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SYNTHESIS OF CONFORMATIONALLY CONSTRAINED 2'-*N*,4'-*C*-ETHYLENE-BRIDGED ADENOSINE (aza-ENA-A)†

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Abstract – The synthesis of conformationally constrained 2'-*N*,4'-*C*-ethylene-bridged adenosine (aza-ENA-A), in which the pentose-sugar is cis-fused with the piperidino skeleton at C2' and C4' centres of the sugar ring, is reported. The corresponding phosphoramidite building block will be used for incorporation into oligo-DNA and -RNA by solid phase synthesis to examine their nuclease stability as well as their application in blocking the translation of the target RNA using the antisense and siRNA approach. 2-Aza-6-oxabicyclo[3.2.1]octane skeleton is assembled through multi-step synthetic manipulation of appropriately protected D-arabinose based sugar precursor. The conversion of appropriate arabino precursor to ribo counterpart was achieved by direct nucleophilic displacement of "*ara*" positioned 2-(trifluoromethanesulfonyloxy) group in the sugar precursor **8.** A high regio- and enhanced stereoselectivity with preferential formation of β anomer in glycosylation reaction was achieved using Vorbrüggen conditions in the absence of any 2-participating group. Coupling step was performed using 1-*O*-acetyl-3,5-di-*O*-benzyl-(2-deoxy-2-azido)-4-*C*-(*p*-toluoyloxyethyl)-D-ribofuranose (**10**) as a glycosyl donor and persilylated N^6-benzoyladenine. Finally, the ring-closure giving the *North*-type conformationally constrained *cis*-fused bicyclic aza-ENA-A have been confirmed unambiguously by the long range ^{1}H-^{13}C NMR correlation (HMBC), TOCSY, COSY and nOe experiments.

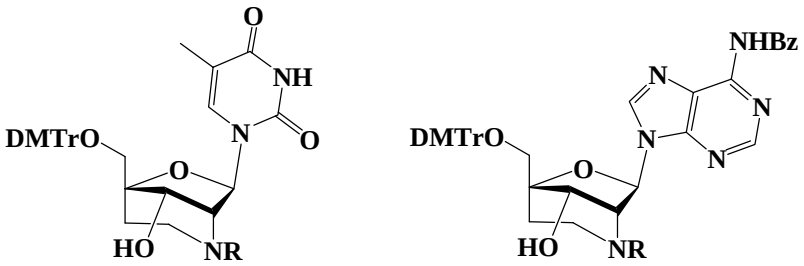
† In memoriam – Professor Ivar Ugi

INTRODUCTION

Antisense oligonucleotides (AONs) incorporated with sugar-modified nucleosides have been successfully used as valuable tools to inhibit gene expression by utilizing various mechanisms of actions.[1-7] Once bound to the target RNA, antisense agent either sterically blocks the synthesis of ribosomal proteins or induces RNase H mediated degradation of the target mRNA. For *in vivo* applications of the oligonucleotide-based approaches, appropriate chemical modifications are used to enhance target affinity, specificity, stability towards the endo- and exonucleases, as well as to achieve tissue-specific delivery in order to improve the overall pharmacokinetic properties.[2, 5, 8-11] Among these, the AONs modified with *North-East* conformationally (-1° < *P* < 34 °)[12] constrained (LNA, ENA, oxetanes and their aza-analogues)[10, 13-20] nucleotide blocks have unique abilities to dictate conformational pre-organization of the AON-RNA duplex to the rigid RNA-RNA type duplex which results in the modulation of the target affinity.[21-24]

We have previously reported synthesis of new 2'-*N*,4'-*C*-ethylene-bridged thymidine (aza-ENA-T) (2-aza-6-oxabicyclo[3.2.1]octane skeleton[20] as sugar part in compound **A** in Figure 1), which has been subsequently incorporated into AONs and their antisense properties have been evaluated *in vitro*. The thermal stability of duplexes involving aza-ENA-T was determined toward the complementary RNA and DNA. Single modification was incorporated at a time at different positions of the 15nt AON sequence, 3'-d(CTTCTTTTTTACTTC)-5', to determine the sequence dependency of the target affinity toward complementary RNA and DNA. The results revealed that the single aza-ENA-T modification enhances the target affinity significantly with complementary RNA (ΔT_m +2.5 to +4 °C) depending upon the site of the modification in the AON strand. This can be attributed to the site-dependency of the variable conformational pre-organization character imparted by the *North*-fused sugar moiety on the single stranded AON. The 2'-amino function of aza-ENA-T is almost 50% protonated at the physiological pH considering its pK_a of 6.66 ± 0.03, and can have electrostatic interaction with the neighboring phosphate, which favors efficient duplex formation as observed in azetidine modified ODNs.[13] On the other hand, with complementary DNA there was significant drop in duplex melting. This can be due to the 2',4'-ethylene-bridge which causes steric clash in the minor groove of the AON-DNA duplex. All of the aza-ENA-T modified AON-RNA hybrid duplexes have been found to be good substrates for the *E. coli* RNase H1 and comparable to that of the native counterpart. They also showed considerably stability in human blood serum (over 48 h) as well as toward snake venom phosphodiesterase compared to the native sequence.[25] Thus the successful design of the conformationally constrained aza-ENA-T modified oligos with the *North*-fused sugar moiety and the protonable endocyclic-2'-aza function at physiological pH, as two key factors enhancing the stability of AON-RNA duplexes, prompted us to investigate the properties of aza-ENA-adenosine analogue (compound **B** in Figure 1). Compared to the earlier report of the thymine

derivative of aza-ENA (aza-ENA-T), the synthesis required completely new and unique approach simply because the assistance by neighboring group participation of a pyrimidine is absent in the purine counterpart. Thus, it is noteworthy that synthesis of all aza- or thio-2',4'-bridged nucleosides[15, 16, 18-20] as well as aza-1',2'-cis-fused nucleoside analogues,[13] which are so far reported in the literature, require an "ara" positioned 2'-hydroxyl group in the sugar, which is first converted into a leaving group for its subsequent displacement with various nucleophiles via an S_N2 reaction. As stated above, this approach is particularly suitable for the synthesize of only pyrimidine analogues of 2'-deoxy-2'-*N*,4'-*C*-constrained nucleosides, because it is easy to form 2,2'-*O*-anhydro analogues by nucleophilic attack of C=O at the C2 centre of uracil or thymine. Recently, the synthesis of 2'-deoxy-2'-*N*,4'-nucleosides by transglycosylation reaction has also been reported; this approach has however severe limitation because of poor accessibility of the appropriate pyrimidine analogues.[18]



R = Pac [-$COCH_2$(OPh)] , TFA [-$COCF_3$]

Figure 1

RESULTS AND DISCUSSION

PART I: SYNTHESIS OF AZA-ENA-A.

We have chosen as our precursor D-arabinose because of the "*ara*" configuration of 2-OH. D-Arabinose was converted to our key compound **1** with free bis-hydroxyl groups (Scheme 1).[26, 27] The next step involves selective benzylation of only 5-hydroxyl group giving compound **2** (45%) with free 6-hydroxyl moiety (Scheme 1). Contrasting to the selective 5-*O*-benzylation of the ribose analogues[14, 28, 29] this reaction (compound **1**→**2**) leads to the mixture of isomeric monobenzylated (ratio 2:1) products, which can however be separated by simple column chromatography. A small amount (≤ 20%) of bis-substituted product is also forming in this reaction. To avoid this, it is crucial to use only 0.8 equiv of benzyl bromide in DMF as a solvent instead of acetonitrile. The unreacted bis-hydroxyl compound **1** can be easily

separated and recycled as a substrate to give desired compound **2** with 45% combined yield. It was important at this stage to separate the monobenzylated (*C*6 versus *C*5) compounds, and verify the assignment of correct 5-*O*-benzylated analog from the one that is benzylated at *C*6 centre. The structure of the 5-*O*-benzylated product **2** has been proved by 1D diff. nOe experiments in which both methyl groups of 1,2-isopropylidene group was irradiated separately (Figure 2).

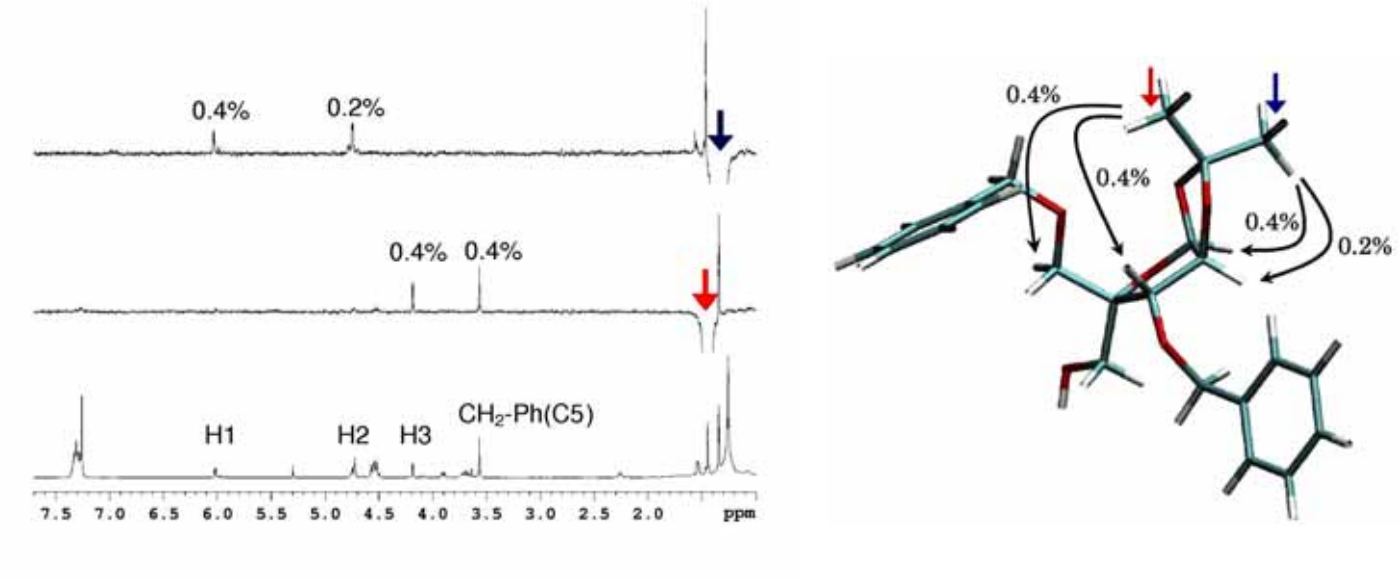
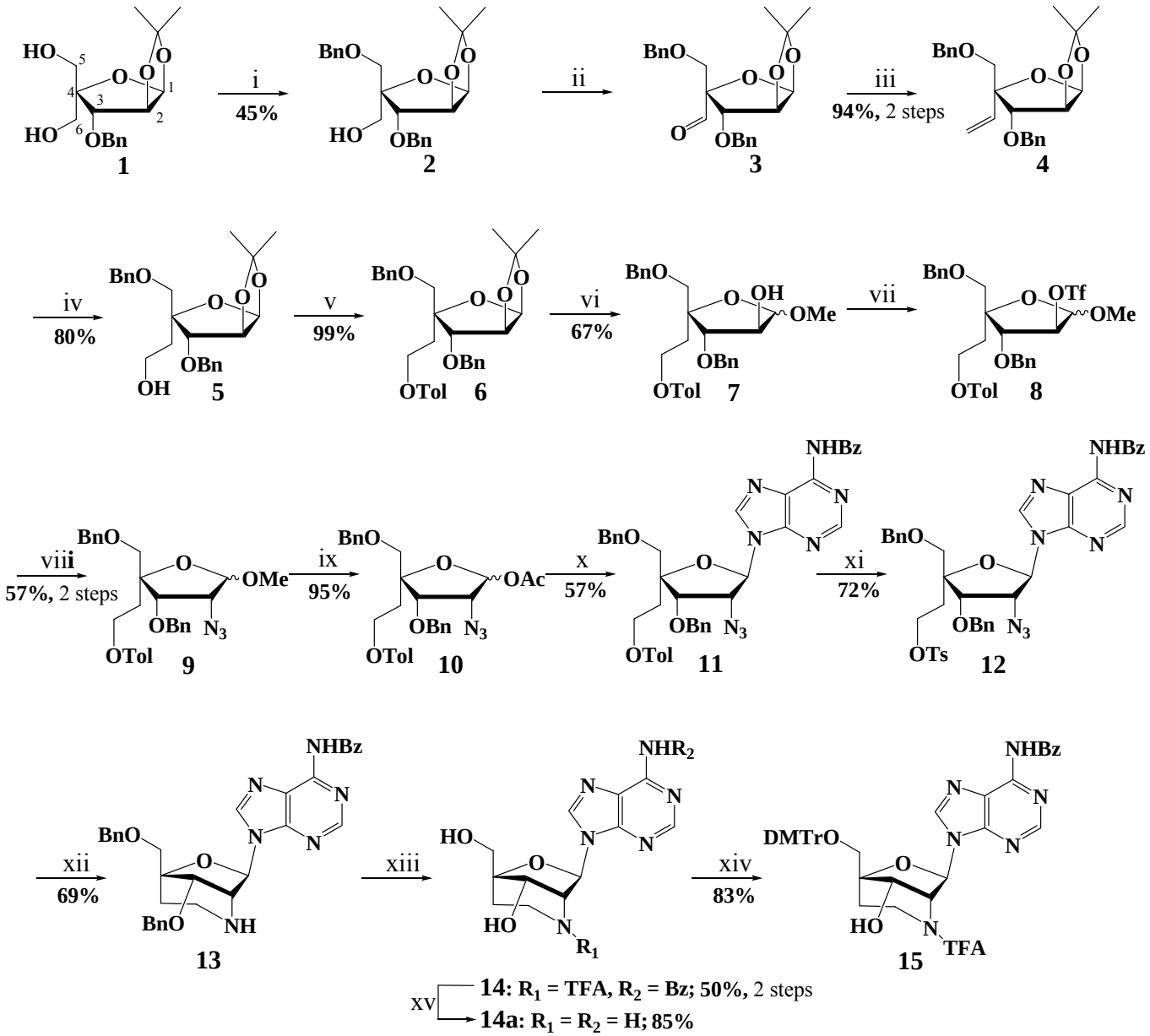


Figure 2. nOe enhancement of protons of the sugar ring after irradiation of methyl groups in 5-*O*-benzylated product **2**.

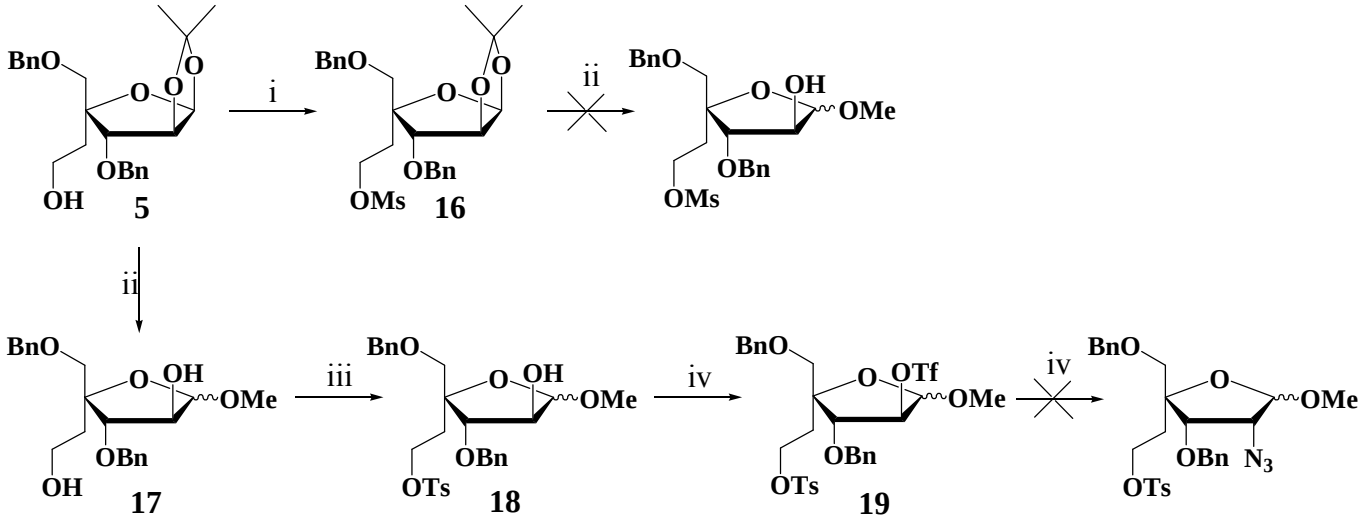
It can clearly be seen that irradiation of one of the methyl groups shows enhancement of the nuclear Overhauser effect for protons H1 and H2 (which are "below" the sugar ring), and irradiation of the other methyl group gives enhancement for protons H3 and the CH_2 group at position 5 (showing that they must be both "above" the sugar ring), which is protected with benzyl group since this CH_2 does not show any coupling with the exchangeable OH proton.

The next step of the synthesis was extension of *C*4-hydroxymethyl group in **2** into its hydroxyethyl analogue **5**. We decided to use Swern oxidation condition of the primary hydroxyl in compound **2** to give the desired aldehyde **3**.[15] This was followed by simple and efficient Wittig reaction on **3**, using carbon-donor generated *in situ* by reaction of methyltriphenylphoshine bromide and butyl lithium, to give compound **4** with double bond at *C*6 position in high yield (94% in two steps).[15] Compound **4** could be easily converted to compound **5** by the hydroboration reaction (using 9-BBN) followed by oxidation (80% in two steps).



Scheme 1. *Reagents and conditions:* (i) NaH, BnBr, DMF, 0 °C, overnight; (ii) oxaloyl chloride, DMSO, CH_2Cl_2, -78 °C, 2 h, then Et_3N, -78 °C to rt; (iii) BuLi, $MeP(Ph)_3Br$, THF, 0 °C to rt, 3 h, then addition of **3** at -78 °C to rt; (iv) 9-BBN, THF, overnight, 3 *N* NaOH, 30% H_2O_2, 30-45 °C, 0.5 h; (v) 4-Tol-Cl, pyridine, rt, 1 h; (vi) MeOH, H_2SO_4, reflux, 0.5 h; (vii) Tf_2O, pyridine, CH_2Cl_2, 0 °C, rt, 0.5 h; (viii) NaN_3, DMF, 40 °C, 48 h; (ix) acetic acid, Ac_2O, H_2SO_4, 0 °C, 3 h; (x) persilylated N^6-benzoyladenine, TMSOTf, CH_3CN, 80 °C, overnight; (xi) NaOH, ethanol, pyridine, rt, 6 min, then pyridine, *p*-toluenosulfonyl chloride, rt, overnight; (xii) PMe_3, 2 *N* NaOH, THF, rt, 3h; (xiii) (a) trifluoroacetic anhydride, pyridine, CH_2Cl_2, 0 °C to rt, 20 min, (b) 1 *M* BCl_3 in CH_2Cl_2, -78 °C to rt, 24 h; (xiv) DMTr-Cl, pyridine, rt, overnight; (xv) 32% aq NH_3, rt, overnight.

Abbreviations: Bn = benzyl, rt = room temperature, Ac = acetyl, Tf = trifluoromethanesulfonyl, Tol = toluoyl, THF = tetrahydrofuran, 9-BBN = 9-borabicyclo[3.3.1]nonane, DMTr = 4,4'-dimethoxytrityl, Bz = benzoyl, TFA = trifluoroacetyl, Me = methyl, DMF = dimethylformamide, Ts = *p*-toluenosulfonyl, DMSO = dimethylsulfoxide.



Scheme 2. *Reagents and conditions:* (i) MsCl, pyridine, 0 °C, 1 h; (ii) MeOH, H_2SO_4, reflux, 0.5 h; (iii) *p*-toluenosulfonyl chloride, pyridine, 0 °C to rt, overnight; (iv) Tf_2O, pyridine, CH_2Cl_2, 0 °C, rt, 0.3 h; then NaN_3, DMF, 40 °C, 48 h.

Abbreviations: Bn = benzyl, rt = room temperature, Me = methyl, DMF = dimethylformamide, Ms = methanesulfonyl, Ts = *p*-toluenosulfonyl.

At this stage, our first attempt was to put activated leaving group (methanesulfonyl or *p*-toluenosulfonyl) at the position 7 in sugar moiety (Scheme 2) without additional protection-deprotection steps involving *p*-toluoyl group. Unfortunately 1,2-*O*-isopropylidene protection in sugar **16** was not possible to remove in acidic condition (refluxing methanol containing sulfuric acid) without formation of side products. This was probably due to the reason that the unhindered and probably more reactive primary 7-*O*-methanesulfonyl leaving group was susceptible to intra-molecular displacement more in 1,2-*β*-*O*-isopropylidene system (compared to that in the 1,2-*α*-*O*-isopropylidene ribose analogue).[15] It was possible, on the other hand, to deprotect first 1,2-*O*-isopropylidene group in compound **5** giving bis-hydroxy compound **17** (80%), and then selectively form 7-*p*-toluenosylfonyl derivative **18** (75%). At this stage we were trying to utilize displacement of highly reactive secondary 2-trifluoromethanesulfonyl derivative in the presence of less reactive but primary 7-*p*-toluenosulfonyl group with sodium azide (compound **19** in Scheme 2). This reaction in our case leads predominantly to bis-azido product, most probably because of much less sterically hindered primary 7-*p*-toluenosulfonyl group (*C*-hydroxymethyl *versus C*-hydroxyethyl) compared to 6-methanosulfonyl group, as it was reported.[18, 19, 29]

Because of the above problems, we decided to start with protection of 7-hydroxy function of **5** with *p*-toluoyl group to form compound **6** (99%), which can be easily deprotected at a later stage. Compound **6** was transformed to its anomeric mixture of 1-methyl glycoside **7** (67%). Standard reaction of **7** with triflic anhydride in pyridine/CH_2Cl_2 mixture as a solvent at 0 °C afforded compound **8**, which can be used,

as a crude, in direct azide displacement reaction to give compound **9** in Scheme 1. Our best result from **7** to **9** was achieved (57%) when reaction was carried out in slightly elevated temperature (40 °C), but for a longer time (48 h).[19, 30] When the reaction was carried out at room temperature there was no indication of any product formation, whereas, at a much higher temperature decomposition of reactive 2-*O*-triflate compound was found to occur. Purification of the desired compound **9** gave basically a single product, not the expected anomeric mixture as in the substrate **8**. This suggests that either one of the anomeric substrates reacted slower, making its reactive 2-*O*-triflate group more vulnerable to decomposition upon prolonged heating or more susceptible to competitive elimination reaction. To form reactive glycosyl donor, compound **9** was subjected to acetolysis to give 1-*O*-acetyl compound **10** (95%) as an anomeric mixture, which, after purification, was used as substrate in coupling reaction with persilylated N^6-benzoyladenine using Vorbrüggen conditions.[31-33] We were expecting that owing to the lack of 2'-participating group in substrate can lead to mixture of α and β anomers (**11**), fortunately we were able to obtain 1:2 ratio of enrichment of required β anomer (57% overall yield). Compound **11** was then converted to its 7'-*O*-*p*-toluenosulfonyl derivative **12** by a two-step reaction sequence: selective deprotection of *p*-toluoyl group (with sodium hydroxide solution in ethanol), followed by usual *p*-toluenosulfonylation in pyridine at room temperature (72% after two steps). Compound **12** is subsequently subjected to reductive cyclization reaction using trimethylphosphine in the presence of 2 *N* sodium hydroxide solution, which gave 2'-amino group *in situ*, which instantaneously cyclized to form the desired 2'-deoxy-2'-*N*,4'-*C*-ethylene-bridged nucleoside **13** (69% after two steps).[34] Compound **13** was then *N*-protected with trifluoroactic anhydride to form mixture of diastereomers (quantitatively), which were then debenzylated to give 3',5'-bis-hydroxy compound **14** (50% after two steps). Deprotection of 3' and 5'-benzyl groups was completed by means of typical reaction with 1 *M* boron trichloride solution.[20] Compound **14** was then 5'-*O*-protected with 4,4'-dimethoxytrityl group to form building block **15** (83%), which can be utilized for preparation of suitably protected phosphoramidite analogues for automatic solid phase oligonucleotide synthesis. Fully deprotected compound **14a** (85%) for characterization was prepared by treatment of compound **14** with 32% aqueous ammonia at room temperature overnight.

PART II: CHARACTERIZATION AND CONFORMATIONAL ANALYSIS OF PROTECTED (13) AND FULLY DEPROTECTED AZA-ENA-A (14A).

The characterization and conformational analysis of the aza-ENA-A, compounds **13** and **14a**, have been performed using NMR spectroscopy (^{1}H at 500 and 600 MHz in $CDCl_3$/DMSO-d_6) obtained by ^{1}H homodecoupling experiments, 1D nuclear Overhauser effect spectroscopy (1D NOESY), 2D total correlation spectroscopy (TOCSY), 2D COSY, and ^{13}C NMR experiments, as well as long-range ^{1}H-^{13}C HMBC correlation ($^2J_{H,C}$ and $^3J_{H,C}$) and a one-bond heteronuclear multiple-quantum coherence (HMQC)

experiments. Coupling constants have been calculated by stepwise single and double decoupling experiments of H6'/H6"/H7'/H7". All chemical shifts assignments and coupling constants on the basis of the NMR data, as well as the actual NMR spectra for the compounds **13** and **14a** are available in Figures 1 – 11 and Tables 1 and 2.

In compound **13**, H7"/ H7' appeared as multiplets at δ 3.22 (ddd) and 3.07 (dd). Irradiation in 1D nOe experiments of H8 (Figure 3, Inset A) shows 2% enhancement on H3' ($d_{H3',H8}$ ≈ ca. 2.4Å) signifying that 9-adeninyl moiety is in the *β*-configuration and *anti* conformation. Irradiation of H1' (Figure 3, Inset B) showed 3% enhancement at the multiplet at δ 3.22 allowing us to assign it to be H7" ($d_{H1',H7"}$ ≈ 2.5Å). This further confirms that the piperidino moiety of the bicyclic sugar is in chair conformation. The multiplicity of the H7" centered at δ 3.22 appears as 8 lines resulting from a doublet of doublet of doublet, which is due to its vicinal coupling to H6' and H6" beside the geminal coupling ($^2J_{H7',H7"}$ = 13.6 Hz, $^3J_{H7",H6'}$ = 11.9 Hz, $^3J_{H7",H6"}$ = 4.9 Hz). The two large couplings (one trans diaxial coupling, $^3J_{H7",H6'}$, beside the geminal coupling) of the multiplet at δ 3.22 further confirms the assignment of H7". The multiplicity of the H7' centered at δ 3.07 appears as 4 lines resulting from a doublet of doublet, which is due to its vicinal coupling to H6' beside the geminal coupling ($^2J_{H7',H7"}$ = 13.6 Hz, $^3J_{H7',H6'}$ = 6.1 Hz). ^{1}H COSY (Figure 4) shows that both H7" and H7' couples to H6'/H6" centered at δ 2.02 and δ 1.37, respectively. H7' shows vicinal coupling to only H6', which is clearly evident from the ^{1}H COSY spectrum. Multiplet centred at δ 2.02 appeared as 8 lines which resulted from a doublet of doublet of doublet ($^2J_{H6',H6"}$ = 13.1 Hz, $^3J_{H6',H7"}$ = 11.7 Hz, $^3J_{H6',H7'}$= 6.6 Hz). Multiplet centred at δ 1.37 appeared as 4 lines because of its vicinal coupling to H7" beside the geminal coupling ($^2J_{H6',H6"}$ = 13.1 Hz, $^3J_{H6",H7"}$ = 4.6 Hz, $^3J_{H6",H7'}$ = 0 Hz). The multiplicity and coupling constants are in agreement with those found for the aza-ENA-T analogue[20] (Table 2). Non-observable $^3J_{H1',H2'}$ and low $^3J_{H2',H3'}$ coupling constants indicate that, similar to that in the ENA,[15] aza-ENA-T,[20] LNA[15, 35] or 2'-amino-LNA,[36] the furanose pucker of aza-ENA-A nucleoside was fixed in *North*-conformation. The molecular structures of the aza-ENA-T monomer units by high-field 1H NMR and theoretical *ab initio* and MD simulations have shown that the piperidino-fused furanose ring is indeed locked in the typical *North*-type conformation, with the pseudorotational phase angle (*P*) and puckering amplitude (ϕ_m) for the *ab initio* optimized geometries (HF, 6-31G**) varying in the ranges 7° < *P* < 27° and 44° < ϕ_m < 52°, respectively.[20] The sugar pucker in aza-ENA-T[20] was found to be close to that of the ENA and LNA (*P* = 12 – 19°),[15] however, with the sugar puckering amplitude, ϕ_m, lower by ~10° compared to that of the LNA.[15, 35] High correspondence in chemical shifts, multiplicity as well as coupling constants between reported earlier aza-ENA-T[20] and those observed for the synthesized aza-ENA-purine analogue (see Table 1 and 2 for comparison) indicates that these two compounds have high structural and conformational similarities.

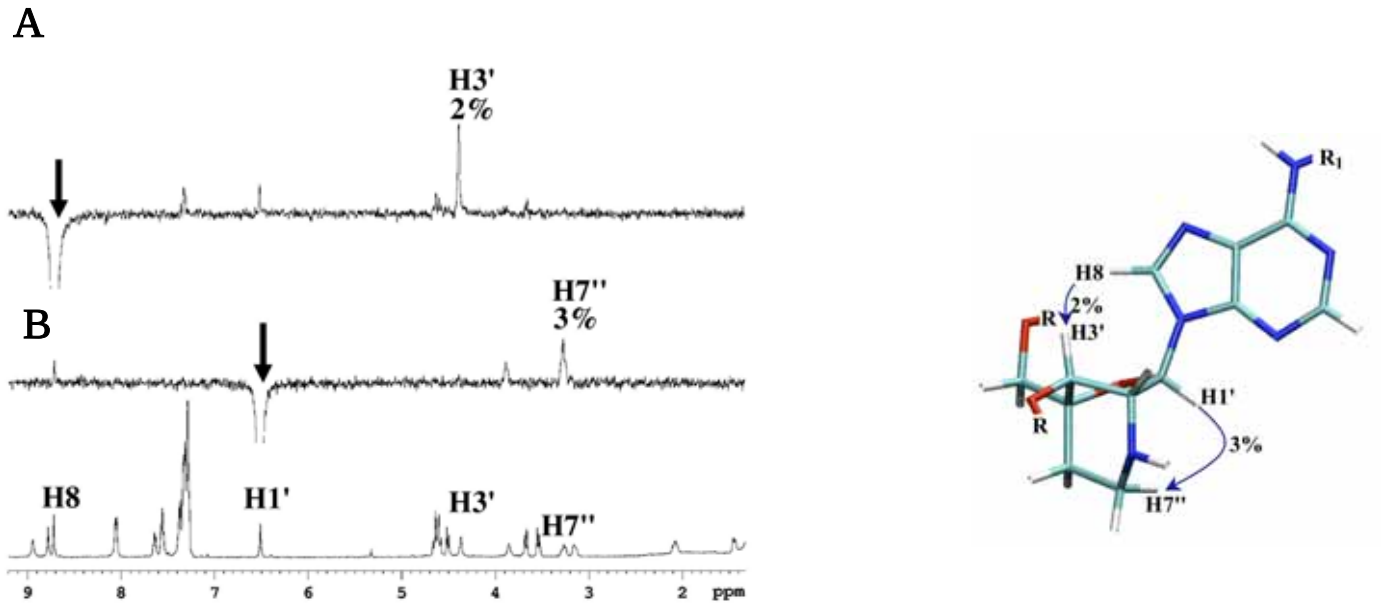


Figure 3. 1D nOe spectrum of Compound 13 (R = Bn, R_1= Bz). Inset (A) Irradiation (shown by thick arrow) of H8 shows 2% enhancement on H3' ($d_{H3',H8} \approx$ ca. 2.4Å) signifying that 9-adeninyl moiety is in the β-configuration and anti conformation. Inset (B) Irradiation of H1' shows 3% enhancement on H7" ($d_{H1',H7''} \approx$ ca. 2.5Å), signifying that H7" is in the axial orientation, hence confirming that the piperidino moiety is in chair conformation.

HMQC (Figure 5) and HMBC (Figure 6) experiments allowed us to assign the ^{13}C chemical shifts. That the sugar fused bicyclic piperidino [3.2.1] octane system in compound **13,** in the ring closure reaction (Scheme I), has indeed been formed is confirmed by the observation of the long range ^{1}H-^{13}C connectivities between H7' with C2′ ($^{3}J_{H7',C2'}$) and H2' with C7′ ($^{3}J_{H2',C7'}$) in HMBC experiment (Figure 7). The fact that only the H7' (not H7") showed HMBC correlation with C-2' ($^{3}J_{H7',C2'}$) indicates that the dihedral angle between H7' and C2', ϕ[H7'-C7'-N-C2'], is closer to 180° whereas between H7" and C2', ϕ[H7"-C7'-N -C2'], is closer to 90°.

The fully deprotected sugar fused bicyclic piperidino[3.2.1]octane compound **14a** showed similar NMR spectra as those for the precursor **13**. The structural integrity of compound **14a** was also confirmed by correct mass measurement by MALDI-TOF mass spectrometry (see EXPERIMENTAL section). For the ^{1}H and ^{13}C assignments supporting the structure of the compound **14a**, see the Figures S1 – S4 in Supporting Information and Tables 1 and 2.

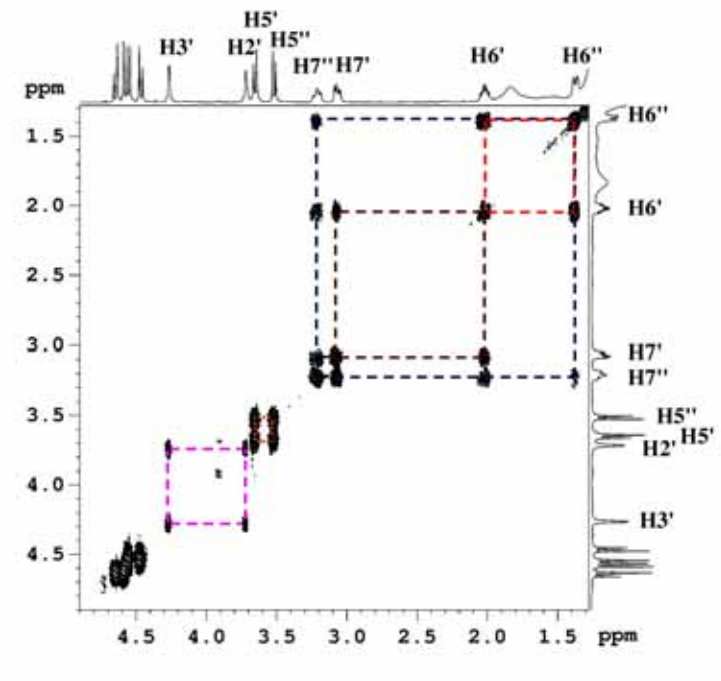


Figure 4. Expansion of the region from 4.9 – 1.3 ppm of COSY spectrum of compound **13**.

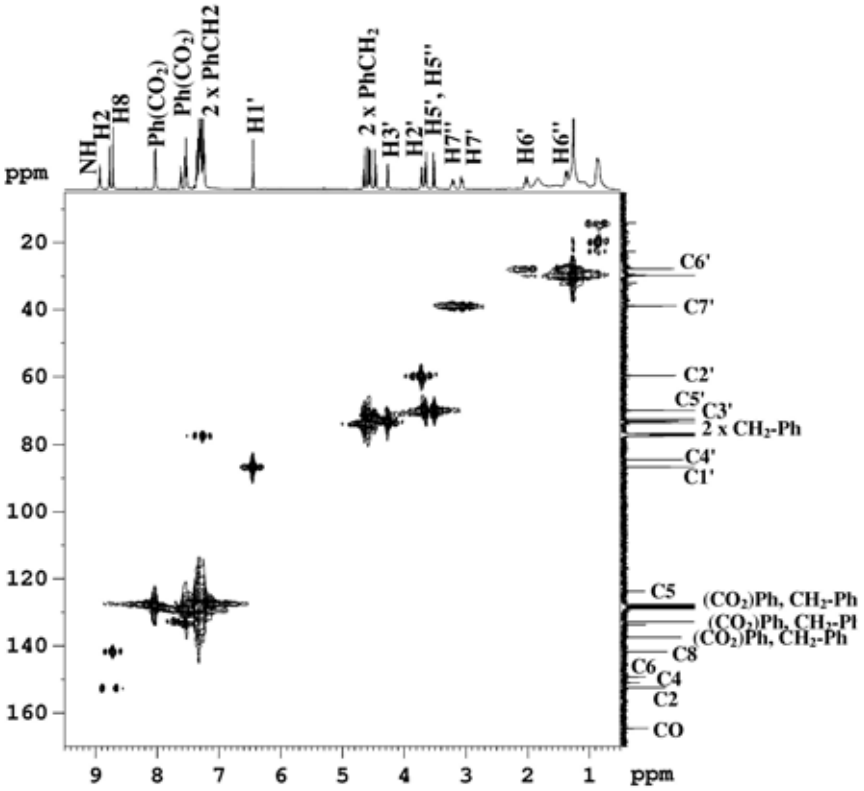


Figure 5. HMQC spectrum of compound **13**.

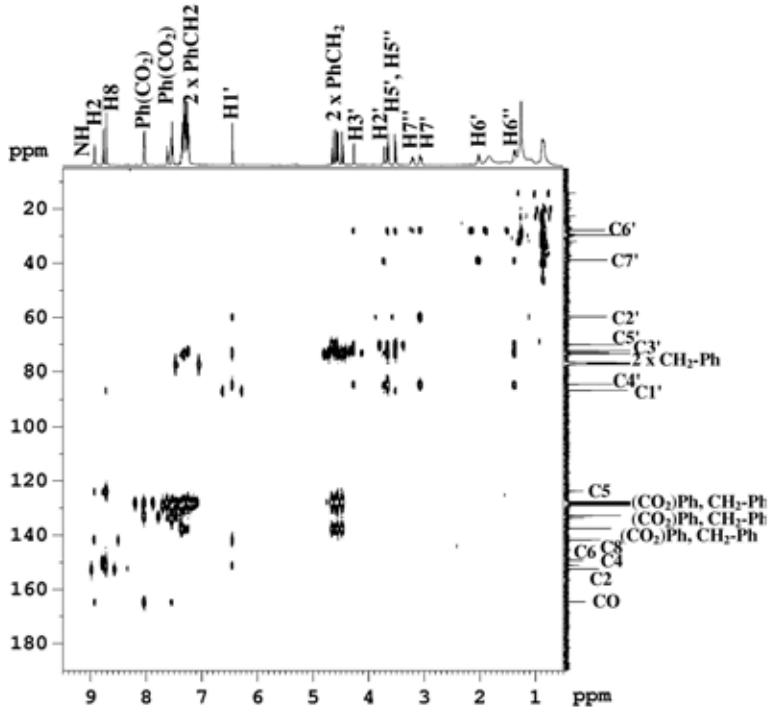


Figure 6. HMBC spectrum of compound 13. Expanded region (in purple box) showing the important long range $^{3}J_{HC}$ connectivities is shown in Figure 7.

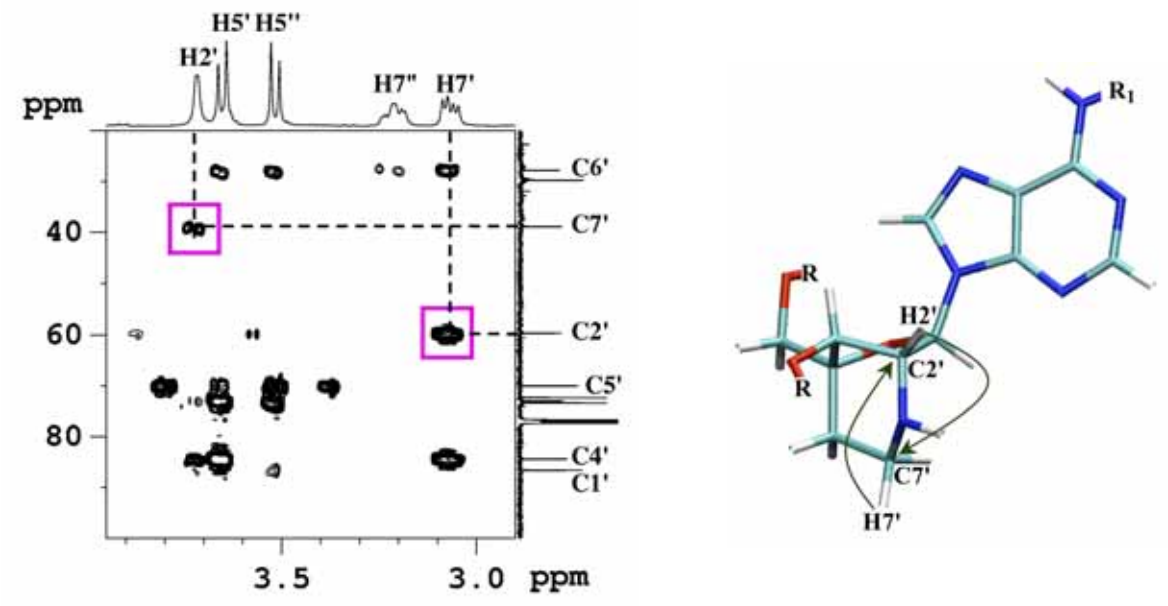


Figure 7. Expansion of HMBC spectrum of **13 (R = Bn, R_1= Bz)** showing important $^{3}J_{HC}$ correlations confirming the ring closure between 2'C and 2'N, forming the 6-membered piperidino ring. H2' shows the $^{3}J_{HC}$ correlation to C7' and H7' shows the $^{3}J_{HC}$ correlation to C2'. The presence of the correlation of only H7' to C2' indicates that the dihedral angle between H7' and C2', ϕ[H7'-C7'-N-C2'], is closer to 180°.

Table 1. Chemical shifts of compounds **13** and **14a**.

^{1}H	**Compound 13**	**Aza-ENA-T (Bn-Protected)***	**Compound 14a**	**Aza-ENA-T (Deprotected)***
N(6) H (A)/ N(6)H_2(A)	8.93	-	7.29	-
H8	8.72	-	8.49	-
H2	8.77	-	8.17	-
Aromatic	8.04, 7.58, 7.44 – 7.17	7.38 – 7.24	-	-
H1'	6.45	5.94	6.24	5.84
OH(C-3')	-	-	5.09	5.16
OH(C-5')	-	-	5.19	5.39
Ph(CH_2)-5'/3'	4.65	4.68	-	-
Ph(CH_2)-5'/3'	4.58	4.52	-	-
Ph(CH_2)-3'/ 5'	4.55	4.57	-	-
Ph(CH_2)-3'/5'	4.47	4.53	-	-
H2'	3.72	3.51	3.41	3.25
H3'	4.26	3.98	4.23	3.99
H5'	3.65	3.71	3.56	3.57
H5"	3.52	3.56	3.51	3.50
H6'	2.02	2.03	1.82	1.78
H6"	1.37	1.30	1.24	1.17
H7'	3.07	3.02	2.91	2.87
H7"	3.22	3.14	3.03	2.95

*Chemical shifts of aza-ENA-T were taken from ref. 20 for comparison.

Table 2. ^{1}H NMR Coupling constants of compounds **13** and **14a**.

$^2J_{HH}$ and $^3J_{HH}$	**Compound 13 *J* (Hz)**	**aza-ENA-T (Bn-Protected)* *J* (Hz)**	**Compound 14a *J* (Hz)**	**aza-ENA-T (Deprotected)* *J* (Hz)**
$^3J_{H1',H2'}$	0	0	0	0
$^3J_{H2',H3'}$	3.6	3.9	3.9	3.9
$^2J_{H5',H5"}$	10.8	10.8	12.2	12.2
$^2J_{HH\ (PhCH2)-3'}$	12.5/11.7	11.8/11.8	-	-
$^2J_{HH\ (PhCH2)-5'}$	11.7/12.5	11.5/11.5	-	-
$^2J_{H7',H7"}$	13.6	13.3	13.3	12.8
$^3J_{H7',H6'}$	6.8	6.5	6.8	6.6
$^3J_{H7',H6"}$	0	0	0	0
$^3J_{H7",H6'}$	11.9	11.6	11.8	13.0
$^3J_{H7",H6"}$	4.9	4.9	4.7	4.8
$^2J_{H6',H6"}$	13.0	13.1	13.0	12.9
$^3J_{H6',H7'}$	6.8	6.7	6.8	6.8
$^3J_{H6',H7"}$	11.7	11.6	11.8	13.0
$^3J_{H6",H7'}$	0	0	0	0
$^3J_{H6",H7"}$	4.6	4.8	4.7	4.6

* ^{1}H NMR Coupling constants of aza-ENA-T were taken from ref. 20 for comparison.

CONCLUSIONS

We have synthesized 2'-deoxy-2'-*N*,4'-*C*-ethylene-bridged adenosine (aza-ENA-A) having 2-aza-6-oxabicyclo[3.2.1]octane skeleton by taking advantage of appropriate stereochemistry of arabinose precursor **5**. We have therefore developed a new method for the synthesis of conformationally constrained purine nucleosides, where nucleophilic S_N2 displacement was performed using D-arabinose derivative **8**. The synthesis involves operationally simple steps, and was optimized with respect to their regio- and stereoselectivity. Crucial coupling step was performed using 1-*O*-acetyl-3,5-di-*O*-benzyl-(2-deoxy-2-azido)-4-*C*-(*p*-toluoyloxyethyl)-D-ribofuranose (**10**) and persilylated N^6-benzoyladenine by means of the Vorbrüggen procedure. Compound **15** will be used as precursor for solid phase synthesis essential for further evaluation of the biological properties of this novel class of conformationally restricted nucleotides.

EXPERIMENTAL

Dichloromethane, pyridine and toluene were dried over calcium hydride, distilled and stored over molecular sieves 4 Å. Anhydrous THF (99.9%) was commercial (Aldrich). Chromatographic separations were performed on Merck G60 silica gel. Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F_{254} glass-backed plates. ^{1}H NMR spectra in $CDCl_3$/DMSO-d_6 were recorded on JEOL GX 270 MHz or Bruker DRX 500/600 MHz NMR spectrometers using TMS (0.0 ppm) as internal standards. ^{13}C NMR spectra were recorded at 67.9 MHz or 125.7 MHz. Mass spectra (MALDI-TOF) were measured using an Ultraflex Tof/Tof instrument (Bruker Daltonics, Germany), and (TOF-MS ES) using Micromass LCT instrument employing electrospray quadropol time-of-flight mass spectrometry (Q-TOF).

3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-*β*-D-arabinofuranose (2). To a stirred suspension of **1** (6.7 g, 21.6 mmol) in anhydrous DMF (400 mL) NaH (0.415 g, 17.3 mmol) was added in portions during 0.5 h at 0 °C. Benzyl bromide (2.05 mL, 17.3 mmol) was added dropwise and reaction mixture kept at 5 °C overnight under nitrogen atmosphere. The reaction was quenched with water and extracted with EtOAc (3x). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-40% EtOAc in cyclohexane v/v), which afforded **2**. Substrate (compound **1**) was recovered and subjected to second benzylation reaction. Combined yield (3.89 g, 9.7 mmol, 45%). R_f = 0.66 (c-hexane/EtOAc 6:4 v/v); TOF-MS ES- *m/z* $[M-H]^-$ found 399.5, calcd 399.2; ^{1}H NMR (270 MHz, $CDCl_3$): 7.31-7.24 (m, 10H, benzyl), 6.01 (d, $J_{H\text{-}1,\,H\text{-}2}$ = 4.32 Hz, 1H, H-1), 4.74-4.70 (m, 2H, H-2 + CH_2Ph), 4.56-4.51 (m, 3H, 1+1/2 x CH_2Ph), 4.17 (d, $J_{H\text{-}2,\,H\text{-}3}$ = 1.59 Hz, 1H, H-3), 3.87-3.62 (m, 2H, H-6', H-6", CH_2OH), 3.55 (s, 2H, H-5', 5-H", CH_2OBn), 2.24 (m, 1H, OH-6'), 1.43 (s, 3H, CH_3, isopropyl), 1.33 (s, 3H, CH_3, isopropyl); ^{13}C

NMR (67.9 MHz, $CDCl_3$): 137.2, 137.0, 128.3, 128.2, 127.7, 127.5, 114.1 (q, isopropyl), 104.6 (C-1), 88.6 (q, C-4), 86.2 (C-2), 85.1 (C-3), 73.5 (CH_2Ph), 72.8 (CH_2Ph), 71.1 (CH_2OBn) 64.5 (CH_2OH) 26.5 (CH_3, isopropyl), 25.6 (CH_3, isopropyl).

3,5-Di-*O*-benzyl-4-*C*-vinyl-1,2-*O*-isopropylidene-*β*-D-arabinofuranose (4). Dry DMSO (2.29 ml, 32.4 mmol) diluted with dry CH_2Cl_2 (30 mL) was added via syringe to the solution of dry CH_2Cl_2 (60 mL) and oxaoyl chloride (1.72 ml, 20.25 mmol) at –78 °C. After stirring reaction for 1.5 h at this temperature under nitrogen atmosphere, sugar **2** (2.5 g, 6.27 mmol) dissolved in anhydrous CH_2Cl_2 (30 mL,) was added slowly, and reaction continued at -70 °C for additional 1h. Then triethylamine was added (8.2 mL, 58.7 mmol) and reaction allowed slowly to warm up to rt The reaction was quenched with cold water and extracted with CH_2Cl_2. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude reaction product **3** was dissolved in 190 mL dry THF and added to cooled to -78 °C solution of $MePPh_3Br$/BuLi and left to react overnight, slowly warming up under nitrogen atmosphere. [$MePPh_3Br$/BuLi was prepared as follows: $MePPh_3Br$ (7.23 g, 20.25 mmol) was suspended in dry THF (60 mL) and put to ice bath, 1.6 *M* solution of BuLi in THF (10.5 mL, 16.9 mmol) was then added and reaction stirred for 0.5 h at 0 °C and 3 h at rt]. Reaction was quenched with saturated aqueous solution of NH_4Cl and extracted with Et_2O (2x). The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-20% EtOAc in cyclohexane v/v), which afforded **4** (2.513 g, 6.35 mmol, 94%). R_f = 0.90 (c-hexane/EtOAc 6:4 v/v); TOF-MS ES- *m/z* $[M-H]^-$ found 396.5, calcd 396.2; ^{1}H NMR (270 MHz, $CDCl_3$): 7.52-7.25 (m, 10H, benzyl), 6.03 (dd, $J_{H\text{-}6,\ H\text{-}7a}$ = 17.3 Hz, $J_{H\text{-}6,\ H\text{-}7b}$ = 10.8 Hz, 1H, H-6), 5.89 (d, $J_{H\text{-}1,\ H\text{-}2}$ = 4.45 Hz, 1H, H-1), 5.52 (dd, $J_{H\text{-}7a,\ H\text{-}7b}$ = 1.6 Hz, $J_{H\text{-}7a,\ H\text{-}6}$ = 17.3 Hz, 1H, H-7a), 5.26 (dd, $J_{H\text{-}7b,\ H\text{-}7a}$ = 1.6 Hz, $J_{H\text{-}7b,\ H\text{-}6}$ = 10.8 Hz, 1H, H-7b), 4.69-4.49 (m, 3H, H-2, 2xCH_2Ph), 4.31 (d, $J_{H\text{-}2,\ H\text{-}3}$ = 2.45 Hz, 1H, H-3), 3.5 (ABq, J_{gem} = 10.39 Hz, 2H, H-5', H-5''), 1.50 (s, 3H, CH_3, isopropyl), 1.36 (s, 3H, CH_3, isopropyl); ^{13}C NMR (67.9 MHz, $CDCl_3$): 138.3, 137.7, 135.18 (C-6), 128.3, 128.2, 127.7, 127.5, 115.7 (C-7), 113.6 (q, isopropyl), 103.7 (C-1), 87.4 (q, C-4), 86.5 (C-2), 84.4 (C-3), 73.3 (CH_2Ph), 72.6 (C-5), 72.3 (CH_2Ph), 27.5 (CH_3, isopropyl), 27.3 (CH_3, isopropyl).

3,5-Di-*O*-benzyl-4-*C*-hydroxyethyl-1,2-*O*-isopropylidene-*β*-D-arabinofuranose (5). To a stirred solution of **4** (2.5 g, 6.35 mmol) in anhydrous THF (80 mL) at rt 0.5 *M* solution of 9-BBN in THF (0.363 g, 11.5 mmol) was added. Reaction was left stirred overnight under nitrogen atmosphere. Water was added till no more gas evolution was observed and then slowly 3 *N* NaOH solution (15 mL) followed by 30% H_2O_2 while keeping temperature between 30-50 °C. Reaction was allowed to react for 30 min, and portioned between water and EtOAc. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-40% EtOAc in cyclohexane v/v), which afforded **5** (2.1 g, 5.08 mmol, 80%). R_f = 0.70 (c-hexane/EtOAc 6:4

v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 414.6, calcd 415.2; ^{1}H NMR (270 MHz, $CDCl_3$): 7.31-7.26 (m, 10H, benzyl), 5.9 (d, $J_{H\text{-}1,\,H\text{-}2}$ = 4.32 Hz, 1H, H-1), 4.67-4.52 (m, 5H, H-2 + 2CH_2Ph), 4.07 (s, 1H, H-3), 3.76-3.65 (m, 2H, H-7', H-7'', CH_2OH), 3.65-3.57 (ABq, J_{gem} = 9.5 Hz, 2H, H-5', H-5'', CH_2OBn), 1.97-2.19 (m, 2H, H-6', H-6'', CH_2), 1.42 (s, 3H, CH_3, isopropyl), 1.25 (s, 3H, CH_3, isopropyl); ^{13}C NMR (67.9 MHz, $CDCl_3$): 137.8, 137.3, 128.5, 128.2, 127.8, 127.5, 112.4 (q, isopropyl), 104.9 (C-1), 89.7 (q, C-4), 85.3 (C-2), 84.9 (C-3), 76.6 (CH_2Ph), 73.5 (CH_2Ph), 72.4 (C-5), 58.8 (C-7), 26.7 (CH_3, isopropyl), 26.2 (CH_3, isopropyl), 22.1 (C-6).

3,5-Di-*O*-benzyl-4-*C*-(*p*-toluoyloxyethyl)-1,2-*O*-isopropylidene-*β*-D-arabinofuranose (6). Compound **5** (2.4 g, 5.8 mmol) was two times co-evaporated with dry pyridine and dissolved in the same solvent. 4-Toluoyl chloride (1.52 mL, 11.5 mmol) was added dropwise under nitrogen atmosphere and stirring was continued for additional 1h. Reaction mixture was cooled down in an ice bath and quenched with saturated aqueous solution of $NaHCO_3$ and extracted (2x) with CH_2Cl_2. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-30% EtOAc in cyclohexane v/v), which afforded **6** (3.05 g, 5.74 mmol, 99%). R_f = 0.87 (CH_2Cl_2/MeOH 97:3 v/v); TOF-MS ES+ *m/z* [M+H]+ found 533.5, calcd 533.2; ^{1}H NMR (270 MHz, $CDCl_3$): 8.04-7.88 (m, 2H, *p*-toluoyl), 7.38-7.31 (m, 12H, 2 x benzyl + *p*-toluoyl), 5.9 (d, $J_{H\text{-}1,\,H\text{-}2}$ = 4.45 Hz, 1H, H-1), 4.67-4.46 (m, 7H, H-2 + 2CH_2Ph + H-7', H-7'',), 4.11 (s, 1H, H-3), 3.63-3.52 (ABq, J_{gem} = 9.63 Hz, 2H, H-5', H-5'', CH_2OBn), 2.28 (s, 3H, OCH_3, *p*-toluoyl), 2.24 (t, 2H, $J_{H\text{-}1,\,H\text{-}2}$ = 7.04 Hz, H-6', H-6'', CH_2), 1.29 (s, 3H, CH_3, isopropyl), 1.25 (s, 3H, CH_3, isopropyl); ^{13}C NMR (67.9 MHz, $CDCl_3$): 165.5 (C=O), 148, 145.1, 138.7, 137.8, 137.3, 128.5, 128.2, 127.8, 127.5, 112.5 (q, isopropyl), 104.7 (C-1), 88.1 (q, C-4), 85.6 (C-2), 84.9 (C-3), 76.6 (CH_2Ph), 73.4 (CH_2Ph), 72.4 (C-5), 61.3 (C-7), 31.1 (C-6), 26.7 (CH_3, isopropyl), 26.2 (CH_3, isopropyl), 21.9 (CH_3, *p*-toluoyl).

3,5-Di-*O*-benzyl-4-*C*-(*p*-toluoyloxyethyl)-1-*O*-methoxy-D-arabinofuranose (7). Compound **6** (3 g, 5.9 mmol) was dissolved in dry MeOH (50 mL) and concentrated sulfuric acid (7 μL) was added. Reaction mixture was refluxed under nitrogen atmosphere for 30 min, after cooled down to rt and quenched with ice-cooled $NaHCO_3$ and extracted (2x) with CH_2Cl_2. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **7** (2.0 g, 3.95 mmol, 67%) as mixture of anomers. R_f = 0.60 (CH_2Cl_2/MeOH 97:3 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 507.4, calcd 507.2; ^{13}C NMR (67.9 MHz, $CDCl_3$): 166.8, 166.4, 143.5, 143.3, 138.2, 138.0, 128.4, 128.3, 127.8, 127.6, 110.4, 100.7, 87.1, 86.9, 86.0, 83.3, 77.9, 75.4, 74.1, 74.0, 73.0, 72.6, 72.4, 62.2, 61.2, 55.3, 55.2, 32.9, 31.9, 26.7, 21.7.

3,5-Di-*O*-benzyl-(2-deoxy-2-azido)-4-*C*-(*p*-toluoyloxyethyl)-1-*O*-methoxy-D-ribofuranose (9). Compound **7** (1.95 g, 3.85 mmol) was co-evaporated with dry pyridine and dissolved in dry CH_2Cl_2 (100

mL). To this solution pyridine (3.7 mL, 46 mmol) was added and reaction mixture was put to the ice bath under nitrogen atmosphere. After cooling down trifluoromethanesulphonic anhydride was added via syringe. Reaction was kept at 0 °C for 10 minutes and allowed to warm up for another 20 minutes. Reaction was quenched with ice-cooled solution of saturated $NaHCO_3$, and compound was extracted (2x) with CH_2Cl_2. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. After drying for 30 min on oil pump crude compound **8** was dissolved in dry DMF (40 mL) and sodium azide (1.5 g, 23 mmol) was added. Reaction was stirred at 40 °C under nitrogen atmosphere for 48 h, and then quenched with ice-cooled solution of saturated $NaHCO_3$. Compound was extracted (2x) with CH_2Cl_2, organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **9** (1.18 g, 2.22 mmol, 57% after two steps) as one anomer. R_f = 0.64 (c-hexane/EtOAc 6:4 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 532.4, calcd 532.2; ^{1}H NMR (270 MHz, $CDCl_3$): 7.94-7.92 (m, 2H, *p*-toluoyl), 7.51-7.20 (m, 12H, 2 x benzyl + *p*-toluoyl), 4.7 (s, 1H, H-1), 4.65-4.46 (m, 6H, $2CH_2Ph$ + H-7', H-7"), 4.31 (d, 1H, $J_{H\text{-}2,\,H\text{-}3}$ = 5.4 Hz, H-3), 3.96 (d, 1H, H-2), 3.55-3.34 (ABq, J_{gem} = 9.26 Hz, 2H, H-5', H-5", CH_2OBn), 3.25 (s, 3H, OCH_3), 2.28 (s, 3H, OCH_3, *p*-toluoyl), 2.38 (m, 2H, H-6', H-6", CH_2); ^{13}C NMR (67.9 MHz, $CDCl_3$): 166.4 (C=O), 143.4, 138.0, 137.6, 128.5, 128.2, 127.8, 127.5, 105.1 (C-1), 84.3 (q, C-4), 82.5 (C-3), 74.7 (CH_2Ph), 73.6 (C-5), 73.3 (CH_2Ph), 66.3 (C-2), 61.2 (C-7), 55.0 (OCH_3), 31.6 (C-6), 21.1 (CH_3, *p*-toluoyl).

1-*O*-Acetyl-3,5-di-*O*-benzyl-(2-deoxy-2-azido)-4-*C*-(*p*-toluoyloxyethyl)-D-ribofuranose (10). Compound **9** (1.0 g, 1.88 mmol) was dissolved in acetic acid (16 mL), acetic anhydride (3.6 mL) was added and reaction was put to ice bath under nitrogen atmosphere. To this solution sulfuric acid (0.24 mL) was added and reaction was stirred at 0 °C for 3 h. Reaction was poured into ice-cooled water/brine solution and extracted with EtOAc, washed with another portion of water and finally with saturated aqueous solution of $NaHCO_3$. Organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **10** (1.0 g, 1.88 mmol, 95%) as mixture of anomers. R_f = 0.33 (c-hexane/EtOAc 7:3 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 560.4, calcd 560.2; ^{13}C NMR (67.9 MHz, $CDCl_3$): 169.0, 167.1, 155.7, 143.4, 137.6, 137.4, 129.0, 128.5, 128.2, 127.8, 127.5, 127.3, 97.9, 96.2, 88.8, 85.9, 80.7, 80.2, 74.8, 73.7, 73.6, 73.4, 65.7, 61.2, 60.8, 31.9, 31.7, 21.7, 21.0, 21.1.

***N*^{6}-Benzoyl-9-[3,5-di-*O*-benzyl-(2-deoxy-2-azido)-4-*C*-(*p*-toluoyloxyethyl)-*β*-D-ribofuranosyl]adenine (11)**. Compound **10** (1.3 g, 2.32 mmol) was co-evaporated with dry acetonitrile, dissolved in the same solvent (35 mL) and added to silylated under standard condition[32, 33] *N*^{6}-benzoyladenine (0.832 g, 3.48 mmol). Trimethylsilyl trifluoromethanesulphonate (0.421 g, 2.55 mmol) was added and reaction was refluxed for 12 h under nitrogen atmosphere, after that cooled to rt and poured into ice-cooled solution of

saturated $NaHCO_3$. Compound was extracted with CH_2Cl_2 (2x), organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **11** (1.18 g, 2.22 mmol, 57%) as *β* anomer. R_f = 0.56 (CH_2Cl_2/MeOH 95:5 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 739.1, calcd 739.3; ^{1}H NMR (270 MHz, $CDCl_3$): 8.95 (s, 1H, N^6-H), 8.75 (s, 1H, H-2), 8.21 (s, 1H, H-8), 8.02-7.10 (m, 19H, *p*-toluoyl + 2 x benzyl + benzoyl), 6.15 (d, $J_{H\text{-}1,\ H\text{-}2}$ = 4.9 Hz, 1H, H-1'), 4.89 (t, 1H, $J_{H\text{-}2,\ H\text{-}3}$ = 5.4 Hz, H-2'), 4.80-4.38 (m, 7H, $2CH_2Ph$ + H-7', H-7'' + H-3'), 3.74-3.44 (ABq, J_{gem} = 10.26 Hz, 2H, H-5', H-5'', CH_2OBn), 2.45 (m, 1H, H-6'), 2.40 (s, 3H, OCH_3, *p*-toluoyl), 2.12 (m, 1H, H-6''); ^{13}C NMR (67.9 MHz, $CDCl_3$): 166.6 (C=O), 164.3 (C=O), 152.8 (C-8), 149.7 (C-4), 143.9 (C-2), 141.6 (C-6), 137.2, 137.0, 133.7, 132.9, 132.8, 129.6, 129.1, 128.9, 128.6, 128.4, 128.3, 128.1, 127.9, 123.3 (C-5), 87.3 (q, C-4'), 86.7 (C-1'), 79.6 (C-3'), 74.5 (CH_2Ph), 73.7 (CH_2Ph), 72.3 (C-5'), 64.8 (C-2'), 60.6 (C-7'), 31.4 (C-6'), 21.7 (CH_3, *p*-toluoyl).

N^6-Benzoyl-9-[3,5-di-*O*-benzyl-(2-deoxy-2-azido)-4-*C*-(*p*-toluenesulfonyloxyethyl)-*β*-D-ribofuranosyl]adenine (12). Compound **11** (0.3 g, 0.39 mmol) was dissolved in mixture of ethanol/pyridine (1:1, v/v, 1.2 mL/1.2 mL) and to that solution 2 *N* NaOH/ethanol (1:1, v/v, 1.6 mL/1.6 mL) was added. Reaction was stirred at rt for 6 min and then Amberlite H^+ Py form was added to neutralize the base. After filtration solution was evaporated to dryness, co-evaporated with dry pyridine (3x) and dissolved in the same solvent (5 mL). *p*-Toluenesulfonyl chloride (81 mg, 0.43 mmol) was added and reaction was stirred at rt overnight under nitrogen atmosphere. Reaction was quenched by addition of ice-cooled solution of saturated $NaHCO_3$. Compound was extracted with CH_2Cl_2 (2x), organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **12** (226 mg, 0.299 mmol, 72% after two steps). R_f = 0.90 (CH_2Cl_2/MeOH 95:5 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 775.1, calcd 775.3; ^{1}H NMR (270 MHz, $CDCl_3$): 8.94 (s, 1H, N^6-H), 8.74 (s, 1H, H-2), 8.18 (s, 1H, H-8), 8.02-7.16 (m, 19H, *p*-toluenosulfonyl + 2 x benzyl + benzoyl), 6.01 (d, $J_{H\text{-}1',\ H\text{-}2'}$ = 5.07 Hz, 1H, H-1'), 4.87 (t, 1H, $J_{H\text{-}2,\ H\text{-}3}$ = 5.56 Hz, H-2'), 4.77-4.40 (m, 5H, $2CH_2Ph$ + H-3'), 4.17 (t, $J_{H\text{-}6,\ H\text{-}7}$ = 6.18 Hz, 2H, H-7', H-7''), 3.60-3.33 (ABq, J_{gem} = 10.36 Hz, 2H, H-5', H-5'', CH_2OBn), 2.40 (m, 4H, OCH_3, *p*-toluenosulfonyl + H-6'), 2.05 (m, 1H, H-6''); ^{13}C NMR (67.9 MHz, $CDCl_3$): 164.1 (C=O), 152.8 (C-8), 151.1, 149.3 (C-4), 143.8 (C-2), 141.9 (C-6), 136.8, 136.7, 133.2, 132.9, 129.9, 129.0, 128.7, 128.3, 127.9, 127.8, 123.1 (C-5), 86.8 (q, C-4'), 86.6 (C-1'), 79.6 (C-3'), 74.5 (CH_2Ph), 73.7 (CH_2Ph), 72.3 (C-5'), 66.4 (C-7'), 64.7 (C-2'), 31.7 (C-6'), 21.7 (CH_3, *p*-toluenosulfonyl).

(1R,5R,7R,8S)-5-Benzyloxymethyl-8-benzyloxy-7-[N^6-benzoyl-(adenin-9-yl)]-2-aza-6-oxabicyclo[3.2 .1]octane (13). Compound **12** (0.175 g, 0.226 mmol) was dissolved in dry THF and to that solution 2 *N* NaOH (5.2 mL) was added followed by addition of 1 M solution of trimethylphosphine in THF (0.452 mL, 0.452 mmol). Reaction was stirred at rt for 3 h, after which time reaction was quenched by adding

ice-cooled solution of brine and compound was extracted with CH_2Cl_2 (3x), organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-10% MeOH in CH_2Cl_2 v/v), which afforded **13** (90 mg, 0.156 mmol, 69%). R_f = 0.32 (CH_2Cl_2/MeOH 95:5 v/v); MALDI - TOF *m/z* $[M+H]^+$ found 577.1, calcd 577.3; ^{1}H NMR (270 MHz, $CDCl_3$): 8.93 (1H, s, NH), 8.77 (1H, s, H2), 8.72 (1H, s, H8), 8.04 (2H, m), 7.58 (3H, m), 7.44 – 7.17 (10H, m), 6.45 (1H, s, H1'), 4.65 (1H, *J* = 12.5 Hz, CH_2Ph), 4.58 (1H, *J* = 12.5 Hz, CH_2Ph), 4.55 (1H, d, *J* = 11.7 Hz, CH_2Ph), 4.47 (1H, d, *J* = 11.7 Hz, CH_2Ph), 4.26 (1H, d, $J_{H\text{-}2,\ H\text{-}3}$ = 3.6 Hz, H3'), 3.72 (1H, d, *J* = 3.6 Hz, H2'), 3.65 (1H, d, *J* = 10.8 Hz, H5'), 3.52 (1H, d, *J* = 10.8 Hz, H5''), 3.22 (1H, ddd, *J* = 4.9, 11.9, 13.6 Hz, H7''), 3.07 (1H, dd, *J* = 6.1, 13.7 Hz, H7'), 2.02 (1H, ddd, *J* = 6.6, 11.7, 13.0 Hz, H6'), 1.37 (1H, dd, *J* = 4.6, 13.1 Hz, H6''); ^{13}C NMR (67.9 MHz, $CDCl_3$): 164.9 (C=O), 152.9 (C-8), 151.4(C-4), 149.6(C-2), 142.1(C-6), 137.9, 134.1, 133.2, 129.3, 129.0, 128.9, 128.5, 128.4, 128.3, 128.2, 128.0, 124.1 (C-5), 87.0 (C-1'), 84.8 (q, C-4'), 73.8 (CH_2Ph), 73.4 (C-3'), 72.7 (CH_2Ph), 70.4 (C-5'), 60.1 (C-2'), 39.3 (C-7'), 28.2 (C-6').

(1R,5R,7R,8S)-8-Hydroxy-5-hydroxymethyl-7-[*N*^{6}-benzoyl-(adenin-9-yl)]-2-aza-2-*N*-trifloroacetic-6-oxabicyclo[3.2.1]octane (14). Compound **13** (0.08 g, 0.139 mmol) was dissolved in dry pyridine/CH_2Cl_2 solution (5/25, v/v, 2 mL) and put to the ice bath. To cooled solution trifluoroacetic anhydride (0.039 mL, 0.278 mmol) was added and after 5 min reaction was warmed to rt and stirred for additional 20 min. After that reaction was quenched by addition of ice-cooled solution of saturated $NaHCO_3$, and compound was extracted with CH_2Cl_2 (3x), organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. After evaporation of solvent the crude product was co-evaporated with dry toluene (2x) and dissolved in dry CH_2Cl_2. Reaction was put to dry ice/EtOH bath (-78 ^{o}C) and 1 *M* BCl_3 solution (1.39 mL, 1.39 mmol) was added. Reaction was stirred for 30 min at -78 ^{o}C and overnight at rt Reaction was quenched by adding 2 mL of MeOH and evaporated. Product was purified by silica gel column chromatography (0-20% MeOH in CH_2Cl_2 v/v), which afforded **14** (34 mg, 0.07 mmol, 50% after two steps) as mixture of diastereomers. R_f = 0.42 (CH_2Cl_2/MeOH 95:5 v/v); MALDI - TOF *m/z* $[M+H]^+$ found 493.0, calcd 493.1; ^{13}C NMR (67.9 MHz, $CDCl_3$): 166.9, 152.4, 150.5, 141.5, 132.9, 132.8, 131.1, 128.7, 128.1, 127.9, 122.2, 122.1, 85.7, 85.3, 84.8, 77.3, 64.5, 63.9, 61.5, 60.1, 59.5, 38.1, 31.2, 26.1, 18.3.

(1R,5R,7R,8S)-5-(4,4'-Dimethoxytrityloxymethyl)-8-hydroxy-7-[*N*^{6}-benzoyl-(adenin-9-yl)]-2-aza-2-*N*-trifloroacetic-6-oxabicyclo[3.2.1]octane (15). Compound **14** (0.03 g, 0.6 mmol) was co-evaporated with dry pyridine to remove traces of water, dissolved in the same solvent (2 mL) and 4,4'-dimethoxytrityl chloride (22.3 mg, 0.66 mmol) was added. Reaction was stirred overnight at rt under nitrogen atmosphere. Reaction was quenched by addition of ice-cooled solution of saturated $NaHCO_3$ and compound was extracted with CH_2Cl_2 (3x), organic phase was dried over anhydrous $MgSO_4$ and

evaporated under reduced pressure. After evaporation of solvent the crude product was co-evaporated with toluene (2x) and purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **15** (41 mg, 0.05 mmol, 83%) as mixture of diastereomers. R_f = 0.64 (CH_2Cl_2/MeOH 95:5 v/v); MALDI-TOF *m/z* $[M + H]^+$ found 795.6, calcd 795.3; ^{13}C NMR (67.9 MHz, $CDCl_3$): 164.7, 158.7, 153.0, 150,5 149.6, 144.2, 141.0, 135.3, 133.7, 132.9, 130.0, 128.9, 128.1, 127.9, 127.2, 123.5, 123.4, 113.4, 87.1, 87.0, 85.6, 84.9, 77.2, 67.0, 66.4, 63.6, 63.5, 59.5, 55.3, 26.9, 26.1, 22.7.

(1R,5R,7R,8S)-8-Hydroxy-5-hydroxymethyl-7-(adenin-9-yl)-2-aza-6-oxabicyclo[3.2.1]octane (14a). Compound **14** (3.5 mg, 0.0072 mmol) was treated with 32% aqueous ammonia solution at rt overnight. The mixture was evaporated and purified by preparative TLC (CH_2Cl_2/MeOH 80:20 v/v) to afford **14a** (1.8 mg, 0.0061 mmol, 85%). R_f = 0.14 (CH_2Cl_2/MeOH 80:20 v/v); MALDI-TOF *m/z* $[M + H]^+$ found 293.2, calcd 293.1; ^1H (500 MHz, DMSO-d_6): 8.49 (1H, s, H2), 8.17 (1H, s, H8), 7.29 (2H, s, NH_2), 6.24 (1H, s, H1'), 5.19 (1H, t, *J* = Hz, OH (C5'), 5.09 (1H, d, *J* = Hz, OH (C3'), 4.23 (1H, t, *J* = 3.9, 3.9 Hz, H3') 3.56 (1H, dd, *J* = 12.2 Hz, H5'), 3.51 (1H, dd, *J* = 12.2 Hz, H5''), 3.41 (1H, d, *J* = 3.9 Hz, H2'), 3.03 (1H, ddd, *J* = 4.7, 11.8, 13.3 Hz, H7''), 2.91 (1H, dd, *J* = 6.8, 13.3Hz, H7'), 1.82 (1H, ddd, *J* = 6.8, 11.8, 13.0 Hz, H6'), 1.24 (1H, dd, *J* = 4.7, 13.0 Hz H6''); ^{13}C (125.7 MHz, DMSO-d_6): 156.9 (C6), 153.3 (C2), 149.3 (C4), 139.8 (C8), 120.3 (C5), 85.9 (C4'), 85.8 (C1'), 65.6 (C3'), 63.0 (C5'), 62.3 (C2'), 39.0 (C7'), 27.4 (C6').

3,5-Di-*O*-benzyl-4-*C*-(methanesulfonyloxyethyl)-1,2-*O*-isopropylidene-*β*-D-arabinofuranose (16). Compound **5** (1.7 g, 4.13 mmol) was two times co-evaporated with dry pyridine and dissolved in the same solvent. Reaction was put to ice bath and methanesulfonyl chloride (0.64 mL, 8.26 mmol) was added under nitrogen atmosphere. Reaction mixture stirred for 1 h at 0 ºC, quenched with saturated solution of $NaHCO_3$ and extracted (2x) with CH_2Cl_2. The organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-30% EtOAc in cyclohexane v/v), which afforded **16** (1.47 g, 2.98 mmol, 72%). R_f = 0.70 (c-hexane/EtOAc 6:4 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 493.1, calcd 493.2; ^1H NMR (270 MHz, $CDCl_3$): 7.32-7.23 (m, 10H, 2 x benzyl), 5.86 (d, $J_{H\text{-}1,\,H\text{-}2}$ = 4.21 Hz, 1H, H-1), 4.79-4.33 (m, 7H, H-2 + $2CH_2Ph$ + H-7', H-7''), 4.03 (s, 1H, H-3), 3.61-3.41 (ABq, J_{gem} = 9.53 Hz, 2H, H-5', H-5'', CH_2OBn), 2.86 (s, 3H, CH_3, methanesulfonyl), 2.21 (m, 2H, H-6', H-6'', CH_2), 1.37 (s, 3H, CH_3, isopropyl), 1.28 (s, 3H, CH_3, isopropyl); ^{13}C NMR (67.9 MHz, $CDCl_3$): 137.2, 128.5, 128.2, 127.8, 112.3 (q, isopropyl), 104.9 (C-1), 88.1 (q, C-4), 85.0 (C-2), 84.8 (C-3), 73.5 (CH_2Ph), 72.4 (CH_2Ph), 72.3 (C-5), 67.3 (C-7), 37.2 (CH_3, methanesulfonyl), 31.9 (C-6), 26.6 (CH_3, isopropyl), 26.0 (CH_3, isopropyl).

3,5-Di-*O*-benzyl-4-*C*-hydroxyethyl-1-*O*-methoxy-D-arabinofuranose (17). Compound **5** (75 mg, 0.18 mmol) was dissolved in dry MeOH (12 mL) and concentrated sulfuric acid (3 μL) was added. Reaction mixture was refluxed under nitrogen atmosphere for 30 min, cooled down to rt and quenched with

ice-cooled solution of saturated $NaHCO_3$. Compound was extracted (2x) with CH_2Cl_2, organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **17** (63 mg, 0.16 mmol, 80%) as mixture of anomers. R_f = 0.57 (CH_2Cl_2/MeOH 95:4 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 389.5, calcd 389.2; ^{13}C NMR (67.9 MHz, $CDCl_3/CD_3OD$): 137.5, 136.8, 128.4, 128.7, 128.5, 128.0, 127.8, 110.4, 100.8, 88.5, 86.9, 87.0, 86.8, 86.1, 78.9, 74.5, 74.0, 72.6, 59.0, 55.7, 55.2, 35.7, 35.2.

3,5-Di-*O*-benzyl-4-*C*-(*p*-toluenesulfonyloxyethyl)-1-*O*-methoxy-D-arabinofuranose (18). Compound **17** (47 mg, 0.12 mmol) was co-evaporeted with dry pyridine and dissolved in the same solvent (1.5 mL). Solution was put to ice bath and *p*-toluenesulphonyl chloride (24 mg, 0.12 mmol) was added and reaction was stirred at 0 ºC for 30 min and at rt overnight under nitrogen atmosphere. Reaction was quenched by addition of ice-cooled solution of saturated $NaHCO_3$. Compound was extracted (2x) with CH_2Cl_2, organic phase was separated, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (0-5% MeOH in CH_2Cl_2 v/v), which afforded **18** (48 mg, 0.09 mmol, 75%) as mixture of anomers. R_f = 0.70 (CH_2Cl_2/MeOH 95:4 v/v); TOF-MS ES+ *m/z* $[M+H]^+$ found 543.4, calcd 543.2; ^{13}C NMR (67.9 MHz, $CDCl_3/CD_3OD$): 144.5, 139.7, 138.1, 133.4, 129.8, 128.4, 128.3, 128.2, 127.9, 127.6, 100.7, 86.8, 83.0, 77.2, 73.6, 72.3, 67.3, 55.2, 55.2, 32.3, 21.6.

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