Tetrahedron 70 (2014) 583–589

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of a bicyclic double-headed nucleoside

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ARTICLE INFO

Article history:
Received 26 September 2013
Received in revised form 21 November 2013
Accepted 2 December 2013
Available online 7 December 2013

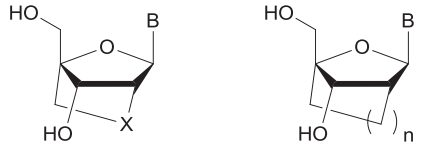
Keywords:
Ring-closing metathesis
Bicyclic nucleosides
Double-headed nucleosides
Vorbrüggen coupling

ABSTRACT

An attempt of preparing a carbocyclic LNA-analogue using different RCM-methods failed. However, a compound with a hemiacetal linker between the C2′ and the C4′-positions was isolated and found to be a suitable substrate for making a conformationally restricted double-headed nucleoside. This contains two uracil nucleobases organized on a bicyclic skeleton and is locked in an *N*-type conformation.

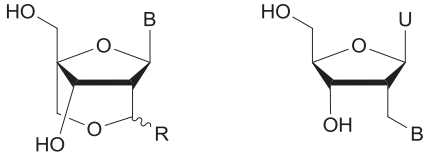
1. Introduction

Chemically modified nucleotide analogues are essential for the design of therapeutically active nucleic acids.[1–3] A large number of modifications have been prepared leading to oligonucleotides that are both physiologically stable and recognize complementary DNA and RNA with high affinity and selectivity.[1–4] Bicyclic nucleoside monomers, which covalently bridge the C2′ with the C4′-position (Fig. 1), have gained particular interest as these are locked in the C3′-*endo* conformation and preorganised for forming thermally very strong duplexes.[4–6] As the first and prime example, the monomer **1** is the building block in LNA (locked nucleic acid),[7–10] which is now a well-established nucleic acid analogue for numerous applications.[11] A number of close analogues of LNA have been investigated,[4–6,12] for instance the 2′-amino-LNA, **2**, which has been used to introduce various groups into nucleic acids via the amino-group leading to interesting components in DNA nanotechnology.[13–15] Recently, carbocyclic analogues of LNA have gained some interest.[4,16–21] As the first of these, we introduced the monomer **3** (B=U),[16] and later some analogues of this,[17] which all contain an all-carbon C2′–C4′ linker, which is one atom longer than the oxymethylene linker in LNA (**1**). The synthesis of **3** was based on ring-closing metathesis (RCM)[22–24] as the key step and was performed in 13 steps and 7% overall yield from uridine.[16] Chattopadhyaya and co-workers have later developed a general method to carbocyclic LNA-analogues based on radical cyclizations.[4] Hence, a series of analogues of both **3** and of the carba-LNA monomer **4** with various substituents at the C2′–C4′ linker have been obtained.[4,18,19] The unsubstituted nucleoside **4** (B=T) was obtained in 20 steps starting from D-allose.[19] Herein, we present our attempts of making **4** (B=U) from a shorter route based on the RCM-strategy. This was absolutely unsuccessful but led to the development of a hemiacetal bicyclic nucleoside structure **5** (in a protected form), that is, a potentially very useful precursor for other analogues, for instance for placing various entities in the DNA duplex in a similar



1 X = O
2 X = NH

3 n = 2
4 n = 1



5 R = OH
6 R = H
7 R = U

8

Fig. 1. Bicyclic and double-headed nucleosides. B=any nucleobase, U=uracil-1-yl.

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http://dx.doi.org/10.1016/j.tet.2013.12.013

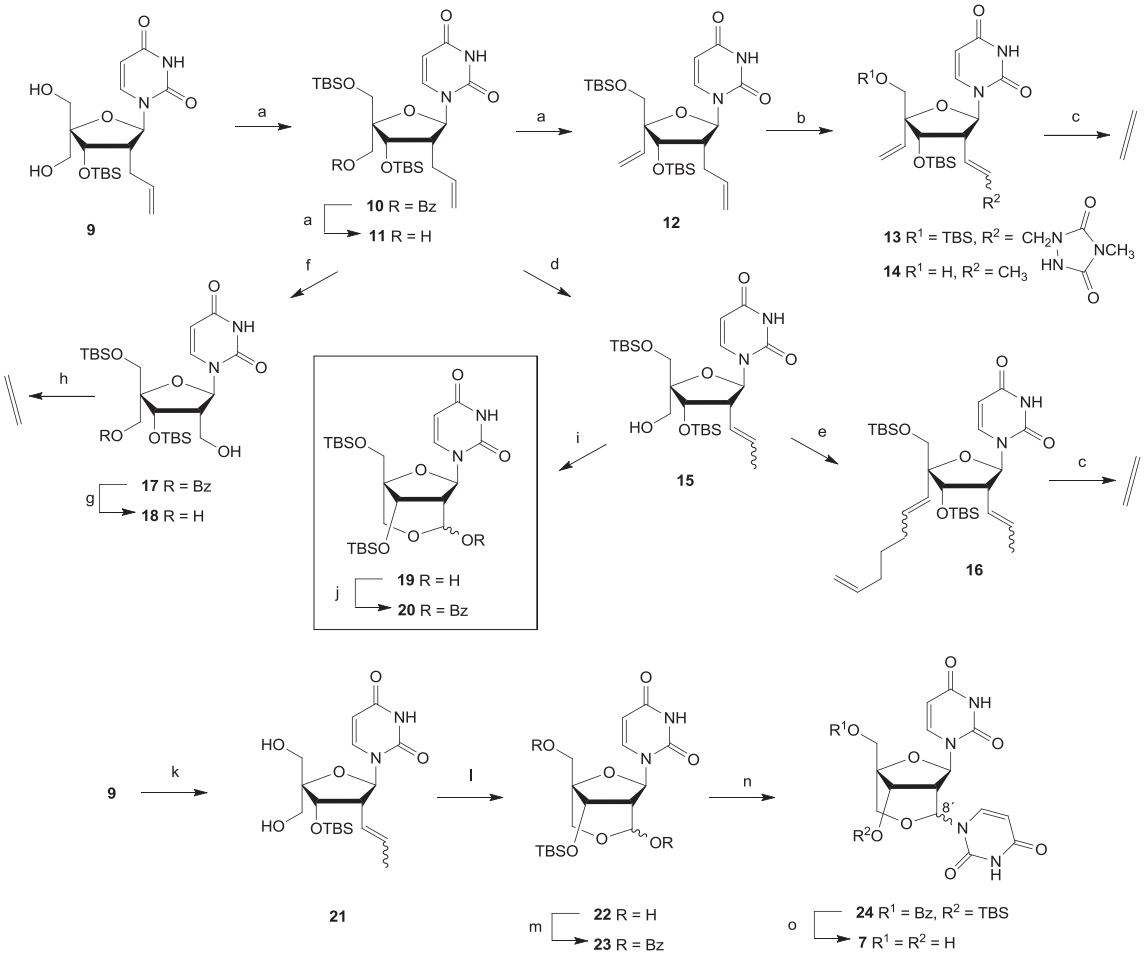
way as from amino-LNA. Analogues made from **5** will be substituted analogues of **6**, which was originally studied by Wang and co-workers.[25] We decided to use **5** as a precursor for a double-headed nucleoside **7**.

Double-headed nucleosides are defined as nucleoside analogues bearing two nucleobases. We[26–33] and others[34–36] have recently studied these as building blocks for oligonucleotides that can either target nucleic acid secondary structures, form interesting new nucleic acid motifs or potentially transfer molecular information in new ways. Recently, the double-headed nucleosides **8** (B=T/A) were synthesized and found to form base-pairs in the core of a duplex hereby extending the duplex with an additional base pair.[31,32] The bicyclic analogue **7** is a conformationally restricted analogue of **8**, but, whereas **8** is preferring a C2′-*endo* conformation,[31] **7** would be conformationally locked in a C3′-*endo* conformation. It is of great interest to study how the restriction itself and the different positioning of the additional nucleobase will influence the properties in forming artificial nucleic acid motifs and targeting other nucleic acids.

2. Results and discussion

2.1. Chemical synthesis

In the original synthesis of the bicyclic nucleoside **3**, the key intermediate **9** (Scheme 1) was first prepared from uridine in six steps.[16] Benzoylation and subsequent silylation gave **10**, debenzoylation afforded **11**, and an oxidation/Wittig sequence gave the nucleoside **12**.[16] With its two terminal alkenes this constituted a perfect substrate for an RCM-reaction, which proceeded in 96% yield affording after hydrogenation and deprotection the bicyclic nucleoside **3**.[16] We envisioned that a similar strategy might also afford the one atom shorter analogue **4** even though ring-closing metathesis might have its limitation in the formation of very constrained ring-systems.[24] Nevertheless, it was relatively straightforward to convert **12** into a substrate for this using a modified ene-reaction.[37] Hence, treatment of **12** with a triazolinedione afforded compound **13** as exclusively the *E*-isomer in a reasonable yield, whereas treatment with $RhCl_3$ mediated a rearrangement of the allyl group[38] and 5′-deprotection to afford **14** as an *E*/*Z*-mixture. However, neither **13** nor **14** worked as substrates for an RCM reaction using Grubbs second generation catalyst. The problem could be the problematic combination of a sterically hindered terminal alkene in the 4′-position and internal alkenes in the 2′-position. In a former study on cyclic dinucleotides, we found a dinucleotide with two 4′-C-vinyl groups to be unreactive towards RCM, whereas cyclizations between a 4′-C-vinyl group and a more reactive 5′-allyl group were possible.[39] Therefore, we could either introduce a terminal alkene (a vinyl group) at the 2′-C-position, where it is expected to be less sterically hindered, or attempt the so-called relay-RCM method.[40,41] The latter strategy was followed by first rearranging the alkene of compound **11** to give **15** (this time optimized



Scheme 1. Reagents and conditions. (a) Ref. 16; (b) 4-methyl-1,2,4-triazoline-3,5-dione, CH_2Cl_2 (65%) or $RhCl_3 \cdot xH_2O$, EtOH; (c) Grubbs second generation catalyst, CH_2Cl_2; (d) $RhCl_3 \cdot xH_2O$, K_2CO_3, EtOH, MW, 80 °C, 61%; (e) i. DMP, CH_2Cl_2; ii. $CH_2{=}CH(CH_2)_4PPh_3Br$, *n*-BuLi, Et_2O; (f) i. 4-methyl-1,2,4-triazolidine-3,5-dione, CH_2Cl_2, (47%); ii. O_3, MeOH, −70 °C; iii. MeOH, $NaBH_4$, 60% (2 steps); (g) $NaOCH_3$, MeOH, 64%; (h) DMP, CH_2Cl_2; (i) i. OsO_4, NMO, acetone, H_2O; ii. $NaIO_4$, dioxane, H_2O, 52%; (j) BzCl, pyridine, 85%; (k) $RhCl_3 \cdot xH_2O$, K_2CO_3, EtOH, MW, 75 °C, 55%; (l) i. OsO_4, NMO, acetone, H_2O; ii. $NaIO_4$, dioxane, H_2O, 62%; (m) BzCl, pyridine, 65%; (n) Uracil, BSA, TMS-OTf, CH_3CN, 58%; (o) i. NH_3, CH_3OH; ii. TBAF, THF, 72%. NMO=*N*-methylmorpholine-*N*-oxide, DMP=Dess–Martin periodinane, TBS=*tert*-butyldimethylsilyl.

with K_2CO_3 to avoid desilylation)[42] and then in an oxidation/Wittig sequence using the hex-5-ene-1-yltriphenylphosphonium bromide[43] to make the substrate **16** as a mixture of *E*/*Z*-isomers. Here the idea is that the RCM starts with excluding cyclopentene and then continues on the hereby activated 4′-C substituent to perform the cyclisation. However, also this attempt failed to give any ring-closed product. In a last attempt to form a carba-LNA-analogue using RCM-methodology, we converted compound **10** to the 2′-hydroxymethyl analogue **17** in first an ene reaction and then an ozonolysis/reduction sequence.[37] Deprotection afforded the 2′,4′-bis-*C*-(hydroxy-methyl) nucleoside **18**. Nevertheless, any attempt of converting this into the 2′,4′-bis-*C*-formyl (to be an intermediate towards a 2′,4′-bis-*C*-vinylnucleoside or a substrate for a McMurry coupling) failed probably due to the 2′-*C*-formyl nucleoside being prone to elimination of the uracil. However, in an attempt to make a sequential oxidation, compound **15** was cleaved in a dihydroxylation/oxidation sequence to give what was found to be the hemiacetal **19** as an almost equimolar mixture of epimers. Trapping the hemiacetal as its benzoic ester afforded, surprisingly, **20** as only one epimer.

We envisioned compound **20** to be a substrate for various coupling reactions, for instance with nucleobases to afford double-headed nucleosides, but with this new target we decided first to avoid some unnecessary protecting steps. Hence, the nucleoside **9** with two primary alcohols was subjected to a $RhCl_3$ mediated allyl rearrangement[42] to give compound **21** as a mixture of *E*/*Z*-isomers. The same oxidative cleavage as before gave the hemiacetal bicyclic nucleoside **22** as a mixture of epimers in a 1:5 ratio. Again the subsequent benzoylation traps the hemiacetal as it benzoic ester **23** as only a single epimer in a good yield. A Vorbrüggen coupling with thymine gave a complicated mixture apparently due to some exchange of the original uracil of **23** with thymine. However, a coupling of **23** with uracil afforded a pure epimeric mixture of double-headed nucleosides **24**, which after deprotection gave the target compound **7** as a mixture of epimers in a 2:3 ratio. In a small amount, the major epimer was isolated for a full NMR characterization.

First of all, the two signals from H1′ and H8′ are both singlets in the ^{1}H NMR spectrum (with H8′ defined as shown in Scheme 1). This indicates the expected locked C3′-*endo* conformation as well as both the H1′–C1′–C2′–H2′ and the H2′–C2′–C8′–H8′ dihedral angles being restricted in close to 90°. However, this information is not conclusive for the 8′-configuration. A ROESY spectrum was hereafter deduced to fully assign the signals and to prove the C8′-configuration to be an (*R*)-configuration (Fig. 2). First of all, a mutual contact between one of the H6′-signals to only one of the H1′/H8′-signals and a mutual contact between the other 6′-proton and the other H1′/H8′-signal proved the 8′(*R*)-configuration. Thus, the opposite 8′(*S*)-configuration would have shown that one of the H6′-signals would see a contact with both H1′/H8′-signals and the other to none of the H1′/H8′-signals. Next, this information was used in the assignment of the two different H6′-signals (hereafter called $H6'_a$ and $H6'_b$), the two different uracils and the two different singlet signals representing H1′ and H8′ (Fig. 2). Thus, one of the H6′-signals showed a mutual contact to one of the H6-signals. With an 8′(*R*)-configuration, this is only possible with this being the H6′ in the pro-*R*-position ($H6'_b$) and the H6 from the uracil in the 8′-position ($H6_b$), respectively. Finally, these assignments and the overall configuration was confirmed by mutual contacts between $H6_a$ and H3′, between $H6_a$ and H1′, as well as between $H6_b$ and both H1′ and H8′. All these conclusive contacts are indicated in Fig. 2, whereas all other expected contacts though found in the ROESY spectrum are omitted for the clarity of the Figure.

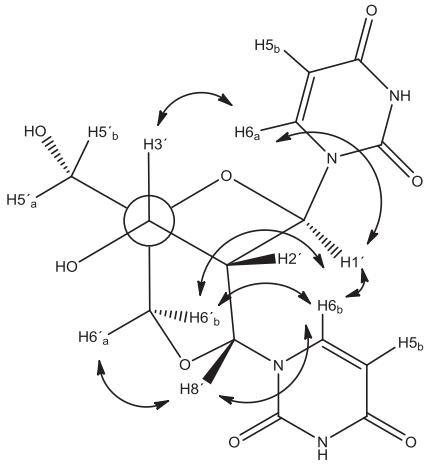


Fig. 2. The conclusive ROESY contacts of the major 8′(*R*)-epimer of **7**. Other contacts are omitted for clarity.

2.2. Discussion

It is clear from the present study that the carbocyclic LNA-analogue **4** and analogues thereof cannot be prepared from an RCM-strategy. The bicyclic ring-system is too constrained, and the radical strategy introduced by Chattopahyaya and co-workers is better for the purpose although the number of steps is significantly larger. Nevertheless, our attempts on RCM were worth the effort as they build mainly on intermediates already prepared in our synthesis of compound **3**, and as the study adds to the general understanding of the scope of the RCM-reaction.

An appealing result of the study is, however, that the 2′-*C*-formyl-4′-*C*-hydroxymethyl nucleosides obtained from the double-bond cleavage of either **15** or **21** readily equilibrates to the hemiacetal analogues **19** or **22**. This shows again that six-membered rings connecting C2′ and C4′ are much easier formed than five-membered rings and less constrained. The benzoic ester of this hemiacetal, **23**, was a good substrate for a Vorbrüggen coupling and probably also for other glycosidation reactions. However, this will be a balance of not also exchanging the uracil of **23**, but with the right optimization, we expect other combinations of nucleobases on the same skeleton as **7** as well as bicyclic nucleoside analogues with other groups attached to the C8′-postion to be obtainable from the key compound **23**.

Both 8′-epimers of the bicyclic double-headed nucleoside **7** are interesting for their potential incorporation into oligonucleotides. The strong conformational restriction, however, will most probably give completely different properties as compared to oligonucleotides containing the native analogue **8** (Fig. 1). Hence, the bicyclic structure will in both epimers prohibit the two nucleobases from the parallel orientation, which is central for **8** in forming two parallel base-pairs in the same duplex.[32] On the other hand, oligonucleotides with a central incorporation of **7** can form other secondary structures and might for instance lead to stable three-way junctions and hereby a tool in the targeting of other secondary structures, as seen before for related double-headed nucleotide analogues.[26,30] They might also be promising building blocks in evolving nucleic acid aptamers.

3. Conclusion

A bicyclic double-headed nucleoside has been synthesized from uridine based on the unexpected formation of a bicyclic hemiacetal structure. This hemiacetal can be an interesting building block for new derivatives, for instance new therapeutically useful LNA-analogues. Furthermore, the bicyclic double-headed nucleosides can constitute a promising new series of nucleic acid analogues with

the ability of forming new nucleic acid motifs, and targeting RNA-secondary structures. Ring-closing metathesis cannot form the bicyclo[2.2.1] skeleton of carba-LNA.

4. Experimental section

4.1. General

Reactions were performed under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out on glass columns using silica gel 60 (0.040–0.063 mm). HRMALDI and ESI mass spectra were recorded in positive-ion mode. NMR spectra were recorded at 300 or 400 MHz for ^{1}H NMR and 75 or 100 MHz for ^{13}C NMR. The δ values are in parts per million relative to tetramethylsilane as internal standard. Assignments of NMR spectra are based on 2D spectra and follow standard carbohydrate and nucleoside style; i.e., the carbon atom next to a nucleobase is assigned C-1′, etc. Compound names for the bicyclic compounds are given according to the von Baeyer nomenclature.

4.2. 3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-2′-[(4-methyl-3,5-dioxo-1,2,4-triazolidin-1-yl)prop-1-en-1-yl]-4′-*C*-vinyluridine (13)

A solution of nucleoside **12** (40 mg, 0.086 mmol) in CH_2Cl_2 (2 mL) was added 4-methyl-1,2,4-trazoline-3,5-dione (10 mg, 0.09 mmol) and stirred for 24 h at room temperature. An additional amount of 4-methyl-1,2,4-trazoline-3,5-dione (7 mg, 0.06 mmol) was added and the mixture was stirred for another 24 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (25–100% EtOAc in petrol ether) to give the product **13** (32 mg, 65%) as an oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.9 (br s, 1H, NH), 7.91 (d, 2H, J=8.2 Hz, H-6), 6.11 (d, 1H, J=7.5 Hz, H-1′), 5.94 (dd, 1H, J=10.8, 15.3 Hz, C*H*=CH$_2$), 5.80 (dd, 1H, J=10.8, 17.2 Hz, C*H*=CHCH$_2$), 5.74 (d, 1H, J=8.2 Hz, H-5), 5.54–5.42 (m, 2H, CH=C*H*CH$_2$, CH=C*H*$_2$), 5.25 (1H, dd, J=1.5, 10.0 Hz, CH=C*H*$_2$), 4.40 (d, 1H, J=6.5 Hz, H-3′), 4.28 (dd, 1H, J=4.6, 14.7 Hz, CH=CHC*H*$_2$), 3.86 (dd, 1H, J=8.2, 14.7 Hz, CH=CHC*H*$_2$), 3.67–3.58 (2H, m, H-5′), 3.04 (s, 3H, CH$_3$), 2.93 (m, 1H, H-2′), 0.92 (s, 9H, SiC(CH$_3$)$_3$), 0.89 (s, 9H, SiC(CH$_3$)$_3$), 0.11 (s, 6H, SiCH$_3$), 0.05 (s, 3H, SiCH$_3$), 0.01 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.1 (C-4), 154.9, 154.4 (CO) 151.6 (C-2), 139.4 (C-6), 134.4, 131.6, 126.7, 116.2 (C=C), 103.2 (C-5), 90.7, 87.9 (C-4′, C-1′), 75.3 (C-3′), 66.8 (C-5′), 55.3 (CH=CHCH$_2$), 48.2 (C-2′), 25.8, 25.7 (SiC(*C*H$_3$)$_3$), 25.3 (CH$_3$), 18.3, 18.1 (Si*C*(CH$_3$)$_3$), −4.1, −4.6, −5.5, −5.6 (SiCH$_3$).

4.3. 3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-2′-(prop-1-en-1-yl)-4′-*C*-vinyluridine (14)

A solution of nucleoside **12** (56 mg, 0.11 mmol) in EtOH (99.9%, 2 mL) was degassed with N_2 for 20 min and added $RhCl_3 \cdot xH_2O$ (4 mg, 0.07 mmol). The mixture was stirred at reflux for 1 h. The mixture was then concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:4 v/v) to give the product **14** (21 mg as a *E*/*Z*-mixture, 48%) as an oil, which was used without further purification in the next step; ^{13}C NMR (100 MHz, $CDCl_3$) δ (major isomer) 163.2 (C-4), 150.2 (C-2), 142.0 (C-6), 135.0, 130.8, 125.2, 115.6 (C=C), 102.7 (C-5), 90.9, 89.9 (C-4′, C-1′), 75.3 (C-3′), 66.2 (C-5′), 52.7 (C-2′), 25.7 (SiC(*C*H$_3$)$_3$), 18.1 (Si*C*(CH$_3$)$_3$), 13.5 (CH$_3$), −4.6, −4.8 (SiCH$_3$).

4.4. 3′,5′-Di-*O*-(*tert*-butyldimethyl-silyl)-2′-deoxy-4′-*C*-hydroxymethyl-2′-(prop-1-en-1-yl)uridine (15)

A solution of nucleoside **11** (400 mg, 0.76 mmol) in EtOH (99.9%, 4 mL) was added anhydrous K_2CO_3 (40 mg, 0.29 mmol), $RhCl_3 \cdot xH_2O$ (34 mg, 0.06 mmol) and stirred in a microwave reactor at 80 °C for 3 h. The mixture was then concentrated under reduced pressure and the residue was added brine (3 mL). The aqueous phase was extracted by EtOAc (2×5 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/petroleum ether, 1:4 v/v) to give the product **15** (230 mg as an *E*/*Z*-mixture, 7:1, 58%) as a white foam; R_f 0.50 (EtOAc/petroleum ether, 1:1 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (br s, 1H, NH), 7.76 (d, 1H, J=8.0 Hz, H-6), 7.67 (d, 1H, J=8.0 Hz, H-6), 6.21 (d, 1H, J=9.6 Hz, H-1′), 5.70 (dd, 1H, J=2.0, 8.0 Hz, H-5), 5.51–5.38 (m, 2H, C*H*=C*H*CH$_3$), 4.38 (d, 1H, J=6.0 Hz, H-3′), 4.35 (d, 1H, J=5.6 Hz, H-3′), 3.88 (dd, 1H, J=3.2, 11.6 Hz, H-5″), 3.78, 3.68 (AB, 2H, J=10.4 Hz, H-5′), 3.51 (dd, 1H, J=10.4, 11.6 Hz, H-5″), 2.87 (m, 1H, H-2′), 2.16 (m, 1H, OH), 1.65 (d, 3H, J=5.6 Hz, CH=CHC*H*$_3$), 1.51 (dd, 3H, J=0.8, 6.8 Hz, CH=CHCH$_3$), 0.93 (s, 18H, SiC(CH$_3$)$_3$), 0.11 (s, 6H, SiCH$_3$), 0.05 (s, 3H, SiCH$_3$), 0.03 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (major isomer) 163.1 (C-4), 150.5 (C-2), 140.0 (C-6), 131.2, 123.9 (*C*H=*C*HCH$_3$), 102.9 (C-5), 88.9, 86.8 (C-4′, C-1′), 77.2 (C-3′), 65.8 (C-5′), 64.0 (C-5″), 54.8 (C-2′), 25.9, 25.9 (SiC(*C*H$_3$)$_3$), 18.4, 18.3 (Si*C*(CH$_3$)$_3$), 13.5 (CH$_3$), −4.3, −4.6, −5.3, −5.5 (SiCH$_3$); HRESI MS m/z (549.2783 [M+Na]$^+$, $C_{25}H_{46}N_2O_6Si_2$–Na$^+$ calcd 549.2787).

4.5. 3′,5′-Di-*O*-(*tert*-butyldi-methyl-silyl)-2′-deoxy-4′-*C*-(hepta-1,6-dienyl)-2′-(prop-1-en-1-yl)uridine (16)

A degassed solution of **15** (122 mg, 0.23 mmol) in CH_2Cl_2 (4 mL) was added Dess–Martin periodinane (135 mg, 0.32 mmol) and the mixture was stirred for 1 h at room temperature. Et_2O was added and the mixture was filtered through a layer of Celite. The filter was washed with EtOAc and the combined organic phase was washed with a mixture of a saturated aqueous solution of $NaHCO_3$ and a 5% aqueous solution of $Na_2S_2O_3$ (1:1 v/v), dried ($MgSO_4$) and concentrated under reduced pressure to give the crude intermediate aldehyde (121 mg, quant). To a stirred solution of (hex-5-en-1yl)triphenyl-phosphonium bromide[43] (259 mg, 0.61 mmol) in anhydrous Et_2O (2 mL) at 0 °C was added a solution of *n*-BuLi in hexane (0.45 mL, 0.50 mmol) and the mixture was stirred at 0 °C for 15 min. A solution of the aldehyde (155 mg, 0.30 mmol) in anhydrous Et_2O (1 mL) was added, and the mixture was stirred at room temperature for 24 h and then diluted with Et_2O (15 mL). The mixture was filtered and washed with a saturated aqueous solution of $NaHCO_3$ (5 mL) and dried ($MgSO_4$). The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (20% EtOAc in petrol ether) to give the product **16** (63 mg, 38%, mixture of *E*/*Z*-diastereomers) as a white foam, which was used without further purification in the next step; R_f 0.75 (EtOAc/petrol ether, 1:1 v/v); HRMALDI MS m/z (613.3458 [M+Na]$^+$, $C_{31}H_{54}N_2O_5Si_2$–Na$^+$ calcd 613.3464).

4.6. 4′-*C*-Benzoyloxymethyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-2′-hydroxymethyluridine (17)

A solution of nucleoside **10** (540 mg, 0.86 mmol) in CH_2Cl_2 (5 mL) was added 4-methyl-1,2,4-trazoline-3,5-dione (116 mg, 1.03 mmol) and stirred for 14 h at room temperature. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (0–3% CH_3OH in CH_2Cl_2) to give the intermediate 4′-*C*-benzoyloxymethyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-2′-[(4-methyl-3,5-dioxo-1,2,4-triazolidin-1-yl)-prop-1-en-1-yl]uridine (297 mg, 47%) as well as unreacted starting material **10** (177 mg, 33%); R_f 0.60 (CH_3OH/dichloromethane, 7.5:92.5 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.79 (br s, 2H, NH), 8.01 (d, 2H, J=7.5 Hz, Ar), 7.71 (d, 1H, J=8.1 Hz, H-6), 7.59–7.54 (m, 1H, Ar), 7.47–7.42 (m,

2H, Ar), 6.25 (d, 1H, J=8.7 Hz, H-1′), 5.98 (dd, 1H, J=9.3, 15.9 Hz, C*H*=CHCH$_2$), 5.75 (d, 1H, J=8.1 Hz, H-5), 5.60–5.52 (m, 1H, CH=C*H*CH$_2$), 4.56, 4.39 (AB, 2H, J=12.0 Hz), 4.46 (d, 1H, J=5.7 Hz, H-3′), 4.27 (dd, 1H, J=4.5, 14.7 Hz, CH=CHC*H*$_2$), 3.95–3.82 (m, 3H, CH=CHC*H*$_2$), 3.04 (s, 3H, CH$_3$), 2.96–2.88 (m, 1H, H-2′), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.92 (s, 9H, SiC(CH$_3$)$_3$), 0.12 (s, 3H, SiCH$_3$), 0.11 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$), 0.04 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 166.3, 162.9, 155.0, 154.7, 151.7 (CO, C-4, C-2), 139.1 (C-6), 133.3, 129.8, 129.7, 128.6 (Ar), 130.5 (*C*H=CHCH$_2$), 127.4 (CH=*C*HCH$_2$), 103.5 (C-5), 88.7, 87.9 (C-4′, C-1′), 76.5, 65.2, 65.2 (C-3′, C-5′, C-5″), 55.0 (C-2′), 48.4 (CH=CH*C*H$_2$), 25.9, 25.8 (SiC(*C*H$_3$)$_3$), 25.3 (CH$_3$), 18.3, 18.3 (Si*C*(CH$_3$)$_3$), −4.1, −4.6, −5.3, −5.4 (SiCH$_3$); HRMALDI MS m/z (766.3254 [M+Na]$^+$, C$_{35}$H$_{53}$N$_5$O$_9$Si$_2$−Na$^+$ calcd 766.3274). A solution of this intermediate (600 mg, 0.81 mmol) in MeOH (6 mL) was bubbled with ozone at −70 °C for 5 min. The stirred solution was allowed to come to room temperature and then poured into water (5 mL). The organic layer was washed with a saturated aqueous solution of NaHCO$_3$ (5 mL) and brine (5 mL), dried (Na$_2$SO$_4$) and then concentrated under reduced pressure. The resulting ozonide was dissolved in MeOH (10 mL) and added NaBH$_4$ (245 mg, 6.45 mmol), and the reaction mixture was stirred for 10 min at room temperature. The organic layer was washed with a saturated aqueous solution of NaHCO$_3$ (2×5 mL) and brine (2×5 mL), and then dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH$_3$OH (0–3.0%) in CH$_2$Cl$_2$) to give the product **17** (299 mg, 60%); R_f 0.70 (CH$_3$OH/CH$_2$Cl$_2$, 1:9 v/v); ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.35 (br s, 1H, NH), 8.02 (d, 2H, J=7.2 Hz, Ar), 7.67 (d, 1H, J=8.1 Hz, H-6), 7.55 (m, 1H, Ar), 7.46–7.41 (m, 2H, Ar), 6.29 (d, 1H, J=8.4 Hz, H-1′), 5.73 (d, 1H, J=8.1 Hz, H-5), 4.66 (d, 1H, J=6.6 Hz, H-3′), 4.55, 4.35 (AB, 2H, J=12.0 Hz), 3.90 (m, 2H), 3.83 (m, 2H), 2.52 (m, 1H, H-2′), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.91 (s, 9H, SiC(CH$_3$)$_3$), 0.15 (s, 3H, SiCH$_3$), 0.14 (s, 3H, SiCH$_3$), 0.11 (s, 3H, SiCH$_3$), 0.09 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 166.4 (CO), 163.4 (C-4), 151.0 (C-2), 140.1 (C-6), 133.2, 129.9, 129.7, 128.5 (Ar), 103.0 (C-5), 88.0, 87.7 (C-4′, C-1′), 74.4 (C-3′), 64.9, 64.5, 59.3 (C-5′, C-5″, CH$_2$OH), 52.1 (C-2′), 25.9, 25.8 (SiC(*C*H$_3$)$_3$), 18.4, 18.2 (Si*C*(CH$_3$)$_3$), −4.3, −4.4, −5.3, −5.4 (SiCH$_3$); HRMALDI MS m/z (643.2838 [M+Na]$^+$, C$_{30}$H$_{48}$N$_2$O$_8$Si$_2$−Na$^+$ calcd 643.2841).

4.7. 3′,5′-Di-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-2′,4′-di-C-(hydroxymethyl)uridine (18)

To a stirred solution of compound **17** (100 mg, 0.16 mmol) in anhydrous CH$_3$OH (1 mL) was added NaOCH$_3$ (52 mg, 0.96 mmol) and the reaction mixture was stirred for 7 h at room temperature. The reaction was quenched by the addition of 80% CH$_3$COOH (1 mL) and then extracted with CH$_2$Cl$_2$ (2×4 mL). The combined extracts were washed with a saturated aqueous solution of NaHCO$_3$ (5 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–5% CH$_3$OH in CH$_2$Cl$_2$) to give the product **18** (53 mg, 64%) as a white foam; R_f 0.50 (CH$_3$OH-dichloromethane, 7.5:92.5 v/v); ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.22 (br s, 1H, NH), 7.88 (d, 1H, J=8.1 Hz, H-6), 6.34 (d, 1H, J=9.0 Hz, H-1′), 5.69 (dd, 1H, J=3.9, 8.1 Hz, H-5), 4.48 (m, 1H, H-3′), 3.92 (m, 2H), 3.85–3.79 (m, 2H), 3.69–3.65 (m, 2H), 3.61 (d, 1H, J=10.2 Hz), 2.84 (s, 1H, OH), 2.50 (m, 1H, H-2′), 0.90 (s, 9H, SiC(CH$_3$)$_3$), 0.89 (s, 9H, SiC(CH$_3$)$_3$), 0.11 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$), 0.09 (s, 3H, SiCH$_3$), 0.07 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 163.3 (C-4), 150.9 (C-2), 140.2 (C-6), 102.9 (C-5), 89.1, 86.8 (C-4′, C-1′), 75.9 (C-3′), 66.6, 64.1, 58.4 (C-5′, C-5″, CH$_2$OH), 52.3 (C-2′), 25.9, 25.8 (SiC(*C*H$_3$)$_3$), 18.4, 18.1 (Si*C*(CH$_3$)$_3$), −5.4, −5.4, −5.5, −5.5 (SiCH$_3$); HRMALDI MS m/z (539.2564 [M+Na]$^+$, C$_{23}$H$_{44}$N$_2$O$_7$Si$_2$−Na$^+$ calcd 539.2579).

4.8. (1*R*,4*R*/*S*,5*R*,6*R*,8*S*)-8-(*tert*-Butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-4-hydroxy-6-(uracil-1-yl)-3,7-dioxabicyclo[3.2.1]octane (19)

To a solution of nucleoside **15** (590 mg, 1.12 mmol) in aqueous acetone (1:1, 15 mL) was added a 2.5% w/v solution of OsO$_4$ in *tert*-butyl alcohol (1.82 mL, 0.18 mmol) and *N*-methylmorpholine-*N*-oxide (164 mg, 1.40 mmol). The reaction mixture was stirred for 17 h at room temperature. A 5% aqueous solution of Na$_2$S$_2$O$_3$ (10 mL) and then EtOAc (10 mL) were added. The mixture was concentrated under reduced pressure to approximately half of the volume and added EtOAc (12 mL). The mixture was washed with a saturated aqueous solution of NaHCO$_3$ (10 mL) and brine (10 mL). The organic phase was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The crude diol was dissolved in aqueous dioxane (20 mL, 1:1), and NaIO$_4$ (1.20 g, 5.61 mmol) was added. The reaction mixture was stirred for 6 h at room temperature. The mixture was added EtOAc (15 mL) and washed with a saturated aqueous solution of NaHCO$_3$ (2×15 mL) and brine (15 mL). The organic phase was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–3% CH$_3$OH in CH$_2$Cl$_2$) to give the hemiacetal product **19** (as an epimeric mixture 1:0.9, 300 mg, 52%); R_f 0.50 (CH$_3$OH/dichloromethane 1:9 v/v); ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.52 (br s, 1H, NH), 9.38 (br s, 1H, NH), 8.09 (d, 1H, J=8.4 Hz, H-6), 8.08 (d, 1H, J=8.0 Hz, H-6), 6.21 (s, 1H, H-1′), 5.84 (s, 1H, H-1′), 5.70–5.67 (m, 2H, H-5), 5.51 (d, 1H, J=11.6 Hz, OH), 5.39 (d, 1H, J=7.6 Hz, H-8′), 5.32–5.29 (m, 1H, H-8′), 4.44 (d, 1H, J=4.8 Hz, H-3′), 4.40 (d, 1H, J=5.6 Hz, H-3′), 4.05, 3.33 (AB, 2H, J=11.6 Hz), 3.94, 3.46 (AB, 2H, J=11.2 Hz), 3.77, 3.56 (AB, 2H, J=11.6 Hz), 3.75, 3.52 (AB, 2H, J=11.2 Hz), 2.59 (dd, 1H, J=2.8, 5.2 Hz, H-2′), 2.49 (d, 1H, J=6.0 Hz, H-2′), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.93 (s, 9H, SiC(CH$_3$)$_3$), 0.93 (s, 9H, SiC(CH$_3$)$_3$), 0.90 (s, 9H, SiC(CH$_3$)$_3$), 0.19 (s, 3H, SiCH$_3$), 0.17 (s, 3H, SiCH$_3$), 0.14 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$), 0.09 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 163.8 (C-4), 150.2, 150.1 (C-2), 139.8, 139.5 (C-6), 101.6, 101.4 (C-5), 94.8, 91.0 (C-8′), 85.0, 83.3, 83.3 (C-1′, C-4′), 67.1, 66.4 (C-3′), 65.1, 60.6, 59.8, 59.4 (C-5′, C-6′), 51.3, 46.9 (C-2′), 26.1, 26.0, 25.7, 25.6 (SiC(*C*H$_3$)$_3$), 18.6, 18.4, 18.0, 17.9 (Si*C*(CH$_3$)$_3$), −4.3, −4.8, −4.9, −5.0, −5.1, −5.3, −5.3, −5.4 (SiCH$_3$); HRMALDI MS m/z (537.2446 [M+Na]$^+$, C$_{23}$H$_{42}$N$_2$O$_7$Si$_2$−Na$^+$ calcd 537.2423).

4.9. (1*R*,4*R*/*S*,5*R*,6*R*,8*S*)-4-Benzoyloxy-8-(*tert*-butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-6-(uracil-1-yl)-3,7-dioxabicyclo[3.2.1]octane (20)

To a solution of the hemiacetal **19** (415 mg, 0.81 mmol) in anhydrous pyridine (2 mL) was added benzoyl chloride (112 μL, 0.97 mmol) dropwise, and the reaction mixture was stirred at room temperature for 1 h and then concentrated under reduced pressure. The residue was partitioned between EtOAc (4 mL) and H$_2$O (4 mL) and the organic phase was washed with a saturated aqueous solution of NaHCO$_3$ (2×3 mL), dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–3% CH$_3$OH in CH$_2$Cl$_2$) to give the product **20** (425 mg, 85%) as a white foam; R_f 0.70 (CH$_3$OH/dichloromethane 1:9 v/v); ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.08 (s, 1H, NH), 8.23 (dd, 2H, J=5.2, 7.2 Hz, Ar), 8.18 (d, 1H, J=8.4 Hz, H-6), 7.57–7.53 (m, 1H, Ar), 7.47–7.43 (m, 2H, Ar), 6.49, 6.44 (s, 2H, H-1′, H-8′), 5.70 (dd, 1H, J=2.0, 8.4 Hz, H-5), 4.51 (d, 1H, J=5.6 Hz, H-3′), 4.10, 3.52 (AB, 2H, J=11.2 Hz), 3.80, 3.58 (AB, 2H, J=11.6 Hz), 2.66 (d, 1H, J=5.6 Hz, H-2′), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.15 (s, 3H, SiCH$_3$), 0.14 (s, 3H, SiCH$_3$), 0.13 (s, 3H, SiCH$_3$), 0.13 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 164.9 (CO), 163.6 (C-4), 149.8 (C-2), 139.9 (C-6), 133.6, 130.3, 129.3, 128.6 (Ar), 101.5 (C-5), 90.3, 83.7, 83.4 (C-1′, C-4′,

C-8′), 66.1 (C-3′), 66.0, 59.3 (C-5′, C-6′), 49.6 (C-2′), 26.0, 25.7 (SiC(CH_3)$_3$), 18.5, 18.0 (SiC(CH$_3$)$_3$), −4.4, −4.9, −5.3, −5.4 (SiCH$_3$); HRMALDI MS m/z (641.2699 [M+Na]$^+$, $C_{30}H_{46}N_2O_8Si_2$−Na$^+$ calcd 641.2685).

4.10. 3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-4′-*C*-hydroxymethyl-2′-(prop-1-en-1-yl)uridine (21)

To a stirred solution of nucleoside **9** (923 mg, 2.24 mmol) in EtOH (99.9%, 7 mL) was added anhydrous K_2CO_3 (62 mg, 0.45 mmol), $RhCl_3 \cdot xH_2O$ (132 mg, 0.22 mmol) and the reaction mixture was stirred at 75 °C for 7 days. The mixture was concentrated under reduced pressure and the residue was dissolved in brine. The aqueous phase was extracted by EtOAc (2×8 mL), and the organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–6% CH_3OH in CH_2Cl_2) to give the product **21** (511 mg, 55%); R_f 0.60 (CH_3OH/dichloromethane 1:9 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (br s, 1H, NH), 7.45 (d, 1H, J=8.0 Hz, H-6), 5.96 (d, 1H, J=9.2 Hz, H-1′), 5.74–5.70 (m, 1H, H-5), 5.53–5.50 (m, 1H, CH=CHCH$_3$), 5.43–5.39 (m, 1H, CH=CHCH$_3$), 4.47 (d, 1H, J=6.4 Hz, H-3′), 3.94–3.89 (m, 1H, CH_2OH), 3.79–3.76 (m, 2H, CH_2OH), 3.70–3.66 (m, 1H, CH_2OH), 3.63–3.56 (m, 2H, CH_2OH), 3.18–3.12 (m, 1H, H-2′), 1.67 (d, 3H, J=6.0 Hz, CH=CHCH_3), 0.93 (s, 9H, SiC(CH$_3$)$_3$), 0.08 (s, 3H, SiCH$_3$), 0.04 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (major isomer) 163.5 (C-4), 150.7 (C-2), 141.6 (C-6), 131.6 (CH=CHCH$_3$), 124.0 (CH=CHCH$_3$), 102.9 (C-5), 90.2 (C-4′), 89.0 (C-1′), 76.7 (C-3′), 64.5, 63.9 (C-5′, C-5″), 52.5 (C-2′), 25.9 (SiC(CH$_3$)$_3$), 18.2 (SiC(CH$_3$)$_3$), −4.5, −4.7 (SiCH$_3$); HRESI MS m/z (435.1935 [M+Na]$^+$, $C_{19}H_{32}N_2O_6Si$−Na$^+$ calcd 435.1922).

4.11. (1*R*,4*R*/*S*,5*R*,6*R*,8*S*)-8-(*tert*-Butyldimethylsilyloxy)-4-hydroxy-1-hydroxymethyl-6-(uracil-1-yl)-3,7-dioxabicyclo [3.2.1] octane (22)

To a stirred solution of nucleoside **21** (525 mg, 1.27 mmol) in aqueous acetone (1:1, 12 mL) was added a 2.5% w/v solution of OsO_4 in *tert*-butyl alcohol (2.59 mL, 0.25 mmol) and *N*-methylmorpholine-*N*-oxide (186 mg, 1.59 mmol). The reaction mixture was stirred for 17 h at room temperature and then added a 5% aqueous solution of $Na_2S_2O_3$ (10 mL) and EtOAc (10 mL). The mixture was concentrated under reduced pressure to approximately half of the volume, added EtOAc (12 mL) and then washed with a saturated aqueous solution of $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude diol was dissolved in aqueous dioxane (18 mL, 1:1) and $NaIO_4$ (1.36 g, 6.37 mmol) was added. The reaction mixture was stirred for 6 h at room temperature and then added EtOAc (15 mL). The mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2×15 mL) and brine (15 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–7.5% CH_3OH in CH_2Cl_2) to give the product **22** (as a mixture of epimers 5:1, 314 mg, 62%); R_f 0.60 (CH_3OH/dichloromethane 1:4 v/v); ^{1}H NMR (400 MHz, CD_3OD) δ (major isomer) 8.21 (d, 1H, J=8.0 Hz, H-6), 6.19, 5.40 (s, 2H, H-1′, H-8′), 5.68 (d, 1H, J=8.0 Hz, H-5), 4.51 (d, 1H, J=4.8 Hz, H-3′), 3.92, 3.43 (AB, 2H, J=11.2 Hz), 3.68, 3.50 (AB, 2H, J=12.0 Hz), 2.47 (d, 1H, J=5.2 Hz, H-2′), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.14 (s, 3H, SiCH$_3$), 0.13 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, CD_3OD) δ (major isomer) 166.4 (C-4), 151.7 (C-2), 142.4 (C-6), 101.6 (C-5), 91.6, 84.9, 84.3 (C-1′, C-4′, C-8′), 68.3 (C-3′), 65.8, 59.2 (C-5′, C-6′), 52.0 (C-2′), 26.1 (SiC(CH$_3$)$_3$), 18.8 (SiC(CH$_3$)$_3$), −4.7, −5.1 (SiCH$_3$); HRESI MS m/z (423.1562 [M+Na]$^+$, $C_{17}H_{28}N_2O_7Si$−Na$^+$ calcd 423.1559).

4.12. (1*R*,4*R*/*S*,5*R*,6*R*,8*S*)-4-Benzoyloxy-1-(benzoyloxymethyl)-8-(*tert*-butyldimethylsilyloxy)-6-(uracil-1-yl)-3,7-dioxabicyclo [3.2.1] octane (23)

A stirred solution of hemiacetal **22** (400 mg, 1.0 mmol) in anhydrous pyridine (3 mL) was added benzoyl chloride (0.29 mL, 2.5 mmol) dropwise and then stirred at room temperature for 45 min. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (0–2.5% CH_3OH in CH_2Cl_2) to give **23** (398 mg, 65%) as a white foam; R_f 0.40 (CH_3OH/dichloromethane 1:19 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (br s, 1H, NH), 8.22 (dd, 2H, J=1.2, 8.8 Hz, Ar), 8.01 (dd, 2H, J=1.2, 8.4 Hz, Ar), 7.88 (d, 1H, J=8.4 Hz, H-6), 7.65 (t, 1H, J=7.6 Hz, Ar), 7.59–7.44 (m, 5H, Ar), 6.54, 6.42 (s, 2H, H-1′, H-8′), 5.50 (dd, 1H, J=2.0, 8.4 Hz, H-5), 4.58, 4.36 (AB, 2H, J=12.8 Hz), 4.53 (d, 1H, J=5.6 Hz, H-3′), 4.26, 3.78 (AB, 2H, J=11.2 Hz), 2.81 (d, 1H, J=5.6 Hz, H-2′), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.15 (s, 3H, SiCH$_3$), 0.09 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 164.8, 163.5 (CO, C-4), 149.7 (C-2), 139.1 (C-6), 134.0, 133.7, 130.3, 129.5, 129.2, 129.2, 129.0, 128.7 (Ar), 101.7 (C-5), 90.3, 84.4 (C-1′, C-8′), 81.9 (C-4′), 67.7 (C-3′), 65.7, 60.6 (C-5′, C-6′), 49.2 (C-2′), 25.7 (SiC(CH$_3$)$_3$), 18.0 (SiC(CH$_3$)$_3$), −4.5, −5.1 (SiCH$_3$); HRESI MS m/z (631.2093 [M+Na]$^+$, $C_{31}H_{36}N_2O_9Si$−Na$^+$ calcd 631.2082).

4.13. (1*R*,4*R*/*S*,5*R*,6*R*,8*S*)-1-(Benzoyloxymethyl)-8-(*tert*-butyldimethylsilyloxy)-4,6-di-(uracil-1-yl)-3,7-dioxabicyclo[3.2.1] octane (24)

To a stirred solution of **23** (412 mg, 0.68 mmol) and uracil (228 mg, 2.03 mmol) in anhydrous CH_3CN (5 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (1.16 mL, 4.74 mmol). The reaction mixture was stirred for 30 min at room temperature and then at 0 °C. Trimethylsilyl triflate (0.6 mL, 3.39 mmol) was added dropwise, and the solution was stirred for 4 days at 60 °C. After cooling to room temperature, the reaction mixture was diluted with EtOAc (15 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2×10 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–5% CH_3OH in CH_2Cl_2) to give product **24** (as a diastereomeric mixture 1:0.70, 233 mg, 58%) as a white foam; R_f 0.35 (CH_3OH/dichloromethane 7.5:92.5 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (br s, 2H, NH), 9.69 (br s, 2H, NH), 8.01–7.98 (m, 4H, Ar), 7.77–7.74 (m, 2H, H-6, Ar), 7.68 (d, 1H, J=8.0 Hz, H-6), 7.65–7.62 (m, 2H, H-6, Ar), 7.53–7.48 (m, 4H, Ar), 7.41 (d, 1H, J=8.0 Hz, H-6), 6.08, 5.99 (s, 2H, H-1′, H-8′), 5.76 (dd, 2H, J=2.0, 8.0 Hz, H-5), 5.70 (s, 1H, H-1′, H-8′), 5.47 (dd, 2H, J=2.0, 8.0 Hz, H-5), 4.60 (d, 1H, J=12.0 Hz), 4.55 (d, 1H, J=12.0 Hz), 4.46 (d, 1H, J=8.0 Hz, H-3′), 4.40 (d, 1H, J=4.0 Hz, H-3′), 4.35–4.25 (m, 4H), 3.96 (d, 1H, J=12.0 Hz), 3.92 (d, 1H, J=12.0 Hz), 3.69 (d, 1H, J=4.0 Hz, H-2′), 3.29 (d, 1H, J=8.0 Hz, H-2′), 0.93 (s, 9H, SiC(CH$_3$)$_3$), 0.80 (s, 9H, SiC(CH$_3$)$_3$), 0.23 (s, 3H, SiCH$_3$), 0.09 (s, 3H, SiCH$_3$), 0.04 (s, 3H, SiCH$_3$), −0.05 (s, 3H, SiCH$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 163.9, 163.7, 163.6, 163.3 (CO, C-4), 150.6, 150.5, 150.2, 149.9 (C-2), 139.2, 138.1 (Ar), 139.9, 139.2, 138.4, 138.1 (C-6), 134.2, 134.1, 129.6, 129.5, 129.1, 129.0, 128.9 (Ar), 102.1, 102.0, 101.7, 100.1 (C-5), 87.0, 84.3, 83.7, 82.2, 81.6, 79.6 (C-1′, C-4′, C-8′), 67.2, 66.1 (C-3′), 66.8, 65.2, 61.2, 60.6 (C-5′, C-6′), 47.1, 45.4 (C-2′), 26.0, 25.7 (SiC(CH$_3$)$_3$), 18.2, 18.0 (SiC(CH$_3$)$_3$), −4.5, −4.6,−5.2, −5.3 (SiCH$_3$); HRESI MS m/z (621.1984 [M+Na]$^+$, $C_{28}H_{34}N_4O_9Si$−Na$^+$ calcd 621.1987).

4.14. (1*R*,4*R*/*S*,5*R*,6*R*,8*S*)-8-Hydroxy-1-hydroxymethyl-4,6-di-(uracil-1-yl)-3,7-dioxabicyclo[3.2.1]octane (7)

To a stirred solution of nucleoside **24** (229 mg, 0.38 mmol) in anhydrous THF (3 mL) was added a 1 M solution of TBAF in anhydrous THF (0.57 mL, 0.57 mmol), and the mixture was stirred at

room temperature for 1 h and then concentrated under reduced pressure. The residue was dissolved in saturated methanolic NH_3 (5 mL), and the reaction mixture was stirred at room temperature for 4 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0–7.5% CH_3OH in CH_2Cl_2) to give the product **7** (as an epimeric mixture 1:0.70, 105 mg, 72%) as a white foam; R_f 0.40 (CH_3OH/dichloromethane 15:85 v/v); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (br s, 4H, NH), 8.08 (d, 1H, J=8.0 Hz, H-6), 7.97 (d, 1H, J=8.4 Hz, H-6), 7.87 (d, 1H, J=8.4 Hz, H-6), 7.58 (d, 1H, J=8.0 Hz, H-6), 6.18 (s, 1H, OH), 5.98, 5.86, 5.75, 5.67 (s, 4H, H-1′, H-8′), 5.59–5.56 (m, 3H, H-5), 5.50 (d, 1H, J=8.0 Hz, H-5), 4.24 (d, 1H, J=5.6 Hz, H-3′), 4.17–4.14 (m, 2H, H-3′, H-6′), 4.0, 3.70 (AB, 2H, J=11.2 Hz, H-6′), 3.66 (d, 1H, J=11.2 Hz, H-6′), 3.61–3.58 (m, 2H, H-5′), 3.50 (dd, 2H, J=5.2, 12.8 Hz, H-5′), 3.10 (dd, 1H, J=1.2, 4.8 Hz, H-2′), 2.70 (d, 1H, J=5.6 Hz, H-2′); HRESI MS m/z (403.0860 [M+Na]$^+$, $C_{15}H_{16}N_4O_8$–Na$^+$ calcd 403.0861). A small amount was separated in a new chromatography to give the pure epimer (1*R*,4*R*,5*R*,6*R*,8*S*)-8-hydroxy-1-hydroxymethyl-4,6-di-(uracil-1-yl)-3,7-dioxabicyclo[3.2.1]octane; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (s, 1H, NH), 11.26 (s, 1H, NH), 7.97 (d, 1H, J=8.8 Hz, H-6$_a$), 7.87 (d, 1H, J=8.4 Hz, H-6$_b$), 6.10 (d, 1H, J=4.4 Hz, 3′-OH), 5.98 (s, 1H, H-8′), 5.67 (s, 1H, H-1′), 5.58 (d, 1H, J=8.8 Hz, H-5$_a$), 5.57 (d, 1H, J=8.4 Hz, H-5$_b$), 5.27 (t, 1H, J=5.2 Hz, 5′-OH), 4.24 (t, 1H, J=5.2 Hz, H-3′), 4.01 (d, 1H, J=10.8 Hz, H-6′$_a$), 3.71 (d, 1H, J=10.8 Hz, H-6′$_b$), 3.61 (dd, 2H, J=5.2, 12.4 Hz, H-5′), 3.51 (dd, 2H, J=5.6, 12.4 Hz, H-5′), 2.71 (d, 1H, J=5.2 Hz, H-2′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.3, 163.1 (C-4), 150.0, 149.9 (C-2), 140.1, 139.2 (C-6), 100.9, 100.5 (C-5), 83.2, 82.2, 78.4 (C-1′, C-4′, C-8′), 66.5 (C-3′), 64.3, 57.7 (C-5′, C-6′), 47.3 (C-2′).

Acknowledgements

The project was supported by The Danish National Research Foundation, The Danish Council for Independent Research Natural Sciences (FNU) and Technology and Production Sciences (FTP) and Villum Kann Rasmussen Fonden.

Supplementary data

Selected NMR spectra can be found in the online version. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.12.013.

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