 mg, 1.05 mmol) was co-evaporated twice with 10 ml dry CH₂Cl₂ and dissolved in 8 ml dry pyridine. DMTr-Cl (482 mg, 1.42 mmol, 1.35 eq.) was added to the solution in three portions over 1.5 h. After 3 h the reaction was quenched with 2 ml MeOH and the solvents were evaporated. The residue was dissolved in 15 ml CH₂Cl₂ and washed with 10 ml saturated NaHCO₃ solution. The organic phase was dried over Na₂SO₄ and the solvent was evaporated. The oily residue was co-evaporated twice with CH₂Cl₂. The crude product was purified by column chromatography (CH₂Cl₂/MeOH 98:2→95:5). The product (400 mg, 0.6 mmol, 57%) was isolated as a white foam. *R*_f = 0.40 (CH₂Cl₂/MeOH 95:5); ¹H NMR (400 MHz, DMSO-d₆) δ 10.69 (s, 1H, H-N(6)), 8.61, 8.60 (2 s, 2H, H-C(2)/H-C(8)), 7.32 (m, 2H, H-DMTr), 7.20 (m, 7H, H-DMTr), 6.80 (m, 4H, H-DMTr), 6.12 (d, $J=4.2$ Hz, 1H, H-C(1')), 6.07 (d, $J=5.7$ Hz, 1H, HO-C(3')), 4.96 (m, 1H, H-C(2')), 4.82 (q, $J=5.8$ Hz, 1H, H-C(3')), 4.10 (m, 1H, H-C(4')), 3.71 (2 s, 6H, OCH₃), 3.24 (m, 2H, H₂-C(5')), 2.97 (sept, $J=6.8$ Hz, 1H, CH(CH₃)₂), 1.13 (d, $J=6.8$ Hz, 1H, CH(CH₃)₂); ¹³C NMR (100 MHz, DMSO-d₆) δ 175.32 (C=O), 158.03, 158.01 (C(ar)-DMTr), 151.77 (C(2)), 151.37 (C(4)), 150.00 (C(6)), 144.75 (C(ar)-DMTr), 143.07 (C(8)), 135.45, 135.43, 129.67, 129.63, 127.74, 127.64, 126.63 (C(ar)-DMTr), 124.13 (C(5)), 113.11, 113.09 (C(ar)-DMTr), 86.09 (C(1')), 85.46 (tert. C-DMTr), 83.16 (C(4')), 70.79 (C(3')), 63.99 (C(2')), 63.05 (C(5')), 54.99 (DMTr-OCH₃), 34.36 (CH(CH₃)₂), 19.24, 19.22 (CH(CH₃)₂); *m/z* calcd for C₃₅H₃₆N₈O₆ + H⁺: 665.28 [M + H]⁺; found: 665.08.

Synthesis of 2'-azido-2'-deoxy-3'-O-[1,6-dioxo-6-(pentafluorophenyl)hexyl]-5'-O-(4,4'-dimethoxytrityl)-6-N-isobutyryl-adenosine (13): Compound 12 (140 mg, 0.21 mmol) and DMAP (26 mg, 0.21 mmol, 1 eq.) were co-evaporated three times with 6 ml dry CH₂Cl₂ and dissolved in 4.8 ml dry DMF/pyridine (1:1). The dipentafluorophenyl adipic acid ester (495 mg, 1.03 mmol, 4.9 eq.) was added and the solution was stirred for 3 h at room temperature. The solvent was removed under high vacuum and the residue was co-evaporated twice with CH₂Cl₂. The crude product was immediately purified by column chromatography (CH₂Cl₂/acetone 96:4). The product (120 mg, 0.125 mmol, 60%) was isolated as white foam. *R*_f = 0.20 (CH₂Cl₂/acetone 96:4); ¹H NMR (600 MHz, DMSO-d₆) δ 10.72 (s, 1H, H-N(6)), 8.63 (s, 1H, H-C(8)), 8.55 (s, 1H, H-C(2)), 7.32 (m, 2H, H-DMTr), 7.21 (m, 7H, H-DMTr), 6.81 (m, 4H, H-DMTr), 6.15 (d, $J=6.1$ Hz, 1H, H-C(1')), 5.71 (dd, $J_1=4.4$ Hz, $J_2=5.8$ Hz, 1H, H-C(3')), 5.47 (t, $J=6.1$ Hz, 1H, H-C(2')), 4.26 (m, 1H, H-C(4')), 3.71 (2 s, 6H, OCH₃), 3.30 (m, 2H, H₂-C(5')), 2.96 (sept, $J=6.9$ Hz, 1H, CH(CH₃)₂), 2.83 (t, $J=7.1$ Hz, 2H, CH₂), 2.52 (t, 2H, CH₂), 1.71 (m, 4H, 2x CH₂), 1.13 (dd, $J_1=1.1$ Hz, $J_2=6.9$ Hz, 6H, CH(CH₃)₂); ¹³C NMR (150 MHz, DMSO-d₆) δ 175.32, 171.89, 169.38 (C=O), 158.08, 158.07 (C(ar)-DMTr), 151.79 (C(2)), 151.54 (C(4)), 150.10 (C(6)), 144.63 (C(ar)-DMTr), 143.23 (C(8)), 135.29, 129.66, 129.61, 127.75, 127.60, 126.68 (C(ar)-DMTr), 124.26 (C(5)), 113.10 (C(ar)-DMTr), 86.04 (C(1')), 85.71 (tert. C-DMTr), 81.37 (C(4')), 72.09 (C(3')), 62.91 (C(2')), 61.86 (C(5')), 54.99, 54.98 (DMTr-OCH₃), 34.36 (CH(CH₃)₂), 32.69 (CH₂), 32.10 (CH₂), 23.52 (CH₂), 23.40 (CH₂), 19.23, 19.20 (CH(CH₃)₂); ¹⁹F NMR (565 MHz, DMSO-d₆) δ -153.55 (F-C(ar)), -158.07 (F-C(ar)), -162.62 (F-C(ar)); *m/z* calcd for C₄₇H₄₃F₅N₈O₉ + H⁺: 959.31 [M + H]⁺; found: 959.83.

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-uridine (15): Uridine (976.8 mg, 4 mmol) was co-evaporated three times with dry CH₂Cl₂ and suspended in 30 ml dry pyridine. DMTr-Cl (1.36 g, 4 mmol, 1 eq.) was added in two portions over 1 h. The reaction was carried out overnight. The next day the reaction was quenched with 12 ml methanol and the solvents were removed. The residue was diluted with CH₂Cl₂ and added to 40 ml saturated NaHCO₃ solution. The aqueous phase was extracted three times with 30 ml CH₂Cl₂. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The residue was co-evaporated twice with 12 ml toluene and the crude product was purified by column chromatography (CH₂Cl₂/MeOH, 98:2→95:5). The product (1.46 g, 2.67 mmol, 67%) was isolated as white foam. *R*_f = 0.30 (CH₂Cl₂/MeOH 95:5); ¹H NMR (600 MHz, DMSO-d₆) δ 11.35 (s, 1H, H-N(3)), 7.71 (d, $J=8.1$ Hz, 1H, H-C(6)), 7.42–7.20 (m, 9H, DMTr H-C(ar)), 6.90 (m, 4H, DMTr H-C(ar)), 5.74 (d, $J=4.3$ Hz, 1H, H-C(1')), 5.5 (d, $J=4.8$ Hz, 1H, HO-C(2')), 5.30 (d, $J=8.1$ Hz, 1H, H-C(5)), 5.17 (d, $J=5.8$ Hz, 1H, HO-C(3')), 4.08 (m, 2H, H-C(2'), H-C(3')), 3.95 (m, 1H, H-C(4')), 3.74 (s, 6H, OCH₃), 3.23 (m, 2H, H₂-C(5)); ¹³C NMR (DMSO-d₆, 150 MHz): δ 163.13 (C(4)), 158.21 (C(ar)-DMTr), 150.56 (C(2)), 144.74 (C(ar)-DMTr), 140.73 (C(6)), 135.47, 135.23, 129.85, 128.01, 127.77, 126.89, 113.34 (C(ar)-DMTr), 101.53 (C(5)), 88.96 (C(1')), 85.93 (tert. C-DMTr), 82.48 (C(4')), 73.57 (C(3')), 69.64 (C(2')), 63.31 (C(5')), 55.14 (OCH₃); *m/z* calcd for C₃₀H₃₀N₂O₈ + Na⁺: 569.19 [M + Na]⁺; found: 569.51.

Synthesis of 2',3'-di-O-(methanesulfonyl)-5'-O-(4,4'-dimethoxytrityl)-uridine (16): Compound 15 (1.4 g, 2.56 mmol) was co-evaporated three times with 15 ml dry CH₂Cl₂ and dissolved in 15 ml dry pyridine. The solution was cooled to 0 °C and methanesulfonyl chloride (2.97 ml, 38.42 mmol, 15 eq.) was added. The reaction was stirred under argon at 0 °C for 30 min and for 1 h at room temperature after which the solution was again cooled to 0 °C, diluted with 40 ml CH₂Cl₂ and treated with 40 ml 5% NaHCO₃ solution for 10 min. During the next step the phases were separated and the aqueous phase was extracted three times with 40 ml CH₂Cl₂. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The oily residue was co-evaporated twice with 10 ml toluene. The crude product was purified by column chromatography (CH₂Cl₂/MeOH 99:1→98:2). The product (1.76 g, 2.5 mmol, 98%) was isolated as yellowish foam. *R*_f = 0.40 (CH₂Cl₂/MeOH 95:5); ¹H NMR (600 MHz, DMSO-d₆) δ 11.54 (s, 1H, H-N(3)), 7.72 (d, $J=8.1$ Hz, 1H, H-C(6)), 7.20–7.42 (m 9H, DMTr H-C(ar)), 6.89 (m, 4H, DMTr H-C(ar)), 5.96 (d, $J=3.7$ Hz, 1H, H-C(1')), 5.59 (m, 1H, H-C(2')), 5.38–5.46 (m, 2H, H-C(3'), H-C(5)), 4.26 (m, 1H, H-C(4')), 3.74 (s, 6H, OCH₃), 3.34–3.46 (m, 2 H, H₂-C(5')), 3.37 (s, 3 H, O-SO₂-CH₃), 3.27 (s, 3 H, O-SO₂-CH₃); ¹³C NMR (DMSO-d₆, 150 MHz): δ 163.02 (C(4)); 157.21 (C(ar)-DMTr), 150.56 (C(2)), 148.39 (C(ar)-DMTr), 140.24 (C(6)), 140.21, 128.89, 127.67, 127.41, 126.43, 112.78 (C(ar)-DMTr), 102.46 (C(5)), 85.98 (C(1')), 82.55 (C(4')), 79.92 (tert. C-DMTr), 76.77 (C(3')), 75.72 (C(2')), 59.48 (C(5')), 55.04 (OCH₃), 37.99, 37.97 (O-SO₂-CH₃); *m/z* calcd for C₃₂H₃₄N₂O₁₂ + Na⁺: 725.15 [M + Na]⁺; found: 725.53.

Synthesis of 2',3'-anhydro-5'-O-(4,4'-dimethoxytrityl)-1-β-D-lyxofuranosyl-uracil (17): Compound 15 (1.75 g, 2.49 mmol) was dissolved in 50 ml ethanol and 21 ml 1 M NaOH. The solution was heated to 60 °C and stirred for 1 h. During the next step the solution was cooled to 20 °C, adjusted to pH 7 and the solvent was evaporated to ca. 20 ml. Following this, the solution was diluted with 30 ml CH₂Cl₂ and treated with 30 ml saturated NaHCO₃ solution. The aqueous phase was extracted three times with 30 ml CH₂Cl₂. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (CH₂Cl₂/MeOH 99:1→98:2). The product (1.17 g, 2.2 mmol, 89%) was isolated as white foam. *R*_f = 0.40 (CH₂Cl₂/MeOH 95:5); ¹H NMR (600 MHz, DMSO-d₆) δ 11.45 (s, 1H, H-N(3)), 7.51 (d, $J=8.1$ Hz, 1H, H-C(6)), 7.20–7.42 (m 9H, DMTr H-C(ar)), 6.89 (m, 4H, DMTr

H–C(ar)), 6.10 (s, 1H, H–C(1')), 5.62 (d, $J=8.1$ Hz, 1H, H–C(5)), 4.24 (t, $J=5.7$ Hz, 1H, H–C(4')), 4.11 (m, 2H, H–C(2'), H–C(3')), 3.74 (s, 6H, OCH₃), 3.19 (m, 2 H, H₂-C(5')); ¹³C NMR (150 MHz, DMSO-d₆) δ 163.05 (C(4)), 158.03 (C(ar)-DMTr), 150.41 (C(2)), 144.69 (C(ar)-DMTr), 140.99 (C(6)), 135.45, 135.27, 129.71, 127.91, 127.64, 126.79, 113.26 (C(ar)-DMTr), 101.98 (C(5)), 85.73 (tert. C–DMTr) 80.94 (C(1')), 75.96 (C(4')), 62.21 (C(5')), 55.71 (C(3')), 55.58 (C(2')), 55.04 (OCH₃); *m/z* calcd for C₃₀H₂₈N₂O₇ + Na⁺: 551.18 [M + Na]⁺; found: 551.38.

Synthesis of 3'-azido-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-1-β-D-arabinofuranosyl-uracil (18): Compound **17** (1.75 g, 2.49 mmol) was dissolved in 12 ml dry DMF and heated to 55 °C. LiN₃ (233 mg, 4.75 mmol, 5 eq.) was added and the reaction solution was stirred for 20 h. After the reaction was completed the solvent was removed under high vacuum. The oily residue was dissolved in 30 ml CH₂Cl₂ and treated with 30 ml saturated NaHCO₃ solution. The aqueous phase was extracted three times with 30 ml CH₂Cl₂. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (CH₂Cl₂/MeOH 99:1→98:2). The product (247 mg, 0.42 mmol, 44%) was isolated as white foam. *R*_f = 0.40 (CH₂Cl₂/MeOH 95:5); ¹H NMR (600 MHz, DMSO-d₆) δ 11.34 (s, 1H, H–N(3)), 7.59 (d, $J=8.1$ Hz, 1H, H–C(6)), 7.20–7.42 (m 9H, DMTr H–C(ar)), 6.89 (m, 4H, DMTr H–C(ar)), 6.15 (d, $J=5.4$ Hz, 1H, HO–C(2')), 6.06 (d, $J=6.1$ Hz, 1H, H–C(1')), 5.37 (d, $J=8.1$ Hz, 1H, H–C(5)), 4.36 (dd, $J_1=6.19$ Hz, $J_2=10.23$ Hz, 1H, H–C(2')), 4.11 (m, 1H, H–C(3')), 3.83 (m, 1H, H–C(4')), 3.74 (s, 6H, OCH₃), 3.30 (m, 2 H, H₂-C(5')); ¹³C NMR (150 MHz, DMSO-d₆) δ 163.05 (C(4)), 158.18 (C(ar)-DMTr), 150.48 (C(2)), 144.55 (C(ar)-DMTr), 141.85 (C(6)), 135.25, 135.15, 129.74, 127.94, 127.64, 126.88, 113.29 (C(ar)-DMTr), 100.54 (C(5)), 85.96 (tert. C–DMTr), 83.38 (C(1')), 78.09 (C(4')), 73.91 (C(2')), 64.82 (C(3')), 62.17 (C(5')), 55.07 (OCH₃); *m/z* calcd for C₃₀H₂₉N₅O₇ + Na⁺: 594.20 [M + Na]⁺; found: 594.25.

Synthesis of 3'-azido-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-uridine (19): Compound **18** (270 mg, 0.47 mmol) and DMAP (172 mg, 1.41 mmol, 3 eq.) were co-evaporated three times with 12 ml dry CH₂Cl₂ and dissolved in 18 ml dry CH₂Cl₂. The solution was cooled to 0 °C and trifluoromethanesulfonyl chloride (76 μl, 0.71 mmol, 1.5 eq.) was added dropwise. After 15 min the solution was diluted with 20 ml CH₂Cl₂, washed with 30 ml saturated NaHCO₃ solution, dried over Na₂SO₄ and evaporated. The intermediate was dissolved in 20 dry toluene and treated with potassium trifluoroacetate (358 mg, 2.35 mmol, 5 eq.), 18-crown-6 (248 mg, 0.94 mmol, 2 eq.) and DIPEA (121 μl, 0.7 mmol, 1.5 eq.) The suspension was stirred for 2 h at 60 °C. After the reaction was completed the solvent was removed under high vacuum. The oily residue was dissolved in 30 ml CH₂Cl₂ and washed with 30 ml saturated NaHCO₃ solution. The phases were separated and the aqueous phase was extracted three times with 30 ml CH₂Cl₂. The organic phases were pooled, dried over Na₂SO₄ and evaporated. The crude product was purified by column chromatography (CH₂Cl₂/MeOH 99:1→98:2). The product (195 mg, 0.34 mmol, 73%) was isolated as white foam. *R*_f = 0.40 (CH₂Cl₂/MeOH 95:5); ¹H NMR (600 MHz, DMSO-d₆) δ 11.39 (s, 1H, H–N(3)), 7.69 (d, $J=8.1$ Hz, 1H, H–C(6)), 7.23–7.41 (m, 9H, DMTr H–C(ar)), 6.91 (m, 4H, DMTr H–C(ar)), 6.26 (d, $J=5.4$ Hz, 1H, HO–C(2')), 5.73 (d, $J=4.1$ Hz, 1H, H–C(1')), 5.35 (d, $J=8.1$ Hz, 1H, H–C(5)), 4.49 (m, 1H, H–C(2')), 4.17 (m, 1H, H–C(3')), 4.01 (m, 1H, H–C(4')), 3.74 (s, 6H, OCH₃), 3.23–3.31 (m, 2 H, H₂-C(5')). ¹³C NMR (150 MHz, DMSO-d₆) δ 162.97 (C(4)), 158.18 (C(ar)-DMTr), 150.48 (C(2)), 144.54 (C(ar)-DMTr), 140.53 (C(6)), 135.20, 134.99, 129.77, 127.96, 127.67, 126.85, 113.31, 113.29 (C(ar)-DMTr), 101.61 (C(5)), 89.03 (C(1')), 86.12 (tert. C–DMTr), 79.70 (C(4')), 73.98 (C(2')), 62.68 (C(5')), 60.30 (C(3')), 55.06 (OCH₃); *m/z* calcd for C₃₀H₂₉N₅O₇ + Na⁺: 594.20 [M + Na]⁺; found: 595.40.

Synthesis of 3'-azido-3'-deoxy-5'-O-(4,4'-dimethoxytrityl)-2'-O-[1,6-dioxo-6-(pentafluoro-phenyloxy)hexyl]-uridine (20): Compound **19** (190 mg, 0.33 mmol) and DMAP (41 mg, 0.33 mmol,

1 eq.) were co-evaporated three times with 10 ml dry CH₂Cl₂ and dissolved in 6 ml dry DMF/pyridine (1:1). Compound **7** (772 mg, 1.61 mmol, 4.89 eq.) was added and the solution was stirred for 3 h at room temperature under argon. The solvent was removed under high vacuum and the residue was co-evaporated twice with dry toluene. The crude product was immediately purified by column chromatography (CH₂Cl₂/acetone 96:4). The product (170 mg, 0.2 mmol, 54%) was isolated as white foam. *R*_f = 0.20 (CH₂Cl₂/acetone 96:4); ¹H NMR (600 MHz, DMSO-d₆) δ 11.44 (s, 1H, H–N(3)), 7.51 (d, $J=8.1$ Hz, 1H, H–C(6)), 7.23–7.41 (m, 9H, DMTr H–C(ar)), 6.91 (m, 4H, DMTr H–C(ar)), 5.83 (d, $J=3.2$ Hz, 1H, H–C(1')), 5.61 (dd, $J_1=3.2$ Hz, $J_2=5.8$ Hz, 1H, H–C(2')), 5.44 (d, $J=8.1$ Hz, 1H, H–C(5)), 4.69 (dd, $J_1=5.9$ Hz, $J_2=7.8$ Hz, 1 H, H–C(3')), 3.96 (m, 1H, H–C(4')), 3.73 (2 s, 6H, OCH₃), 3.26–3.31 (m, 2H, H₂-C(5')), 2.82 (t, $J=6.9$ Hz, 2H, CH₂), 1.69 (m, 4H, CH₂); 2.51 (m, 2H, CH₂). ¹³C NMR 150 MHz, DMSO-d₆) δ 171.68, 169.38 (C=O), 162.30 (C(4)), 158.18 (C(ar)-DMTr), 150.18 (C(2)), 144.46 (C(ar)-DMTr), 140.90 (C(6)), 135.11, 134.97, 129.74, 127.92, 127.66, 126.84, 113.27 (C(ar)-DMTr), 101.78 (C(5)), 87.94 (C(1')), 86.10 (tert. C–DMTr), 80.06 (C(4')), 74.66 (C(2')), 61.90 (C(5')), 59.23 (C(3')), 55.04 (OCH₃), 32.63 (CH₂), 32.09 (CH₂), 23.47, 23.40 (CH₂); ¹⁹F NMR (565 MHz, DMSO-d₆) δ –153.56 (F–C(ar)), –158.09 (F–C(ar)), –162.63 (F–C(ar)); *m/z* calcd for C₄₂H₃₆F₅N₅O₁₀ + Na⁺: 888.23 [M + Na]⁺; found: 888.59.

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Conflict of Interest

The authors declare no conflict of interest.

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