A Simplified and Efficient Route to 2′-*O*, 4′-*C*-Methylene-Linked Bicyclic Ribonucleosides (Locked Nucleic Acid)

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A novel efficient method for the synthesis of locked nucleic acid (LNA) monomers is described. The LNA 5′,3′-diols containing thymine, 4-*N*-acetyl- and 4-*N*-benzoylcytosine, 6-*N*-benzoyladenine, and 2-*N*-isobutyrylguanine as nucleobases were prepared via convergent syntheses. The method is based on the use of the common sugar intermediate 1,2-di-*O*-acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-D-erythro-pentofuranose (**8**) that easily can be prepared from D-glucose in multigram scale. Four different nucleobases were stereoselectively coupled to **8** using a modified Vorbrüggen procedure to give the corresponding 4′-*C*-branched nucleoside derivatives. Subsequent ring closing furnished the protected LNA nucleosides. The 5′-*O*-mesyl groups were efficiently displaced by nucleophilic substitution using sodium benzoate. Saponification of the 5′-benzoates followed by catalytic removal of the 3′-*O*-benzyl groups afforded the free LNA diols. The exocyclic amino groups of adenosine and cytidine were selectively acylated to give 4-*N*-acetyl- or 4-*N*-benzoyl-LNA-C and 6-*N*-benzoyl-LNA-A. The isobutyryl group of guanine was retained during the preparation of 2-*N*-isobutyryl-LNA-G. The LNA-T diol and base-protected LNA diols can be directly converted into LNA-phosphoramidites for automated chemical synthesis of LNA containing oligonucleotides.

Introduction

In recent years, much effort has been put into the design and synthesis of modified oligonucleotides (ONs) with the aim of obtaining strong recognition of complementary DNA and/or RNA sequences.[1−3] Among others, conformationally restricted ONs containing bi- and tricyclic carbohydrate-modified nucleotides have been studied with a number of these showing increased stabilities of duplexes with complementary nucleic acids, as compared to the corresponding unmodified duplexes.[3]

ONs containing 2′-*O*,4′-*C*-methylene-linked bicyclic ribonucleosides termed LNA (locked nucleic acid, Figure 1) were introduced a few years ago[4,5] showing unprecedented thermal stabilities toward complementary DNA and RNA with excellent mismatch discrimination.[4] In addition, LNA is fully compatible with conventional DNA/RNA chemistry. Thus, fully or partly modified LNA ONs can efficiently be oligomerized using commercially available automatic DNA synthesizers. The very high thermal stabilities of duplexes containing LNA were attributed to the fixed *N*-type [3′-endo/^3E] conformation of the bicyclic carbohydrate units.[6] The *N*-conformation of the LNA monomers in ONs has been verified by NMR[6a−d] as well as X-ray crystallography.[6e]

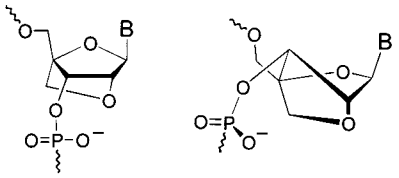

Figure 1. Chemical structure of a LNA nucleotide. The pentofuranose moiety is effectively locked in an N-type (north type) conformation. B = nucleobase.

On the basis of the exciting properties of LNA, a large variety of applications in molecular diagnostics including gene array analysis can be envisioned.[7] In fact, 5′-anthraquinone conjugated 8-mer LNAs have been photochemically immobilized in microtiter plates for use in

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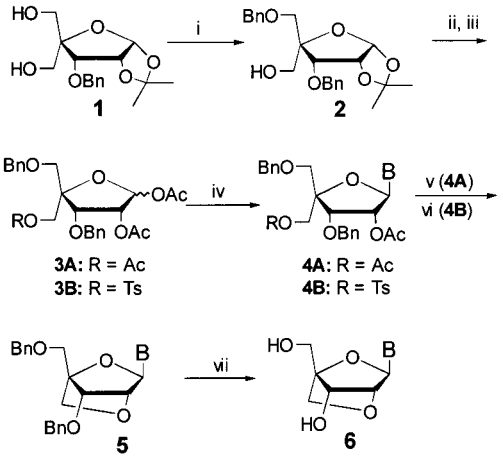
diagnostic genotyping including, for example, the detection of the prothrombic mutation factor V Leiden[8] and the detection of the apolipoprotein B3500 polymorphism causing hypercholesterolemia.[7b] Furthermore, LNA ONs have shown very promising properties as antisense therapeutics. Thus, short sequences of LNA exhibited potent antisense activity in a G-protein coupled receptor assay system in living rats without detectable toxic reactions and were not readily degraded in blood serum and cell extracts.[9]

Although a few novel LNA-like nucleoside analogues were reported to be already synthesized after introduction of LNA,[10] the production of the 2′-*O*,4′-*C*-methylene-ribonucleosides has until now not been a routine procedure. As a result of our continued endeavors for optimizing the synthesis of 2′-*O*, 4′-*C*-methylene-linked nucleosides, we hereby present an improved convergent synthesis of the LNA monomers.

For the synthesis of LNA monomers, two strategies can be envisioned: a linear strategy using nucleosides as the starting material or a convergent strategy where an appropriate modified glycosyl donor is synthesized and then coupled to the nucleobase affording the modified nucleoside. Taking advantage of the former strategy, Obika et al. were the first to report the synthesis of LNA-U and LNA-C nucleosides with uridine as the starting material.[11] We have used a similar strategy for the synthesis of LNA-A.[12] Despite some advantages such as a relatively short chain of chemical transformations and high availability of the starting RNA nucleosides, the linear strategy has turned out not to be effective in terms of scaling up possibilities. A key reaction in the synthetic pathway is the introduction of the additional oxymethyl group into the 4′-*C*-position of the protected RNA nucleosides. This can be carried out via oxidation of the 5′-OH group to the corresponding aldehyde followed by a crossed aldol condensation with formaldehyde and consecutive reduction. All methods reported so far for preparation of 5′-aldehydonucleosides from natural ribonucleosides required handling of complicated reaction mixtures which proved to be difficult to work up, and fine-tuning of the reaction conditions were complicated due to low stability of the intermediates. Moreover, the obtained results were very dependent on the nature of the nucleosides, and no universal method was developed.

Alternatively, a convergent strategy for the synthesis of LNA monomers was explored by Wengel and coworkers.[4a,b] Using the previously described 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (**1**)[13a] (Scheme 1) as a starting material, LNA monomers with all the natural nucleobases were synthesized and incorporated into DNA.[4a,b] Importantly, **1** can readily be synthesized in multigram scale from D-glucose.[13] However, the use of **1** was complicated by the presence of two diastereotopic hydroxymethyl groups. Even though the regioselective benzylation of **1** to **2** has been described,[4a,b,14] it was impossible for us to obtain the reported 71% yield when performing the reaction on a larger scale. Additionally, all attempts to increase the regioselectivity by varying the reaction conditions (e.g., temperature and concentration) failed as well, as did the use of the dibutyltin oxide method.[15] Thus, chromatographic purification of the reaction mixture remained necessary. Acetylation of **2** followed by acetolysis and subsequent acetylation afforded **3A** that was used as a glycosyl donor for coupling to the appropriate protected nucleobases to give the 4′-*C*-branched nucleoside derivatives **4A**. Deacetylation with sodium methoxide and monotosylation followed by a ring closing reaction gave the protected LNA nucleosides **5**. The free diols **6** were finally obtained after debenzylation of **5**.

Scheme 1[a]



[a] Reagents: (i) NaH, BnBr, DMF; (ii) Ac_2O (for **3A**) or TsCl (for **3B**), pyridine; (iii) (a) 80% AcOH, (b) Ac_2O, pyridine; (iv) nucleobase, BSA, TMSOTf, MeCN or 1,2-dichloroethane; (v) (a) MeONa, MeOH, (b) TsCl, pyridine, (c) NaH, DMF; (vi) (a) NH_3, methanol, (b) NaH, DMF; (vii) debenzylation. B = nucleobase.

Later, Wengel reported[12] an improvement for this procedure, taking advantage of another glycosyl donor. Using the coupling sugar **3B** (Scheme 1) containing tosyl instead of acetyl on the 4-*C*-hydroxymethyl group, the nucleoside **4B** could be obtained. In contrast to **4A**, **4B** was converted into protected LNA nucleoside **5** via a simple two-step ring closing procedure, which was performed by deacetylation with methanolic ammonia followed by reaction with sodium hydride in DMF. However, this method was only exemplified by the synthesis of the LNA-T nucleoside.

To synthesize LNA monomers on a larger scale, the synthetic pathway based on the selective benzylation of **1** is not ideal. In this paper, we present a new strategy for synthesis of LNA monomers based on a new di-*O*-mesylated glycosyl donor. Using this strategy, LNA

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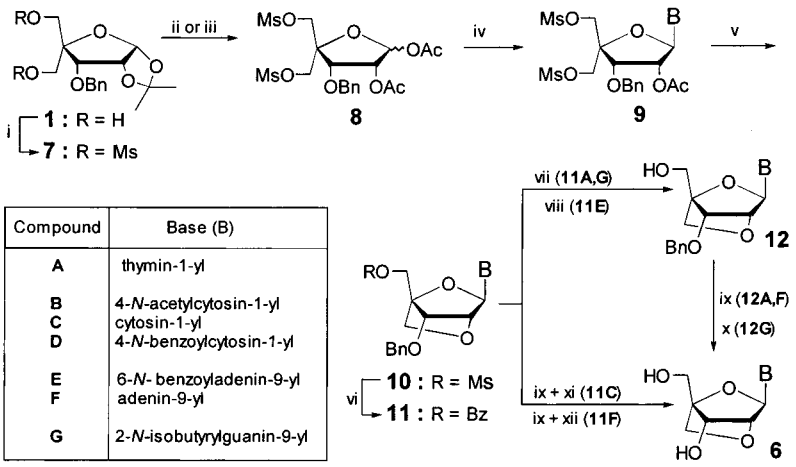
Scheme 2[a]



Compound	Base (B)
A	thymin-1-yl
B	4-*N*-acetylcytosin-1-yl
C	cytosin-1-yl
D	4-*N*-benzoylcytosin-1-yl
E	6-*N*- benzoyladenin-9-yl
F	adenin-9-yl
G	2-*N*-isobutyrylguanin-9-yl

[a] Reagents: (i) MsCl, pyridine; (ii) (a) 80% TFA; (b) Ac_2O, pyridine; (iii) AcOH, Ac_2O, concd H_2SO_4; (iv) nucleobase, BSA, TMSOTf, MeCN or 1,2-dichloroethane; (v) NaOH or LiOH, THF or 1,4-dioxane (+NH_4OH for **10F**); (vi) NaOBz, DMF; (vii) NaOH, THF or EtOH/pyridine; (viii) NH_4OH, $MeNH_2$, MeOH; (ix) 20% $Pd(OH)_2$/C, HCO_2NH_4, MeOH; (x) 10% Pd/C, HCO_2H, MeOH; (xi) NH_4OH, (xii) (a) BzCl, pyridine; (b) NaOH, EtOH/pyridine.

nucleosides with the four natural nucleobases thymine, adenine, cytosine, and guanine have effectively been synthesized.

Results and Discussion

On the basis of the convergent strategy published by Wengel and co-workers (Scheme 1),[4b,12] we developed a more efficient method for the synthesis of all four LNA nucleosides. The LNA nucleosides containing thymine, Bz-protected adenine, Bz- or Ac-protected cytosine, and isobutyryl-protected guanine were synthesized. The nucleobase protecting groups of the LNA nucleosides were chosen to make the monomers fully compatible with chemical oligomerization based on the phosphite triester methodology.

To overcome the early chromatographic purification step and essentially increase the overall yields of the syntheses, we were interested in replacing the benzyl group on the 5-hydroxy functionality. Encouraged by the positive result obtained with the glycosyl donor **3B**, we envisioned that it would be possible to convert the furanose **1** into 1,2-di-*O*-acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-D-*erythro*-pentofuranose **8** (Scheme 2) and use this as a universal glycosyl donor. Indeed, permesylation of the ribofuranose **1** gave the di-*O*-mesylated compound **7**, and subsequent acetolysis and acetylation in a one-pot procedure afforded the anomeric mixture **8** in nearly quantitative yield without any chromatographic purification.

The anomeric mixture **8** was then used as a common glycosyl donor for coupling reactions with protected nucleobases by the method of Vorbrüggen and co-workers.[16] Thus, stereoselective reaction of **8** with silylated thymine afforded nucleoside **9A** in 88% yield after chromatographic purification. The compound was subsequently converted into the 3′,5′-di-*O*-protected LNA-T nucleoside **10A** in a one-pot deacetylation and ring closing procedure after treatment of **9A** with aqueous sodium hydroxide. This fast conversion proceeded very smoothly, and no traces of side products from 5′-*O*-demesylation or reaction(s) with the nucleobase were detected. Recently, an analogous method was used for the ring closing procedure during the synthesis of the 3′-azido LNA-T nucleoside using a methanolic solution of potassium carbonate.[17] To deprotect the 5′-hydroxy group, we attempted a direct hydrolysis of mesylate **10A**. The consecutive heating of the same reaction mixture that was used for the ring closing reaction resulted in hydrolysis (or nucleophilic substitution) of the 5′-*O*-mesylate, and nucleoside **12A** was isolated in 78% yield. However, removal of the 5′-*O*-mesyl group using this method was time-consuming as it was necessary to reflux the reaction mixture for 1−2 days. Furthermore, the reaction was accompanied by the formation of a number of side products. We therefore investigated nucleophilic displacement of the mesyl group by sodium benzoate.[18] Thus, after treatment of **10A** with sodium benzoate in DMF, the benzoylated nucleoside **11A** was easily obtained after crystallization from ethanol in 86% yield. It is worth noticing that the 5′-mesylate likely can be substituted by other benzoate-like nucleophilic reagents, allowing the synthesis of other 5′-modified LNA nucleosides, for example, LNA triphosphates. Saponification of the 5′-*O*-benzoyl ester of **11A** afforded the 3′-*O*-benzylated LNA-T nucleoside **12A** in 91% yield. Subsequent catalytic removal of the 3′-*O*-benzyl group afforded the desired unprotected LNA-T nucleoside **6A** as a crystalline product in 83% yield (Scheme 2).

Following the principle of a convergent synthesis strategy, analogous synthetic procedures were used for the synthesis of the other LNA monomers. The cytidine derivative **9B** was furnished in 82% yield via stereoselective coupling of **8** with 4-*N*-acetylcytosine. Treatment of **9B** with sodium hydroxide resulted in a 2′-*O*-deprotection and ring closing reaction in a similar way as described for **9A**. Additionally, due to the lability of the 4-*N*-acetyl group of **9B**, the reaction was allowed to proceed until complete nucleobase deprotection was

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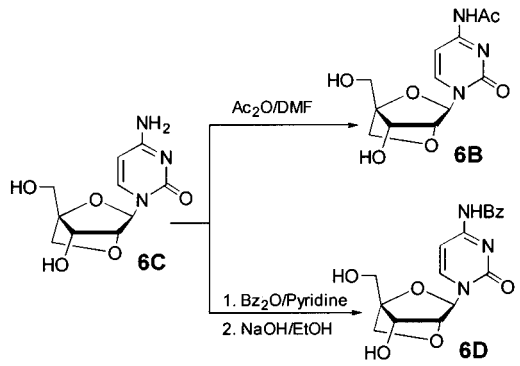
Scheme 3



accomplished, thus giving **10C**, which could be isolated as a white solid material in 87% yield. Nucleophilic displacement of the 5′-mesylate group with benzoate was performed by treatment of **10C** with sodium benzoate in DMF at 100 °C and afforded the benzoylated nucleoside **11C** in 71% yield. Similar reactions of the mesyloxy groups have been reported by Kellogg et al.[19] taking advantage of cesium acetate rather than sodium benzoate. However, a similar nucleophilic substitution of the mesylate **10C** by the acetate was unsuccessful in our hands. To obtain the fully unprotected LNA-C nucleoside **6C**, the 5′-*O*-benzoyl-3′-*O*-benzyl-LNA-C nucleoside **11C** was subjected to a one-pot procedure involving catalytic hydrogenation and debenzoylation reactions. Thus, treatment of **11C** with ammonium formate and $Pd(OH)_2/C$ in refluxing methanol followed by treatment with ammonium hydroxide afforded the free nucleoside **6C** as a white solid material in 77% yield (Scheme 2). To use this in ON synthesis, the exocyclic amine needed to be protected. This can be done selectively by treatment of the unprotected nucleoside with acetic anhydride in DMF.[20] Thus, **6C** was treated with 1.2 equiv of acetic anhydride in DMF overnight giving the *N*-acetylated derivative **6B** after crystallization from methanol in 65% yield (Scheme 3). For the synthesis of 4-*N*-benzoyl LNA-C **6D**, nucleoside **6C** was reacted with 2 equiv of benzoic anhydride in anhydrous pyridine affording a mixture of mono-, di-, and tribenzoylated nucleosides, which subsequently was partially hydrolyzed after addition of sodium hydroxide to the reaction mixture. Following this reaction sequence, the 4-*N*-benzoylated LNA-C nucleoside **6D** was obtained in 93% yield as a crystalline product (Scheme 3).

It is commonly known that the two major products formed during a Vorbrüggen coupling reaction between a glycosyl donor and a properly protected purine are the *N*-7 and the *N*-9 isomers, where the *N*-9 isomer is the naturally occurring isomer. The latter isomer is the thermodynamic product of the reaction, and hence prolonged reaction time and elevated temperature can increase the yield of this desired isomer.[16] Thus, reactions between silylated purines and **8** were performed under conditions favoring the thermodynamic products. It has previously been shown[16b,21] that the coupling reactions of 6-*N*-benzoyl adenine with different glycosyl donors have to be conducted for longer time than in cases of other purine nucleobases.[16b] The long reaction time was explained by slow conversion of the kinetically favored *N*-3 and *N*-7 products into the thermodynamically favorable *N*-9 isomer. Following this method, the isomerically pure adenosine nucleoside **9E** was isolated by column chromatography in 68% yield after refluxing the anomeric mixture **8** and 6-*N*-benzoyl adenine for 29 h under Vorbrüggen coupling conditions. The deacetylation and ring closing reaction steps were performed as described for the pyrimidine derivatives **9A** and **9B**. Thus, the bicyclic nucleoside **10E** was isolated in 78% yield from **9E** after treatment with aqueous alkali. Both sodium and lithium hydroxide were successfully used to promote the reaction. Since all our attempts to develop a method for selective *O*-debenzylation of the LNA-A nucleosides in the presence of the 6-*N*-benzoyl group failed, we alternatively attempted to remove the 6-*N*-benzoyl group with ammonium hydroxide during the cyclization of **9E** giving derivative **10F** in 78% yield. The 5′-*O*-benzoyl nucleosides **11E** and **11F** were obtained via benzoate displacement of 5′-mesylates **10E** and **10F** in 88% and 84% yield, respectively. The free diol **6F** was obtained in 78% yield after total deprotection of **11E** using a mixture of ammonia and methylamine in methanol followed by catalytic transfer hydrogenation. On the contrary, derivative **11F** was first debenzylated, and the crude product subsequently reacted with a 5-fold excess of benzoyl chloride to give perbenzoylated LNA-A nucleoside. The base-protected diol **6E** was finally obtained in 75% yield after selective saponification of the 3′- and 5′-benzoates with sodium hydroxide (Scheme 2).

The most problematic chemistry encountered during the convergent synthesis of nucleosides with all the naturally occurring nucleic bases usually occurs with guanine. Coupling of guanine-type bases with sugar derivatives produces an isomeric mixture of *N*-9/*N*-7 nucleosides even when the reaction is performed under thermodynamic control. Additionally, the two isomers are often difficult to separate.[21−23] Furthermore, the ratio of produced isomers frequently depends on reaction conditions and the nature of protecting groups on the nucleobases and the glycosyl donors. In the first published synthesis of the LNA-G nucleoside (Scheme 1),[4b] the formation of three isomers (ratio 25:5:2) was detected during the coupling of 2-*N*-isobutyrylguanine with sugar derivative **3A**. Our attempts to couple 2-*N*-isobutyrylguanine with the new coupling sugar **8** resulted in the formation of a mixture of two isomers (ratio ca. 9:1). Further investigations demonstrated that replacement of 2-*N*-isobutyrylguanine by 2-*N*-acetylguanine in the coupling reaction with **8** did not improve the regioselectivity as the corresponding two isomers were obtained in a ratio of ca. 1:1. Furthermore, the glycosidation of 2-*N*-isobutyrylguanine with 1,2-di-*O*-benzoyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-D-*erythro*-pentofuranose gave a mixture with an isomeric ratio of *N*-9/*N*-7 = 6:1 (data not shown). Another method reported for increasing the ratio of *N*-9/*N*-7 isomers in the glycosylation of guanines is constriction of the guanine system into "6-enolate" derivatives.[23] For that purpose, several 6-*O*-protecting groups of guanine, such as diphenylcarbamoyl,[23] benzyl,[24] and *p*-nitrophenyl-

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ethyl[25] protective groups were suggested. However, coupling reactions of **8** with 6-*O*-benzyl-2-*N*-isobutyrylguanine or 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine under standard conditions did not furnish the desired *N*-9 products in satisfactory yields (data not shown). Thus, in this specific case, the best results were obtained when the coupling was performed with **8** and *N*-2-isobutyryl guanine, and this method was chosen for the synthesis of the LNA-G monomer.

The mixture of isomers containing ca. 90% of **9G** was obtained from **8** in 84% yield and used in the next step. An isomerically pure compound was obtained after the ring closing reaction, and hence **10G** was obtained as the pure *N*-9 isomer in 85% yield after chromatographic purification. The nucleoside derivative **10G** was then converted into the diol **6G** via nucleophilic replacement of the mesyl group with benzoyl and a final deprotection of the 5′- and 3′-hydroxy groups generally proceeding as described for the other LNA nucleosides. However, some additional precautions for the handling of the LNA-G nucleoside were required due to the presence of the relatively labile 2-*N*-isobutyryl group. In fact, we had to use an EtOH/pyridine mixture as a solvent during treatment of **11G** with aqueous NaOH for 5′-hydroxyl deprotection. Second, a mixture of Pd/C + HCO_2H was used for the final removal of the 3′-*O*-benzyl group from **12G**. The use of formic acid as a hydrogen donor in the reaction prevented removal of the 2-*N*-isobutyryl group, which took place when ammonium formate was used as the H donor (Scheme 2).

Conclusion

Following the universal convergent scheme as described above, it was possible to efficiently synthesize LNA monomers with all four naturally occurring nucleobases. These can be converted into their corresponding phosphoramidites to be used in LNA synthesis as previously described.[4b,11] The method introduced herein has three key points which make it considerably more useful in comparison to the procedures reported so far: (i) no regioselective benzylation reaction is needed, and the easily produced di-*O*-mesyl furanose **8** is used as a glycosyl donor, (ii) simplified deacetylation and ring closing of nucleosides **9A**, **9B**, **9E**, and **9G** in aqueous solutions, and (iii) effective substitution of 5′-mesylates in all LNA nucleosides by sodium benzoate. The hereby reported results exemplify the power of the convergent strategy, and this strategy can likewise be applied for the syntheses of LNA monomers containing other nucleobases or heterocycles. The synthesis of LNA monomers with other base moieties is currently being investigated and will be presented elsewhere.

Experimental Section

General Procedures. All chemicals were obtained from commercial suppliers and were used without additional purification. An atmosphere of nitrogen was applied when reactions were conducted in anhydrous solvents. Column chromatography was performed using Silica gel 60 (0.063−0.200 mm) from Merck, and HPLC was performed using a column Prep Nova-Pak HR Silica 60 (6 μm, 30 × 300 mm) from Waters. After column chromatography, appropriate fractions were pooled out and concentrated under reduced pressure. Melting points were determined on a Büchi B-540 melting point apparatus and are uncorrected. All ^{1}H NMR spectra were recorded at 400 MHz; ^{13}C NMR spectra were recorded at 62.9 MHz for **11A**, **10C**, **9G**, and **11G** and at 100.6 MHz for all other compounds. The values for chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as the internal standard. Matrix-assisted laser desorption ionization−time of flight (MALDI−TOF) mass spectra were recorded in positive ion mode on a Voyager-DE PRO spectrometer from PerSeptive Biosystems using 2,5-dihydroxybenzoic acid as matrix. Elemental analyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

3-*O*-Benzyl-4-*C*-methanesulfonoxymethyl-5-methanesulfonyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (7). A solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose **1**[13a] (11.1 g, 35.8 mmol) in anhyd pyridine (30 mL) was cooled in an ice bath, and MsCl (8.3 mL, 107.2 mmol) was added. The mixture was stirred for 1 h at room temperature, diluted with Et_2O (200 mL), and washed with H_2O (3 × 200 mL). The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, coevaporated with toluene (2 × 100 mL), and dried in vacuo to yield 16.4 g (98%) of compound **7** as a white solid material. mp 108−110 °C (MeOH). ^{1}H NMR ($CDCl_3$): δ 7.38−7.26 (m, 5H), 5.79 (d, J = 4.0 Hz, 1H), 4.88 (d, J = 11.9 Hz, 1H), 4.77 (d, J = 11.5 Hz, 1H), 4.65 (dd, J = 5.1 and 3.8 Hz, 1H), 4.57 (d, J = 11.5, 1H), 4.42 (d, J = 12.0 Hz, 1H), 4.32 (d, J = 11.0 Hz, 1H), 4.19 (d, J = 5.1 Hz, 1H), 4.15 (d, J = 11.1 Hz, 1H), 3.08 (s, 3H), 2.98 (s, 3H), 1.69 (s, 3H), 1.34 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 136.5, 128.5, 128.3, 128.0, 113.9, 104.4, 83.1, 78.3, 77.7, 72.7, 69.3, 68.5, 37.9, 37.3, 26.1, 25.5. MALDI-MS *m*/*z*: 489.3 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{26}O_{10}S_2$: C, 46.34; H, 5.62. Found: C, 46.33; H, 5.52.

1,2-Di-*O*-acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-D-*erythro*-pentofuranose (8). Method 1. Ac_2O (28 mL, 297 mmol) and concd H_2SO_4 (28 μL) were added to a solution of compound **7** (18.6 g, 39.9 mmol) in AcOH (280 mL), and the mixture was stirred overnight at room temperature. More concd H_2SO_4 (5 μL) was added, and the reaction was continued for 24 h. H_2O (200 mL) was added, and the mixture was stirred for 3 h and washed twice with CH_2Cl_2 (250 and 100 mL). The combined organic layers were washed with saturated $NaHCO_3$ (4 × 200 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give compound **8** (19.7 g, 97%) as a colorless syrup (two anomers; ratio ∼1:5).

Method 2. A solution of compound **7** (16 g, 34.3 mmol) in 80% aqueous trifluoroacetic acid (100 mL) was stirred at room temperature for 1 h. The solvents were removed under reduced pressure, and the residue was dissolved in dichloromethane (200 mL) and washed with saturated $NaHCO_3$ (2 × 200 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a colorless oily intermediate. The intermediate was coevaporated with anhyd pyridine (2 × 50 mL), dissolved in anhyd pyridine, and treated with Ac_2O (12 mL, 127 mmol) overnight. The reaction mixture was quenched by addition of saturated $NaHCO_3$ (250 mL) and washed with EtOAc (2 × 200 mL). The organic layers were combined, washed with brine (150 mL), dried (Na_2SO_4), and concentrated under reduced pressure to yield compound **8** (15.9 g, 91%) as a mixture of two stereoisomers (ratio ∼1:7 by ^{1}H NMR). The product was pure by NMR spectra and was used in the next step without further purification. The analytical pure sample was obtained by silica gel column chromatography (20−50% v/v EtOAc/hexane). ^{13}C NMR ($CDCl_3$, main isomer): δ 169.1, 168.6, 136.3, 128.5−128.0 (m), 97.2, 82.6, 78.6, 73.9, 73.3, 68.8, 68.4, 37.5, 37.3, 20.8, 20.5. MALDI-MS *m*/*z*: 533.7 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{26}O_{12}S_2$: C, 44.70; H, 5.13. Found: C, 44.80; H, 5.06.

1-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl)thymine (9A). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) (70 mL, 283 mmol) was added to a mixture of **8** (52 g, 102 mmol) and thymine (16 g, 127 mmol) in anhyd MeCN (250 mL). The reaction mixture was refluxed for 1 h to get a clear solution.

(25) Jenny, T. F.; Schneider, K. C.; Benner, S. A. *Nucleosides Nucleotides* **1992**, *11*, 1257.

Trimethylsilyl triflate (TMSOTf) (24 mL, 133 mmol) was added, and refluxing was continued further for 4 h. The solution was cooled to room temperature, diluted with CH_2Cl_2 (200 mL), and washed with saturated $NaHCO_3$ (2 × 250 mL). The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (0−2% v/v MeOH/CH_2Cl_2) to give **9A** (51.6 g, 88%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 9.33 (br s, 1H), 7.40−7.28 (m, 5H), 7.08 (d, J = 1.2 Hz, 1H), 5.71 (d, J = 3.3 Hz, 1H), 5.58 (dd, J = 6.4 and 3.3 Hz, 1H), 4.70 (d, J = 6.4 Hz, 1H), 4.60 (d, J = 10.8 Hz, 1H), 4.55 (d, J = 10.8 Hz, 1H), 4.53 (d, J = 11.7 Hz, 1H), 4.38 (d, J = 10.8 Hz, 1H), 4.34 (d, J = 10.8 Hz, 1H), 4.32 (d, J = 11.7 Hz, 1H), 3.02 (s, 3H), 3.00 (s, 3H), 2.11 (s, 3H), 1.92 (d, J = 1.1 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 170.0, 163.7, 150.1, 137.9, 136.6, 128.6, 128.5, 128.4, 111.8, 92.4, 84.0, 77.9, 74.8, 73.7, 68.4, 67.5, 37.7, 37.6, 20.7, 12.6. MALDI-MS *m*/*z*: 598.8 $[M + Na]^+$. Anal. Calcd for $C_{22}H_{28}N_2O_{12}S_2$: C, 45.83; H, 4.89; N, 4.86. Found: C, 45.80; H, 4.83; N, 4.65.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-methanesulfonoxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (10A). To a solution of compound **9A** (44 g, 76.3 mmol) in 1,4-dioxane/H_2O (1/1 v/v; 200 mL) was added 2 M NaOH (200 mL). The mixture was stirred for 1 h at room temperature, diluted with saturated $NaHCO_3$ (200 mL), and extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were dried (Na_2SO_4), concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (1−3% v/v MeOH/CH_2Cl_2) to give **10A** (31.5 g, 94%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 9.24 (br s, 1H), 7.41−7.22 (m, 6H), 5.68 (s, 1H), 4.66 (d, J = 11.5 Hz, 1H), 4.61 (s, 1H), 4.59 (d, J = 12.1 Hz, 1H), 4.56 (d, J = 11.5 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 4.08 (d, J = 7.9 Hz, 1H), 3.93 (s, 1H), 3.87 (d, J = 7.9 Hz, 1H), 3.08 (s, 3H), 1.93 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 163.6, 149.6, 136.3, 134.0, 128.4, 128.2, 127.8, 110.7, 87.5, 85.5, 76.6, 75.9, 72.3, 71.5, 64.0, 37.8, 12.4. MALDI-MS *m*/*z*: 439.7 $[M + H]^+$. Anal. Calcd for $C_{19}H_{22}N_2O_8S\cdot{}^1/_6\ H_2O$: C, 51.70; H, 5.10; N, 6.35. Found: C, 51.73; H, 5.11; N, 6.45.

(1*S*,3*R*,4*R*,7*S*)-1-Benzoyloxymethyl-7-benzyloxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (11A). NaOBz (5.4 g, 37.5 mmol) was added to a solution of compound **10A** (8.2 g, 18.7 mmol) in anhyd DMF (400 mL). The mixture was stirred for 5 h at 100 °C, cooled to room temperature, and filtrated. The solvent was evaporated under reduced pressure, and the residue was suspended in EtOAc (150 mL), washed with H_2O (3 × 100 mL), dried (Na_2SO_4), and concentrated to a solid residue. Crystallization from EtOH afforded **11A** (7.55 g, 86%) as a white solid material. mp 164−166 °C. ^{1}H NMR ($CDCl_3$): δ 8.78 (br s, 1H), 7.94 (m, 2H), 7.61 (m, 1H), 7.45 (m, 2H), 7.30−7.20 (m, 6H), 5.63 (s, 1H), 4.83 (d, J = 12.6 Hz, 1H), 4.73 (d, J = 11.9 Hz, 1H), 4.66 (s, 1H), 4.56 (d, J = 12.6 Hz, 1H), 4.52 (d, J = 11.9 Hz, 1H), 4.18 (d, J = 7.7 Hz, 1H), 3.97 (d, J = 7.8, 1H), 3.91 (s, 1H), 1.58 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 165.5, 163.4, 149.5, 136.4, 133.7, 133.6, 129.5, 129.1, 128.6, 128.5, 128.2, 127.8, 110.3, 87.5, 86.2, 76.6, 75.7, 72.1, 72.0, 58.9, 12.1. MALDI-MS *m*/*z*: 487.3 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{24}N_2O_7\cdot{}^1/_4H_2O$: C, 64.03; H, 5.26; N, 5.97. Found: C, 63.88; H, 5.18; N, 5.95.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (12A). Method 1. H_2O (50 mL) and 2 M NaOH (200 mL) were added to a solution of compound **10A** (22 g, 50 mmol) in 1,4-dioxane (200 mL). The reaction mixture was refluxed for 24 h, cooled to room temperature, and neutralized with AcOH (25 mL). Saturated $NaHCO_3$ (200 mL) was added, and the mixture was washed with CH_2Cl_2 (2 × 200 mL). Organic layers were combined, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (1−3% v/v MeOH/CH_2Cl_2) afforded **12A** (14.1 g, 78%) as a white solid material.

Method 2. To a solution of compound **11A** (1.05 g, 2.24 mmol) in THF/H_2O (1:1 v/v; 16 mL) was added 2 M NaOH (4 mL). After stirring for 1 h, AcOH (1 mL) was added, and the solvents were removed under reduced pressure. The residue was suspended in dichloromethane (100 mL) and washed with saturated $NaHCO_3$ (2 × 100 mL). The aqueous layers were combined and washed with dichloromethane (100 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel HPLC (2−3% v/v MeOH/CH_2Cl_2) to give **12A** (0.74 g, 91%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 9.28 (br s, 1H), 7.45 (d, J = 1.1 Hz, 1H), 7.38−7.22 (m, 5H), 5.66 (s, 1H), 4.67 (d, J = 11.6 Hz, 1H), 4.56 (d, J = 11.7 Hz, 1H), 4.54 (s, 1H), 4.05 (d, J = 7.9 Hz, 1H), 4.01 (d, J = 12.5 Hz, 1H), 3.96 (s, 1H), 3.95 (d, J = 12.6 Hz, 1H), 3.83 (d, J = 7.9 Hz, 1H), 1.88 (d, J = 1.1 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 163.9, 149.8, 137.0, 134.7, 128.5, 128.2, 127.8, 110.3, 88.2, 87.3, 76.9, 75.9, 72.3, 72.0, 57.6, 12.7. MALDI-MS *m*/*z*: 383.3 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{20}N_2O_6\cdot{}^1/_5\ H_2O$: C, 59.40; H, 5.65; N, 7.70. Found: C, 59.33; H, 5.41; N, 7.68.

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6A). A mixture of compound **12A** (3 g, 8.24 mmol), 20% $Pd(OH)_2$/C (1.5 g), and HCO_2NH_4 (1.6 g, 25.4 mmol) was suspended in MeOH (20 mL). After refluxing the mixture for 10 min, the catalyst was filtered off and washed with MeOH. The combined filtrates were concentrated to a white solid residue. Crystallization from 15% MeOH/CH_2Cl_2 (v/v) gave **6A** (1.9 g, 83%) as a white solid material. mp 196−198 °C. ^{1}H NMR (DMSO-d_6): δ 11.32 (br s, 1H), 7.60 (d, J = 1.1 Hz, 1H), 5.68 (d, J = 4.1 Hz, 1H), 5.38 (s, 1H), 5.20 (t, J = 5.6 Hz, 1H), 4.09 (s, 1H), 3.89 (d, J = 4.0 Hz, 1H), 3.80 (d, J = 7.8 Hz, 1H), 3.74 (d, J = 5.5 Hz, 2H), 3.61 (d, J = 7.8 Hz, 1H), 1.75 (d, J = 1.1 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 164.1, 150.1, 135.1, 108.6, 89.0, 86.5, 79.1, 71.2, 68.9, 56.2, 12.6. MALDI-MS *m*/*z*: 293.2 $[M + Na]^+$. Anal. Calcd for $C_{11}H_{14}N_2O_6\cdot{}^1/_2\ H_2O$: C, 47.31; H, 5.41; N, 10.03. Found: C, 47.39; H, 5.16; N, 10.01.

1-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-*β*-D-*erythro*-pentofuranosyl)-4-*N*-acetylcytosine (9B). BSA (23 mL, 93.0 mmol) was added to a suspension of compound **8** (24 g, 47.0 mmol) and 4-*N*-acetylcytosine (11 g, 71.8 mmol) in anhyd MeCN (300 mL). The mixture was refluxed for 1 h and cooled to room temperature. TMSOTf (10 mL, 55.3 mmol) was added dropwise, and the resulting mixture was refluxed for 3 h and stirred overnight at room temperature. EtOAc (250 mL) was added, and the solution was washed with saturated $NaHCO_3$ (2 × 300 mL) and brine (250 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1−4% v/v MeOH/CH_2Cl_2) to give **9B** (23.5 g, 82%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 9.79 (br s, 1H), 7.73 (d, J = 7.5 Hz, 1H), 7.41 (d, J = 7.5 Hz, 1H), 7.37−7.27 (m, 5H), 5.76−5.72 (m, 2H), 4.78 (d, J = 5.7 Hz, 1H), 4.60−4.53 (m, 3H), 4.45 (d, J = 10.6 Hz, 1H), 4.41 (d, J = 10.7 Hz, 1H), 4.37 (d, J = 11.5 Hz, 1H), 3.01 (s, 3H), 3.00 (s, 3H), 2.26 (s, 3H), 2.12 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.9, 169.8, 163.4, 154.6, 146.6, 136.7, 128.6, 128.4, 128.3, 97.0, 94.4, 84.7, 77.9, 74.4, 73.9, 68.1, 67.8, 37.6, 37.5, 24.9, 20.8. MALDI-MS *m*/*z*: 626.5 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{29}N_3O_{12}S_2\cdot{}^1/_5\ H_2O$: C, 45.49; H, 4.88; N, 6.92. Found: C, 45.54; H, 4.91; N, 6.65.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-methanesulfonoxymethyl-3-(cytosin-1-yl)-2,5- dioxabicyclo[2.2.1]heptane (10C). Nucleoside **9B** (22.5 g, 37.1 mmol) was dissolved in THF/MeOH (2:1 v/v, 300 mL), and 1 M NaOH (200 mL) was added to the solution. After stirring for 1 h, AcOH (10 mL) was added, and the mixture was concentrated under reduced pressure to ca. $^1/_2$ of its volume and cooled in an ice bath. The precipitate was filtered off, washed with ice-cold H_2O, and dried in vacuo. Purification by silica gel column chromatography (2−5% v/v MeOH/CH_2Cl_2) afforded **10C** (14.1 g, 87%) as a white solid material. ^{1}H NMR (CD_3OD): δ 7.70 (d, J = 7.7 Hz, 1H), 7.32−7.28 (m, 5H), 5.89 (d, J = 7.6 Hz, 1H), 5.63 (s, 1H), 4.67 (d, J = 12.1 Hz, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.56 (d, J = 11.6 Hz, 1H), 4.52 (s, 1H), 4.06 (d, J = 7.9 Hz, 1H), 3.92 (s, 1H), 3.89 (d, J = 8.0 Hz, 1H), 3.15 (s, 3H). ^{13}C NMR (CD_3OD): δ 167.9, 157.7, 141.1, 138.6, 129.6, 129.4, 129.3, 95.9, 89.6, 87.0, 78.3, 77.3, 73.3, 73.0, 66.2, 37.5. MALDI-MS *m*/*z*: 446.4 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{21}N_3O_7S\cdot{}^5/_6\ H_2O$: C, 49.31; H, 5.17; N, 9.52. Found: C, 49.31; H, 5.12; N, 9.43.

(1*S*,3*R*,4*R*,7*S*)-1-Benzoyloxymethyl-7-benzyloxy-3-(cytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (11C). A mixture of compound **10C** (8.5 g, 19.4 mmol) and NaOBz (5.79 g, 40.2 mmol) was suspended in anhyd DMF (300 mL), stirred for 5 h at 100 °C, and cooled to room temperature. The solution was filtrated, and the solvent was removed under reduced pressure. The residue was redissolved in CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (2 × 200 mL), dried (Na_2SO_4), and concentrated. The residue was crystallized from EtOH to give **11C** (6.2 g, 71%) as a white solid material. mp 199−200 °C. The mother liquor was concentrated under reduced pressure, and the residue was purified by silica gel HPLC (2−4% v/v MeOH/CH_2Cl_2) to give 1.9 g more of **11C** (total yield 93%). ^{1}H NMR ($CDCl_3$): δ 7.95 (m, 2H), 7.66−7.47 (m, 4H), 7.26−7.22 (m, 5H), 5.76 (d, J = 7.5 Hz, 1H), 5.69 (s, 1H), 4.76 (d, J = 12.5 Hz, 1H), 4.75 (s, 1H), 4.66 (d, J = 11.6 Hz, 1H), 4.61 (d, J = 12.5 Hz, 1H), 4.45 (d, J = 11.6 Hz, 1H), 4.18 (d, J = 7.7 Hz, 1H), 3.97 (d, J = 7.7 Hz, 1H), 3.90 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 165.8, 165.5, 155.2, 139.2, 136.5, 133.5, 129.4, 129.1, 128.5, 128.4, 128.0, 127.6, 94.6, 88.0, 85.7, 76.5, 75.9, 72.0, 71.9, 59.5. MALDI-MS *m*/*z*: 472.6 $[M + Na]^+$. Anal. Calcd for $C_{24}H_{23}N_3O_6$: C, 64.14; H, 5.16; N, 9.35. Found: C, 64.07; H, 4.96; N, 9.27.

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(cytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6C). A mixture of compound **11C** (6.44 g, 14.3 mmol) and 20% $Pd(OH)_2$/C (3 g) was suspended in MeOH (100 mL), and HCO_2NH_4 (6 g, 95.1 mmol) was added. The mixture was refluxed for 1 h, diluted with concd NH_4OH (50 mL), and stirred overnight at 50 °C. The catalyst was filtered off and washed with boiling H_2O. The combined filtrates were concentrated under reduced pressure to a solid residue and crystallized from H_2O/EtOH (1:1 v/v) to give **6C** (2.95 g, 77%) as a white solid material. mp 275−278 °C (dec). ^{1}H NMR (DMSO-d_6): δ 7.74 (d, J = 7.5 Hz, 1H), 7.15 (br s, 1H), 7.19 (br s, 1H), 5.75 (d, J = 7.5 Hz, 1H), 5.39 (s, 1H), 4.09 (s, 1H), 3.82 (d, J = 7.8 Hz, 1H), 3.81 (s, 1H), 3.75 (s, 2H), 3.63 (d, J = 7.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 165.9, 154.8, 139.9, 93.4, 88.6, 87.0, 79.1, 71.1, 68.5, 56.3. MALDI-MS *m*/*z*: 256.0 $[M + H]^+$. Anal. Calcd for $C_{10}H_{13}N_3O_5 \cdot {}^{11}/_{16}$ H_2O: C, 44.88; H, 5.37; N, 15.70. Found: C, 44.78; H, 5.10; N, 15.45.

(1*S*,3*R*,4*R*,7*S*)-3-(4-*N*-Acetylcytosin-1-yl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (6B). To a solution of compound **6C** (0.42 g, 1.57 mmol) in anhyd DMF (20 mL) was added Ac_2O (0.18 mL, 1.9 mmol). The mixture was stirred at room temperature overnight, and the solvent was removed under reduced pressure. Crystallization from MeOH gave **6B** (0.32 g, 65%) as a white solid material. mp 223−225 °C (dec). ^{1}H NMR (DMSO-d_6): δ 10.88 (br s, 1H), 8.17 (d, J = 7.5 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 5.67 (d, J = 4.0 Hz, 1H), 5.47 (s, 1H), 5.14 (t, J = 5.7 Hz, 1H), 4.20 (s, 1H), 3.86 (d, J = 7.8 Hz, 1H), 3.82 (d, J = 4.0 Hz, 1H), 3.80−3.76 (m, 2H), 3.68 (d, J = 7.9 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 171.4, 162.9, 154.5, 144.6, 95.5, 89.5, 87.7, 78.9, 71.4, 68.8, 56.6, 24.7. MALDI-MS *m*/*z*: 297.9 $[M + H]^+$. Anal. Calcd for $C_{12}H_{15}N_3O_6 \cdot {}^1/_3$ H_2O: C, 47.52; H, 5.21; N, 13.86. Found: C, 47.69; H, 5.11; N, 13.69.

(1*S*,3*R*,4*R*,7*S*)-3-(4-*N*-Benzoylcytosin-1-yl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (6D). To a suspension of compound **6C** (3.85 g, 14.4 mmol) in anhyd pyridine (50 mL) was added Bz_2O (6.7 g, 29.6 mmol). The mixture was stirred overnight, diluted with EtOH (50 mL), and 2 M NaOH (75 mL) was added. After stirring for 1 h, AcOH (10 mL) was added to the solution, and the solvents were removed under reduced pressure. The residue was crystallized from H_2O to give **6D** (4.88 g, 93%) as a white solid material. mp 221−223 (dec). ^{1}H NMR (DMSO-d_6): δ 11.26 (br s, 1H), 8.25 (d, J = 7.5 Hz, 1H), 7.99 (m, 2H), 7.65−7.50 (m, 3H), 7.42 (d, J = 7.5 Hz, 1H), 5.68 (d, J = 4.2 Hz, 1H), 5.53 (s, 1H), 5.17 (t, J = 5.7 Hz, 1H), 4.24 (s, 1H), 3.89−3.79 (m, 4H), 3.71 (d, J = 7.7 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 167.7, 163.6, 154.4, 144.6, 133.5, 133.1, 128.8, 96.0, 89.3, 87.5, 78.7, 71.2, 68.5, 56.3. MALDI-MS *m*/*z*: 360.5 $[M + H]^+$. Anal. Calcd for $C_{17}H_{17}N_3O_6 \cdot {}^1/_6$ H_2O: C, 56.35; H, 4.82; N, 11.60. Found: C, 56.37; H, 4.68; N, 11.62.

9-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-methanesulfonoxymethyl-5-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl)-6-*N*-benzoyladenine (9E). To a suspension of compound **8** (19.6 g, 38.4 mmol) and 6-*N*-benzoyladenine (11.02 g, 46.1 mmol) in anhyd 1,2-dichloroethane (175 mL) was added BSA (25.1 mL, 101 mmol). The mixture was refluxed for 1 h and cooled to room temperature. TMSOTf (13.9 mL, 76.8 mmol) was added, and the solution was refluxed for 5 h, stirred at room temperature overnight, and refluxed further for 24 h. The reaction mixture was poured into ice-cold saturated $NaHCO_3$ (200 mL), stirred for 0.5 h, and filtrated. The phases were separated, and the organic phase was washed with saturated $NaHCO_3$ (3 × 150 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (1−1.5% v/v MeOH/CH_2Cl_2) afforded **9E** (18.1 g, 68%) as a slightly yellow solid material. ^{1}H NMR ($CDCl_3$): δ 8.76 (s, 1H), 8.12 (s, 1H), 8.02 (m, 2H), 7.61 (m, 1H), 7.51 (m, 2H), 7.40−7.34 (m, 5H), 6.23 (d, J = 3.5 Hz, 1H), 6.08 (dd, J = 5.9 and 3.5 Hz, 1H), 5.12 (d, J = 6.0 Hz, 1H), 4.68 (d, J = 11.1 Hz, 1H), 4.67 (d, J = 11.6 Hz, 1H), 4.64 (d, J = 11.0 Hz, 1H), 4.44 (d, J = 10.8 Hz, 1H), 4.39 (d, J = 11.7 Hz, 1H), 4.36 (d, J = 11.0 Hz, 1H), 3.03 (s, 3H), 2.87 (s, 3H), 2.13 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 169.5, 164.5, 152.5, 150.9, 149.7, 142.4, 136.3, 133.2, 132.8, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 123.5, 87.8, 84.1, 77.3, 74.6, 73.4, 67.3, 67.2, 37.6, 37.3, 20.5. MALDI-MS *m*/*z*: 712.4 $[M + Na]^+$.

(1*S*,3*R*,4*R*,7*S*)-3-(6-*N*-Benzoyladenine-9-yl)-7-benzyloxy-1-methanesulfonyloxymethyl-2,5-dioxabicyclo[2.2.1]-heptane (10E). Compound **9E** (17.9 g, 26.0 mmol) was dissolved in a mixture of THF (160 mL) and H_2O (110 mL). LiOH·H_2O (5.5 g, 131 mmol) was added, and the mixture was stirred for 3.5 h at room temperature. The solution was neutralized with AcOH (6 mL) to give a precipitate. The precipitate was filtered off and washed with water to give **10E** (11.6 g, 78%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 8.72 (s, 1H), 8.15 (s, 1H), 8.02 (m, 2H), 7.63−7.59 (m, 1H), 7.54−7.50 (m, 2H), 7.32−7.27 (m, 5H), 6.10 (s, 1H), 4.95 (s, 1H), 4.67 (d, J = 11.7 Hz, 1H), 4.62 (d, J = 12.0 Hz, 1H), 4.60 (d, J = 11.7 Hz, 1H), 4.57 (d, J = 11.7 Hz, 1H), 4.33 (s, 1H), 4.21 (d, J = 7.9 Hz, 1H), 4.01 (d, J = 7.9 Hz, 1H), 3.04 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 164.2, 152.5, 150.6, 149.5, 140.5, 136.4, 133.3, 132.8, 128.7, 128.4, 128.2, 127.8, 127.7, 123.3, 86.7, 85.3, 77.4, 76.8, 72.5, 72.2, 64.1, 37.7. MALDI-MS *m*/*z*: 575.0 $[M + Na]^+$. Anal. Calcd for $C_{26}H_{25}N_5O_7S \cdot H_2O$: C, 54.83; H, 4.78; N, 12.30. Found: C, 54.89; H, 4.85; N, 12.42.

(1*S*,3*R*,4*R*,7*S*)-3-(6-*N*-Benzoyladenine-9-yl)-1-benzoyloxymethyl-7-benzyloxy-2,5-dioxabicyclo[2.2.1]heptan (11E). Compound **10E** (11.5 g, 20.2 mmol) was dissolved in anhyd DMF (450 mL). NaOBz (5.40 g, 37.4 mmol) was added, and the mixture was stirred at 90 °C for 7 h, cooled to room temperature, filtrated, concentrated under reduced pressure, and coevaporated with MeCN. The residue was redissolved in CH_2Cl_2 (150 mL), washed with saturated $NaHCO_3$ (3 × 100 mL) and brine (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Crystallization from H_2O/EtOH (1:1 v/v) gave **11E** (10.6 g, 88%) as a white solid material. mp 112−115 °C. ^{1}H NMR (DMSO-d_6): δ 11.2 (br s, 1H), 8.72 (s, 1H), 8.48 (s, 1H), 8.06 (m, 2H), 7.94 (m, 2H), 7.66 (m, 2H), 7.54 (m, 4H), 7.36−7.26 (m, 5H), 6.11 (s, 1H), 4.97 (s, 1H), 4.82 (s, 2H), 4.77 (s, 1H), 4.75 (d, J = 12.4 Hz, 1H), 4.69 (d, J = 11.9 Hz, 1H), 4.19 (d, J = 8.0 Hz, 1H), 4.07 (d, J = 7.9 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 165.6, 165.3, 151.8, 151.6, 150.5, 141.9, 137.7, 133.7, 133.6, 132.5, 129.3, 129.1, 128.9, 128.6, 128.5, 128.3, 127.7, 127.6, 125.7, 85.9, 85.3, 77.9, 77.1, 72.0, 71.3, 60.6. MALDI-MS *m*/*z*: 600.7 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{27}N_5O_6 \cdot H_2O$: C, 64.53; H, 4.91; N, 11.76. Found: C, 64.77; H, 5.01; N, 11.85.

(1*S*,3*R*,4*R*,7*S*)-3-(Adenin-9-yl)-7-benzyloxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (12F). Compound **11E** (15.5 g, 26.8 mmol) was suspended in a mixture of MeOH (150 mL) and concd NH_4OH (200 mL). The solution was stirred for 2 days at room temperature, whereafter $MeNH_2$ (40%, 20 mL) was added, and the mixture was stirred overnight. The precipitate was filtered off, dried in vacuo, and crystallized from EtOH to give **12F** (8.55 g, 86%) as a white solid material.

mp 218−219.5 °C. ^{1}H NMR (DMSO-d_6): δ 8.18 (s, 1H), 8.14 (s, 1H), 7.33−7.30 (m, 5H), 5.97 (s, 1H), 5.17 (t, J = 5.8 Hz, 1H), 4.73 (s, 1H), 4.63 (s, 2H), 4.35 (s, 1H), 3.95 (d, J = 7.9 Hz, 1H), 3.84−3.81 (m, 3H). ^{13}C NMR (DMSO-d_6): δ 156.1, 152.8, 148.6, 138.0, 137.9, 128.3, 127.7, 127.6, 119.1, 88.0, 85.4, 77.3, 77.0, 72.1, 71.3, 56.8. MALDI-MS m/z: 370.7 $[M + H]^+$. Anal. Calcd for $C_{18}H_{19}N_5O_4 \cdot {}^1/_3\ H_2O$: C, 57.59; H, 5.28; N, 18.66. Found: C, 57.58; H, 5.40; N, 18.43.

(1*S*,3*R*,4*R*,7*S*)-3-(Adenin-9-yl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (6F). To a suspension of compound **12F** (3.69 g, 10.0 mmol) in EtOH (50 mL) were added 20% $Pd(OH)_2$/C (1 g) and HCO_2NH_4 (3.2 g, 50.4 mmol). The solution was refluxed for 3 h, and more HCO_2NH_4 (1 g, 15.9 mmol) was added. After 2 h, the hot solution was filtrated through a Celite pad, and Celite was washed with boiling EtOH/H_2O (200 mL). Evaporation of the combined filtrates gave **6F** (2.54 g, 91%) as white crystals. mp 266−268 °C (dec). ^{1}H NMR (DMSO-d_6): δ 8.22 (s, 1H), 8.15 (s, 1H), 7.30 (br s, 2H), 5.89 (s, 1H), 5.68 (d, J = 4.2 Hz, 1H), 5.05 (t, J = 5.8 Hz, 1H), 4.41 (s, 1H), 4.25 (d, J = 3.7 Hz, 1H), 3.92 (d, J = 7.8 Hz, 1H), 3.82 (m, 2H), 3.76 (d, J = 7.9, 2H). ^{13}C NMR (DMSO-d_6): δ 156.1, 152.8, 148.5, 137.9, 119.1, 88.6, 85.4, 79.3, 71.5, 70.0, 56.8. MALDI-MS m/z: 280.6 $[M + H]^+$ Anal. Calcd for $C_{11}H_{13}N_5O_4 \cdot {}^1/_8\ H_2O$: C, 46.93; H, 4.74; N, 24.88. Found: C, 47.04; H, 4.56; N, 24.74.

(1*S*,3*R*,4*R*,7*S*)-3-(Adenin-9-yl)-7-benzyloxy-1-methanesulfonoxymethyl-2,5-dioxabicyclo[2.2.1]heptane (10F). To a solution of **9E** (2.5 g, 3.6 mmol) in 1,4-dioxane (20 mL) was added concd NH_4OH (20 mL). The solution was stirred at room temperature overnight and diluted with 2 M NaOH (5 mL). After 30 min, the solvents were removed under reduced pressure, and the residue was resuspended in CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ (100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification by silica gel column chromatography (2−5% v/v MeOH/CH_2Cl_2) gave **10F** (1.26 g, 78%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 1H), 7.90 (s, 1H), 7.31−7.27 (m, 5H), 6.04 (s, 1H), 4.93 (s, 1H), 4.68 (d, J = 11.7 Hz, 1H), 4.60 (d, J = 11.7, 1H), 4.59 (d, J = 11.7 Hz, 1H), 4.57 (d, J = 11.9 Hz, 1H), 4.35 (s, 1H), 4.19 (d, J = 7.9 Hz, 1H), 4.02 (d, J = 7.9 Hz, 1H), 3.03 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 155.4, 152.9, 148.6, 138.0, 136.4, 128.4, 128.2, 127.8, 119.7, 86.6, 85.1, 77.5, 76.8, 72.4, 72.2, 64.4, 37.7. MALDI-MS m/z: 470.4 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{21}N_5O_6S \cdot {}^1/_8\ H_2O$: C, 50.75; H, 4.76; N, 15.57. Found: C, 50.64; H, 4.51; N, 15.52.

(1*S*,3*R*,4*R*,7*S*)-3-(Adenin-9-yl)-7-benzyloxy-1-benzoyloxymethyl-2,5-dioxabicyclo[2.2.1]heptane (11F). NaOBz (5.36 g, 37.2 mmol) was added to a solution of compound **10F** (8.3 g, 18.6 mmol) in anhyd DMF (250 mL). The mixture was stirred at 100 °C for 4 h, cooled to room temperature, filtrated, and concentrated under reduced pressure. The residue was suspended in EtOAc (200 mL), washed with saturated $NaHCO_3$ (3 × 200 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Crystallization from EtOH gave **11F** (7.4 g, 84%) as a white solid material. mp 169−171 °C. ^{1}H NMR ($CDCl_3$): δ 8.31 (s, 1H), 7.96 (m, 2H), 7.84 (s, 1H), 7.57 (m, 1H), 7.43 (m, 2H), 7.28−7.21 (m, 5H), 6.23 (br s, 2H), 6.05 (s, 1H), 4.96 (s, 1H), 4.81 (d, J = 12.6 Hz, 1H), 4.70 (d, J = 11.8 Hz, 1H), 4.65 (d, J = 12.6 Hz, 1H), 4.55 (d, J = 11.9 Hz, 1H), 4.35 (s, 1H), 4.28 (d, J = 7.9 Hz, 1H), 4.10 (d, J = 7.9 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 165.7, 155.5, 153.0, 148.6, 137.6, 136.5, 133.3, 129.4, 129.1, 128.4, 128.3, 128.0, 127.7, 119.9, 86.7, 85.6, 77.4, 76.8, 72.5, 72.2, 59.8. MALDI-MS m/z: 496.6 $[M + Na]^+$. Anal. Calcd for $C_{25}H_{23}N_5O_5$: C, 63.42; H, 4.90; N, 14.79. Found: C, 63.21; H, 4.93; N, 14.84.

(1*S*,3*R*,4*R*,7*S*)-3-(6-*N*-Benzoyladenin-9-yl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (6E). A mixture of compound **11F** (5.8 g, 12.2 mmol) and 20% $Pd(OH)_2$/C (3 g) was suspended in MeOH/1,4-dioxane (9:1, 150 mL), and HCO_2NH_4 (8 g, 126.9 mmol) was added. The mixture was refluxed overnight, cooled to room temperature, and filtrated through a Celite pad. The filtrate was concentrated under reduced pressure, coevaporated with anhyd pyridine (2 × 50 mL), dissolved in anhyd pyridine (100 mL), and cooled in an ice bath. Benzoyl chloride (7.1 mL, 61.5 mmol) was added dropwise, and the mixture was stirred for 20 h at room temperature. The mixture was diluted with EtOAc (200 mL), washed with saturated $NaHCO_3$ (3 × 200 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The oily residue was dissolved in pyridine/EtOH (1:1 v/v, 200 mL), and 2 M NaOH (50 mL) was added. After 15 min, AcOH (9 mL) was added, and the solvents were removed under reduced pressure. The residue was suspended in 20% EtOH/CH_2Cl_2 and filtrated through a short silica gel column. The filtrate was concentrated to a yellow solid residue and purified by silica gel HPLC (5−15% v/v MeOH/CH_2Cl_2) to give **6E** (3.75 g, 75%) as a white solid material. ^{1}H NMR (CD_3OD): δ 8.73 (s, 1H), 8.57 (s, 1H), 8.11 (m, 2H), 7.69 (m, 1H), 7.59 (m, 2H), 6.16 (s, 1H), 4.67 (s, 1H), 4.42 (s, 1H), 4.12 (d, J = 8.0 Hz, 1H), 4.01 (s, 2H), 3.95 (d, J = 7.9 Hz, 1H). Dichloromethane (s, 5.53 ppm) was detected as an impurity. ^{13}C NMR (CD_3OD): δ 168.1, 153.3, 152.4, 151.1, 143.0, 134.9, 134.0, 129.8, 129.5, 125.3, 90.4, 87.8, 81.0, 73.0, 71.6, 58.3. MALDI-MS m/z: 406.5 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{17}N_5O_5 \cdot {}^7/_{24}\ CH_2Cl_2$: C, 53.83; H, 4.34; N, 17.16. Found: C, 53.81; H, 4.39; N, 17.04.

9-(2-*O*-Acetyl-3-*O*-benzyl-4-*C*-methanesulfoxymethyl-5-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl)-2-*N*-isobutyrylguanine (9G). To a suspension of **8** (18.5 g, 36.3 mmol) and 2-*N*-isobutyrylguanine (9.15 g, 41.4 mmol) in anhyd 1,2-dichloroethane (150 mL) was added BSA (30 mL, 122 mmol). The mixture was refluxed for 1.5 h, and TMSOTf (13.5 mL, 74.3 mmol) was added. After refluxing further for 2 h, the reaction mixture was cooled to room temperature, diluted with CH_2Cl_2 (200 mL), washed with saturated $NaHCO_3$ (2 × 200 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1−2% v/v MeOH/CH_2Cl_2) to give a mixture of two isomers (20.6 g, 84%) as a white solid material (ratio 1:10 by ^{1}H NMR). The mixture was used in the next step without separation of isomers. For the main isomer assigned as **9G**, ^{1}H NMR ($CDCl_3$): δ 12.22 (br s, 1H), 9.34 (br s, 1H), 7.76 (s, 1H), 7.40−7.30 (m, 5H), 6.03 (d, J = 3.9 Hz, 1H), 5.76 (dd, J = 6.0 and 3.9 Hz, 1H), 5.08 (d, J = 6.0 Hz, 1H), 4.91 (d, J = 10.5 Hz, 1H), 4.67 (d, J = 10.9), 4.61 (d, J = 11.1 Hz, 2H), 4.49 (d, J = 10.5 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 4.32 (d, J = 11.7 Hz, 1H), 3.14 (s, 3H), 3.02 (s, 3H), 2.70 (m, 1H), 2.09 (s, 3H) 1.24 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 179.3, 169.7, 154.7, 148.2, 147.1, 138.8, 136.5, 128.5, 128.3, 128.1, 121.5, 88.7, 84.4, 78.1, 74.7, 74.2, 67.9, 67.3, 37.6, 37.5, 36.1, 20.5, 18.9, 18.8. MALDI-MS m/z: 694.4 $[M + Na]^+$. Anal. Calcd for $C_{26}H_{33}N_5O_{12}S_2 \cdot {}^1/_3\ H_2O$: C, 46.08; H, 5.01; N, 10.33. Found: C, 46.10; H, 4.98; N, 10.27.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-methanesulfonoxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]-heptane (10G). To a solution of compound **9G** (10.2 g, 15.1 mmol) in THF (100 mL) was added 1 M NaOH (100 mL), and the mixture was stirred at 0 °C for 1 h. Acetic acid (6 mL) was added, and the solution was concentrated to ca. $^1/_2$ of its volume under reduced pressure. The formed precipitate was filtered off, washed with water, and dried in vacuo. Purification by silica gel column chromatography (0.8−3.5% v/v MeOH/CH_2Cl_2) gave **10G** (6.9 g, 85%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 12.14 (br s, 1H), 9.51 (br s, 1H), 7.77 (s, 1H), 7.30−7.26 (m, 5H), 5.84 (s, 1H), 4.67 (d, J = 11.5 Hz, 1H), 4.63 (d, J = 12.0 Hz, 1H), 4.62 (s, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.56 (d, J = 11.9 Hz, 1H), 4.50 (s, 1H), 4.12 (d, J = 8.0, 1H), 3.93 (d, J = 7.9 Hz, 1H), 3.06 (s, 3H), 2.78 (m, 1H), 1.26 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 179.3, 155.3, 147.7, 147.3, 136.7, 136.3, 128.3, 128.0, 127.7, 121.2, 86.8, 85.3, 77.5, 76.9, 72.3, 72.0, 64.6, 37.5, 36.0, 18.9, 18.8. MALDI-MS m/z: 556.8 $[M + Na]^+$. Anal. Calcd for $C_{23}H_{27}N_5O_8S \cdot {}^1/_3\ H_2O$: C, 51.20; H, 5.17; N, 13.00. Found: C, 51.02; H, 5.09; N, 13.08.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-benzoyloxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (11G). A mixture of compound **10G** (12.5 g, 23.4 mmol) and NaOBz (6.8 g, 47.2 mmol) was suspended in anhyd DMF (25 mL) and was stirred for 2 h at 100 °C and overnight at 80 °C. The mixture was cooled to room temperature, filtrated, and concentrated under reduced pressure. The residue was redissolved in EtOAc (200 mL) and washed with saturated $NaHCO_3$

(2 × 200 mL) and H_2O (200 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0.9−3.5% v/v MeOH/CH_2Cl_2) to give **11G** (12.1 g, 92%) as a white solid material. ^{1}H NMR ($CDCl_3$): δ 12.12 (br s, 1H), 9.30 (br s, 1H), 7.92 (m, 2H), 7.72 (s, 1H), 7.57 (m, 1H), 7.42 (m, 2H), 7.24−7.20 (m, 5H), 5.81 (s, 1H), 4.80 (d, J = 12.6 Hz, 1H), 4.66 (s, 1H), 4.64 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 12.7 Hz, 1H), 4.21 (d, J = 8.1 Hz, 1H), 4.20 (s, 1H), 4.00 (d, J = 7.9 Hz, 1H), 2.77 (m, 1H), 1.27 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 179.3, 165.6, 155.2, 148.0, 147.1, 136.4, 135.4, 133.3, 129.3, 128.9, 128.4, 128.3, 127.9, 127.5, 120.9, 86.2, 85.5, 76.9, 72.3, 72.1, 59.4, 35.9, 18.8. MALDI-MS m/z: 582.5 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{29}N_5O_7 \cdot {}^1/_4\ H_2O$: C, 61.75; H, 5.27; N, 12.41. Found: C, 61.80; H, 5.33; N, 12.31.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-hydroxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (12G). To a solution of compound **11G** (8.2 g, 14.5 mmol) in EtOH/pyridine (8:1 v/v, 450 mL) was added 2 M NaOH (15.5 mL), and the mixture was stirred for 30 min at room temperature. AcOH (25 mL) was added, and the solvents were removed under reduced pressure. The residue was crystallized from H_2O/EtOH (1:1 v/v) to give **12G** (5.8 g, 88%) as a white solid material. mp 231−233 °C. ^{1}H NMR (DMSO-d_6): δ 8.05 (s, 1H), 7.33−7.26 (m, 5H), 5.85 (s, 1H), 5.17 (t, J = 5.4 Hz, 1H), 4.69 (s, 1H), 4.64 (s, 2H), 4.23 (s, 1H), 3.95 (d, J = 7.9 Hz, 1H), 3.83 (m, 2H), 3.80 (d, J = 8.0 Hz, 1H), 2.78 (m, 1H), 1.12 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 180.2, 154.8, 148.2, 147.7, 137.9, 136.3, 128.3, 127.6, 127.5, 120.5, 88.2, 85.2, 76.9, 72.1, 71.3, 56.7, 34.8, 18.9. MALDI-MS m/z: 478.5 $[M + Na]^+$. Anal. Calcd for $C_{22}H_{25}N_5O_6$: C, 58.02; H, 5.53; N, 15.38. Found: C, 57.91; H, 5.35; N, 15.24.

(1*S*,3*R*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(2-*N*-isobutyrylguanin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (6G). To a solution of compound **12G** (5.8 g, 12.7 mmol) in MeOH (50 mL) were added 10% Pd/C (2 g) and HCO_2H (3 mL, 79.5 mmol). The mixture was refluxed for 5 h, cooled to room temperature, and filtrated through a Celite pad. The filtrate was concentrated under reduced pressure to afford a glasslike residue. Crystallization from H_2O gave **6G** (3.9 g, 80%) as a white solid material. mp 196−199 °C. ^{1}H NMR (DMSO-d_6): δ 7.80 (s, 1H), 5.51 (s, 1H), 5.44 (br s, 1H), 4.77 (br s, 1H), 4.12 (s, 1H), 3.88 (s, 1H), 3.65 (d, J = 7.7 Hz, 1H), 3.53 (m, 2H), 3.47 (d, J = 7.7 Hz, 1H), 2.50 (m, 1H), 0.84 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 180.3, 154.8, 148.3, 147.6, 136.4, 120.6, 88.8, 85.3, 79.3, 71.6, 69.8, 56.7, 34.8, 19.0, 18.9. MALDI-MS m/z: 388.4 $[M + Na]^+$. Anal. Calcd for $C_{15}H_{19}N_5O_6 \cdot H_2O$: C, 47.00; H, 5.52; N, 18.27. Found: C, 46.90; H, 5.30; N, 18.37.

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Supporting Information Available: Copies of ^{13}C NMR spectra for all reported compounds and copies of ^{1}H NMR spectra for **8**, **6E**, and **9E**. This material is available free of charge via Internet at http://pubs.acs.org.

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