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Synthesis and biological evaluation of branched and conformationally restricted analogs of the anticancer compounds 3′-*C*-ethynyluridine (EUrd) and 3′-*C*-ethynylcytidine (ECyd)

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Abstract—The synthesis of branched and conformationally restricted analogs of the anticancer nucleosides 3′-*C*-ethynyluridine (EUrd) and 3′-*C*-ethynylcytidine (ECyd) is presented. Molecular modeling and ^{1}H NMR coupling constant analysis revealed that the furanose rings of all analogs except the LNA analog are conformationally biased towards South conformation, and are thus mimicking the structure of ECyd. All target nucleosides were devoid of anti-HIV or anticancer activity.

1. Introduction

3′-*C*-Ethynyluridine (EUrd, **1**) and its cytidine analog (ECyd, **2**) (Fig. 1) display excellent broad-spectrum antitumor activities in vitro as well as in tumor models.[1] EUrd and ECyd undergo phosphorylation catalyzed by uridine–cytidine kinase (EC 2.7.1.48, UCK) to give the corresponding 5′-monophosphates, which are further phosphorylated by nucleotide kinases to the pharmacologically active triphosphates. The triphosphates of EUrd and ECyd competitively inhibit human RNA polymerases I, II, III, and thereby DNA templated RNA synthesis, which leads to cell apoptosis presumably via RNase L catalyzed rRNA fragmentation.[2–4] Both nucleosides are distributed very selectively into tumor tissue,[5,6] which accounts for the absence of severe toxicities in nude rat models,[2,7] and renders them as promising agents in the therapy of solid human cancers.

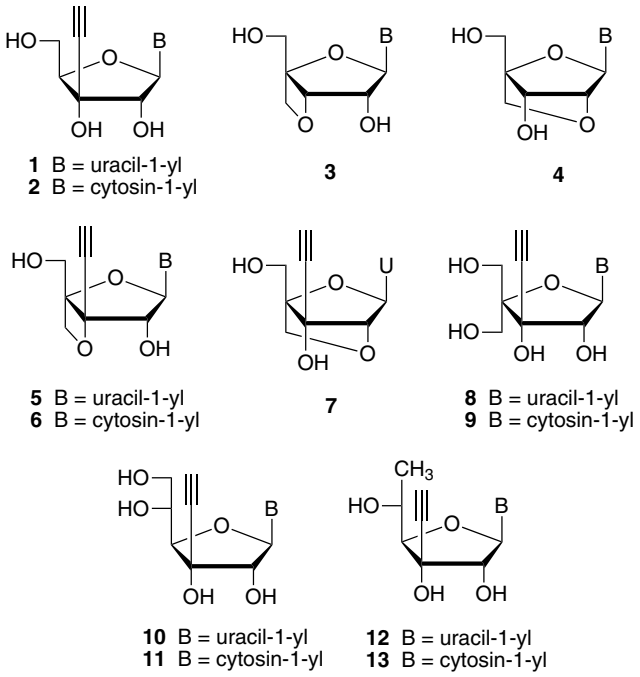

Figure 1. EUrd **1**, ECyd **2**, conformationally restricted nucleosides **3**–**4** and target nucleosides **5**–**13**.

Keywords: Bicyclic nucleosides; Branched nucleosides; EUrd; TAS-1-06; LNA.
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ECyd, which is more potent than EUrd, is currently in Phase I clinical trials.

Several ECyd-analogs have been synthesized, which has clarified the importance of the nucleobase,[1] the C3′-substituent,[8–10] the configurational requirements at the C2′- and C3′-positions,[8] and the 4′-oxo function[11] for anticancer activity. The major conclusion from these structure–activity relationship (SAR) studies is that UCK has a very strict substrate specificity, which has precluded development of even more potent analogs. While EUrd/ECyd are sufficiently phosphorylated, analogs carrying bulkier C3′-substituents such as ethyl, ethenyl, or cyclopropyl are not.[8] 3′-Deoxy and *xylo*-EUrd/ECyd-analogs are also inactive implying that *ribo*-configuration of the furanose moiety is important for UCK-catalyzed phosphorylation.[8]

The furanose ring of nucleosides in solution normally adopts a number of conformations, which can be described by the phase angle P in the pseudorotational cycle (Fig. 2).[12] In solid state, however, nucleosides typically cluster in two antipodal domains centered around $P = 0°$ (North) and $P = 180°$ (South).[13] The furanose ring of ECyd is known to adopt a South conformation in crystalline state ($P = 182°$) as well as in solution.[11] Most enzymes involved in nucleoside metabolism and nucleotide polymerization have strict conformational preferences for the furanose ring of their substrates. Conformational restriction of the furanose ring has been a successful strategy to probe for conformational preferences of such enzymes, and has revealed that activating kinases and the final target enzymes may have antipodal conformational requirements for the furanose ring.[14–17] 3′-*O*,4′-*C*-methylene ribonucleosides (**3**, Fig. 1)[18] and 2′-*O*,4′-*C*-Methylene ribonucleosides (locked nucleic acid nucleosides, i.e., LNA nucleosides) (**4**, Fig. 1),[19] represent two classes of conformationally restricted nucleosides where the furanose ring is fixed in South and North conformations, respectively.

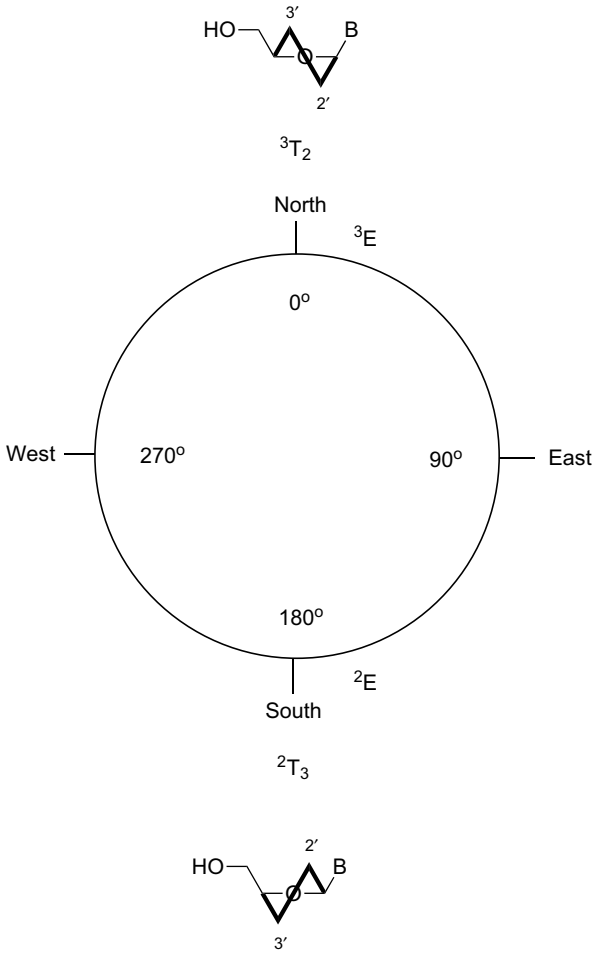


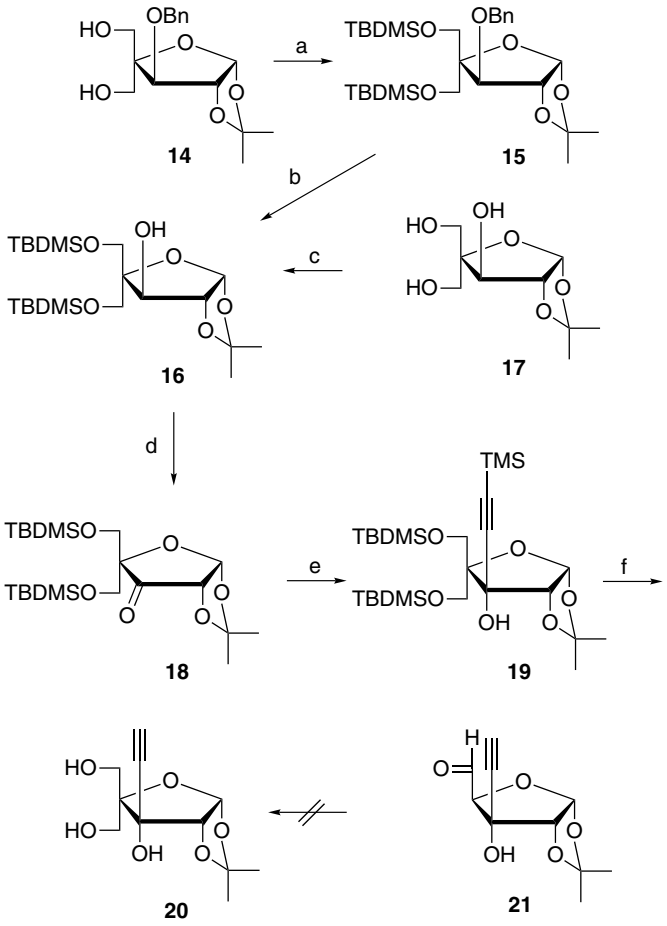
Figure 2. Pseudorotational cycle of the furanose ring in nucleosides.

A set of EUrd/ECyd analogs with the furanose rings restricted in *S*-type or *N*-type sugar conformations may elucidate the substrate preference of UCK. Furthermore, if UCK, nucleotide kinases and RNA polymerase have similar preferences for the sugar conformation of their substrates, the favorable entropic gain arising from preorganization of the furanose ring, may potentially lead to stronger binding and increased anticancer activity. We therefore chose to evaluate 3′-*O*,4′-*C*-methylene-linked bicyclic EUrd/ECyd analogs **5**–**6** and LNA–EUrd analog **7** (Fig. 1). Furthermore, since no SAR studies have evaluated the effect of additional branching at the C-4′- and C-5′-position on anticancer activity, we addressed this shortcoming by synthesizing and evaluating 4′-*C*-hydroxymethyl EUrd/ECyd-analogs **8**–**9**, 5′-*C*-hydroxymethyl EUrd/ECyd-analogs **10**–**11** and 5′-*C*-methyl EUrd/ECyd-analogs **12**–**13** (Fig. 1).

2. Synthesis

2.1. Synthesis of key intermediate *20* toward 4′-*C*-hydroxymethyl, 3′-*O*,4′-*C*-methylene-linked, and LNA-type EUrd/ECyd analogs

Triol **20** was identified as a suitable divergence point toward the bicyclic nucleosides **5**–**7** and the 4-*C*-hydroxymethyl nucleosides **8**–**9** (Scheme 1). The starting material for synthesis of triol **20**, 4-*C*-hydroxymethyl furanose **14**,[20] was conveniently obtained in four steps from inexpensive 1,2;5,6-di-*O*-isopropylidene-α-D-glucose without purification of the intermediates in 64% overall yield. Protection of the hydroxyl groups of furanose **14** as *tert*-butyldimethylsilyl (TBDMS) ethers furnished furanose **15** in 71% yield. Subsequent debenzylation of **15** by catalytic hydrogenation using H_2 and $Pd(OH)_2/C$ in ethanol afforded alcohol **16** in 76% yield along with a by-product which NMR (results not shown) and MALDI-MS (*m*/*z* 357 $[M+Na]^+$) confirmed to arise from the cleavage of one of the TBDMS groups.[21] Prolonged exposure (>36 h) of furanose **15** to debenzylation conditions resulted in undesired cleavage of the benzyl group and both TBDMS groups to furnish the known triol **17**,[22] verifying the recently reported solvent-dependent lability of TBDMS *O*-ethers under Pd/C-catalyzed hydrogenation conditions.[23,24] Triol **17**,[22] more conveniently obtained from 1,2;5,6-di-*O*-isopropylidene-α-D-glucose in three steps without purifica-



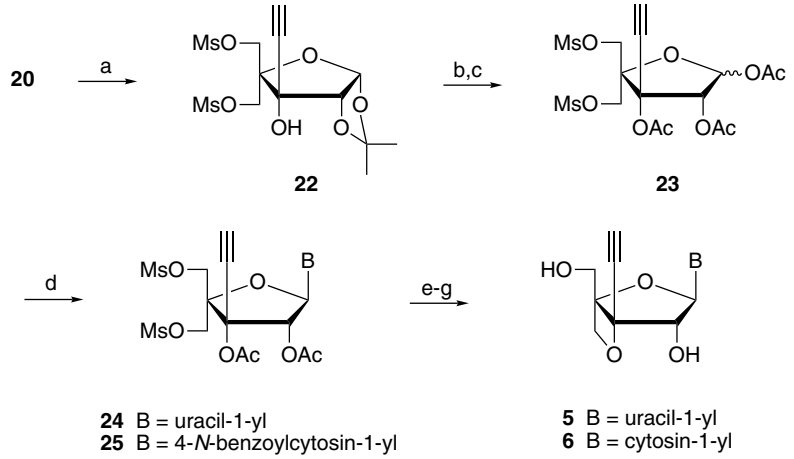
Scheme 1. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt, 71%; (b) H_2, 20% $Pd(OH)_2$/C, EtOH, rt, 76%; (c) TBDMSCl, imidazole, DMF, 0 °C to rt, 71%; (d) Dess–Martin periodinane, CH_2Cl_2, 0 °C to rt, 97% or TEMPO, BAIB, CH_2Cl_2, rt, 91%; (e) TMSC≡CH, *n*-BuLi, THF, −78 °C, 90%; (f) TBAF, THF, rt, 74%.

tion of intermediates (64% overall yield), was converted to furanose **16** in 71% yield by selective TBDMS protection of the primary hydroxyl groups. Oxidation of furanose **16** to 3-ulose **18** was accomplished with Dess–Martin periodinane[25] or with 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO)[26] and [(diacetoxy)iodo]benzene (BAIB) as co-oxidant in excellent yields (97% and 91%, respectively). Nucleophilic addition of LiC≡CTMS (prepared in situ from trimethylsilylacetylene and *n*-butyllithium in THF) to 3-ulose **18**, occurred from the sterically less hindered β face to exclusively give *ribo*-configured furanose **19** in 90% yield.[27] Desilylation of **19** with tetrabutylammonium fluoride (TBAF) furnished the key intermediate **20** in 74% yield.

Attempts to shorten the synthetic route by subjecting the known hydroxy aldehyde **21**[28] to typical crossed aldol condensation and crossed Cannizzaro reduction conditions (37% aq formaldehyde, 2 M NaOH, 1,4-dioxane),[20] failed.

2.2. Synthesis of 3′-*O*,4′-*C*-methylene-linked EUrd/ECyd analogs 5 and 6

Inspired by the improved synthetic route to LNA,[29] we decided to install methanesulfonate groups at C5′/C5″ prior to glycosylation, and triol **20** was therefore selectively mesylated to afford bis-sulfonic ester **22** in 77% yield (Scheme 2). Cleavage of the 1,2–*O*-isopropylidene group of **22** by treatment with aqueous trifluoroacetic acid (TFA) furnished the anomeric triol, which upon treatment with acetic anhydride and DMAP at elevated temperatures resulted in a complicated reaction mixture from which glycosyl donor **23** was isolated in relatively low yield (<50%). Instead, the crude triol was peracetylated using acetic anhydride and catalytic trimethylsilyl triflate (TMSOTf).[30] Interestingly, the choice of solvent was critical for the outcome of this reaction. Desired glycosyl donor **23** was not obtained when the reaction was carried out in acetonitrile, a solvent recommended for this type of reaction,[30] whereas excellent yield (88%, from **22**) of **23** was observed when the reaction was carried out in dichloromethane. Subsequent one-pot reactions of glycosyl donor **23** with uracil or 4-*N*-benzoylcytosine, *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and TMSOTf,[31] stereoselectively afforded the N1-linked β-nucleosides via anchimeric assistance of the O2-acetyl group in 74% yield for both **24** and **25**. Treatment of nucleosides **24** and **25** with saturated methanolic ammonia resulted in tandem deacylation and selective 3′-*O*,4′-*C* ring closure. Subsequent nucleophilic substitution of the remaining C5′-mesylate group



Scheme 2. Reagents and conditions: (a) MsCl, pyridine, 0 °C to rt, 77%; (b) 80% aq TFA, 0 °C; (c) Ac_2O, TMSOTf, CH_2Cl_2, 0 °C, 88% (two steps); (d) uracil/4-*N*-benzoylcytosine, BSA, TMSOTf, 1,2-dichloroethane, reflux, 74% for **24**, 74% for **25**; (e) satd NH_3/MeOH, rt; (f) NaOBz, DMF, 110 °C; (g) satd NH_3/MeOH, rt, 62% for **5** (three steps), 26% for **6** (three steps).

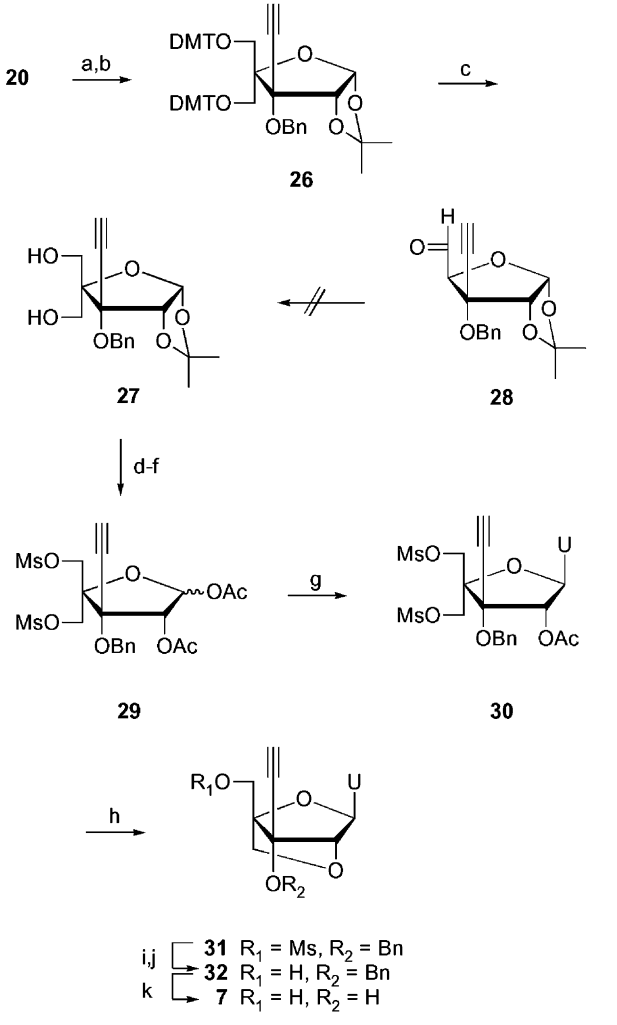
with sodium benzoate in DMF afforded benzoates, which were deacylated with saturated methanolic ammonia to furnish the bicyclic nucleosides **5** and **6** in modest yields (62% of **5** from **24** and 26% of **6** from **25**, respectively).

Selective 3′-*O*,4′-*C* ring closure as observed during synthesis of **5** and **6** rather than 2′-*O*,4′-*C* ring closure (which would furnish LNA analogs), has also been observed during synthesis of 3′-*O*,4′-*C*-methyleneribonucleosides.[18] This selectivity has been proposed to arise from preorganization of the nucleosides in a South conformation, by which the 3′-OH group is in closer proximity to the leaving group at the C5″-position than the 2′-OH group.

Evidence for the proposed dioxabicyclo[3.2.0]heptane skeleton of **5** and **6** was obtained by ^{1}H NMR (DMSO-d_6) experiments. The signal of the 2′-OH group of nucleoside **6** appeared at 5.87 ppm as an exchangeable doublet. Furthermore, the signal of the 5′-OH group appeared as an exchangeable triplet at 4.93 ppm, which coupled to H-5′ (3.70 ppm, doublet). The oxetane ring protons H-5″ appeared as two doublets at 4.29 and 4.70 ppm. Also, key NOE contacts between H-5″$_B$/H-1′ (7%) and H2′/H-6 (12%) verified the dioxabicyclo[3.2.0]heptane skeleton and β-configuration of **6**, respectively. Similar conclusions were made from ^{1}H NMR- and NOE-experiments of nucleoside **5**.

2.3. Synthesis of EUrd–LNA-type analog 7

Based on the observations during synthesis of **5** and **6**, it was obvious that synthesis of EUrd–LNA-analog **7** (Scheme 3) would require a suitable protection of the O3-hydroxyl group of triol **20** to prevent undesired 3′-*O*,4′-*C* ring closure at a later stage. Protection of the O3-hydroxyl group as a benzyl ether was chosen since this protecting group has been successfully employed at a similar stage in the synthesis of LNA[29] and it was therefore expected to be fully compatible with the subsequent synthetic steps. Furthermore, deprotection of



Scheme 3. Reagents and conditions: (a) DMTCl, DMAP, pyridine, rt; (b) NaH, BnBr, Bu_4I, THF; (c) 80% aq AcOH, rt, 55% (three steps); (d) MsCl, pyridine, rt; (e) 80% aq TFA, rt; (f) Ac_2O, pyridine, rt, 89% (three steps); (g) uracil, BSA, TMSOTf, CH_3CN, 50 °C, 88%; (h) 2 M NaOH, 1,4-dioxane–H_2O (2:1, v/v), rt, 94%; (i) NaOBz, DMF, 110–140 °C; (j) satd NH_3/MeOH, rt, 62% (two steps); (k) BCl_3, CH_2Cl_2, hexanes, −78 °C to rt, 63%. U = uracil-1-yl.

benzyl ethers is known to be orthogonal with the alkyne functionality in related systems.[28] The primary hydroxyl groups of triol **20** were therefore transiently protected as the 4,4′-dimethoxytrityl (DMT) ethers and subsequent O3-benzylation furnished fully protected furanose **26**. Deprotection of the DMT ethers with acetic acid afforded furanose **27** in 55% yield over three steps.

Attempts to shorten the route to furanose **27** by subjecting known O3-benzylated aldehyde **28**[28] to tandem crossed aldol condensation and crossed Cannizzaro reduction conditions, failed. This is quite surprising since the corresponding reaction with a C3-allyl group is known to occur satisfactorily.[32]

Permesylation of furanose **27** followed by isopropylidene cleavage and peracetylation using standard conditions furnished glycosyl donor **29** in 89% yield over three steps. One-pot reaction of glycosyl donor **29** with uracil, BSA and TMSOTf [31] exclusively gave β-nucleoside **30** in 88% yield. Treatment of nucleoside **30** with saturated methanolic ammonia resulted merely in O2′-deacylation (results not shown) whereas subjecting **30** to aqueous sodium hydroxide in 1,4-dioxane,[29] resulted in tandem deacetylation and ring closure to give LNA-type derivative **31** in excellent 94% yield. Nucleophilic displacement of the remaining mesylate group with sodium benzoate required harsh conditions (140 °C, 3 days), which resulted in partial decomposition as seen by the formation of tars. The crude benzoate was reacted with saturated methanolic ammonia to give nucleoside **32** in 62% yield over two steps. Final debenzylation of nucleoside **32** with boron trichloride afforded EUrd–LNA-type analog **7** in 63% yield without affecting the alkyne functionality.

The dioxabicyclo[2.2.1]heptane skeleton of LNA–EUrd-analog **7** was ascertained by ^{1}H NMR experiments. The signals of H-1′ and H-2′ appeared as singlets, which is a typical observation for the locked dioxabicyclo[2.2.1]heptane skeleton of LNA.[29,33] The signals of the 3′-OH and 5′-OH groups appeared as an exchangeable singlet and triplet, respectively, while no signals for a secondary hydroxyl group (e.g., a 2′-OH group) were observed.

2.4. Synthesis of 4′-*C*-hydroxymethyl EUrd/ECyd analogs 8 and 9

Selective acetylation of triol **20** was followed by 1,2-*O*-isopropylidene cleavage of resulting alcohol **3** by treatment with aqueous trifluoroacetic acid (Scheme 4). The crude triol was peracetylated using acetic anhydride and catalytic DMAP to give the glycosyl donor **34** in 55% yield from **20**. Elevated temperatures were required to facilitate acetylation of the tertiary hydroxyl group. Reaction of glycosyl donor **34** with persilylated uracil or cytosine under modified Vorbrüggen conditions,[31] stereoselectively afforded β-nucleosides **35** and **36** in 77% and 54% yield, respectively. Subsequent treatment of nucleosides **35** and **36** with saturated methanolic ammonia gave 4′-*C*-hydroxymethyl EUrd/ECyd analogs **8** and **9** in 97% and 75% yield, respectively.

The relative configuration of the nucleobase and 3′-*C*-ethynyl group was verified by single crystal X-ray diffraction studies of the 4′-*C*-hydroxymethyl nucleosides **8** and **9** (Fig. 3). The absolute configuration follows from the stereochemically pure starting materials and the applied synthetic route (Schemes 1 and 4).

2.5. Synthesis of 5′-*C*-hydroxymethyl EUrd/ECyd analogs 10 and 11

Pyridinium dichromate (PDC) oxidation of commercially available 1,2;5,6-di-*O*-isopropylidene-α-D-glucose **37** to the known 3-ulose[34] followed by nucleophilic addition of LiC≡CTMS formed in situ, furnished alcohol **38** in 73% yield over two steps (Scheme 5). Desilylation of alcohol **37** with TBAF to afford known furanose **39**[35] in 76% yield confirmed the exclusive formation of *allo*-configured alcohol **38** during acetylide addition. Selective 5,6-*O*-isopropylidene cleavage of furanose **38** afforded crystalline triol **40** (82% yield), which was enough reactive to undergo peracetylation with excess acetic anhydride and catalytic DMAP at room temperature

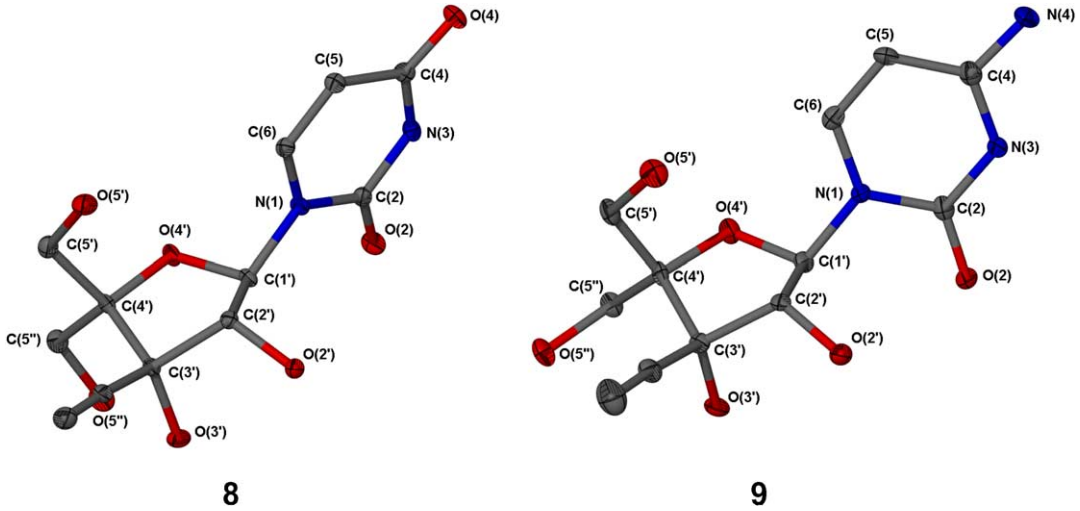
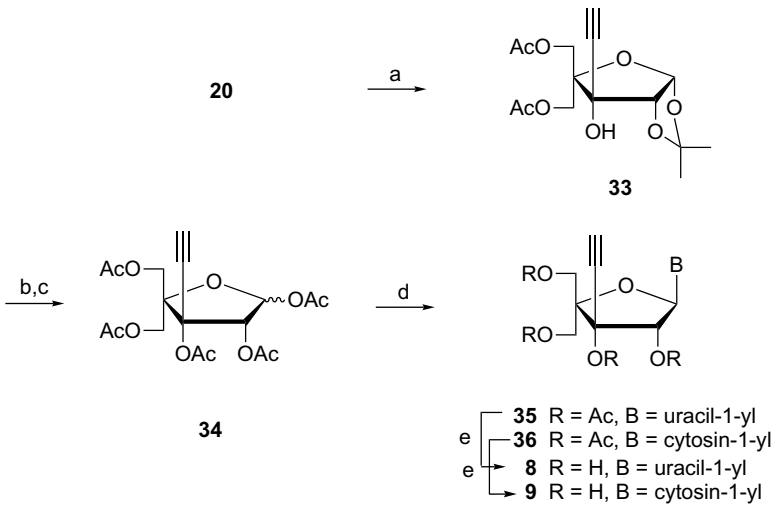
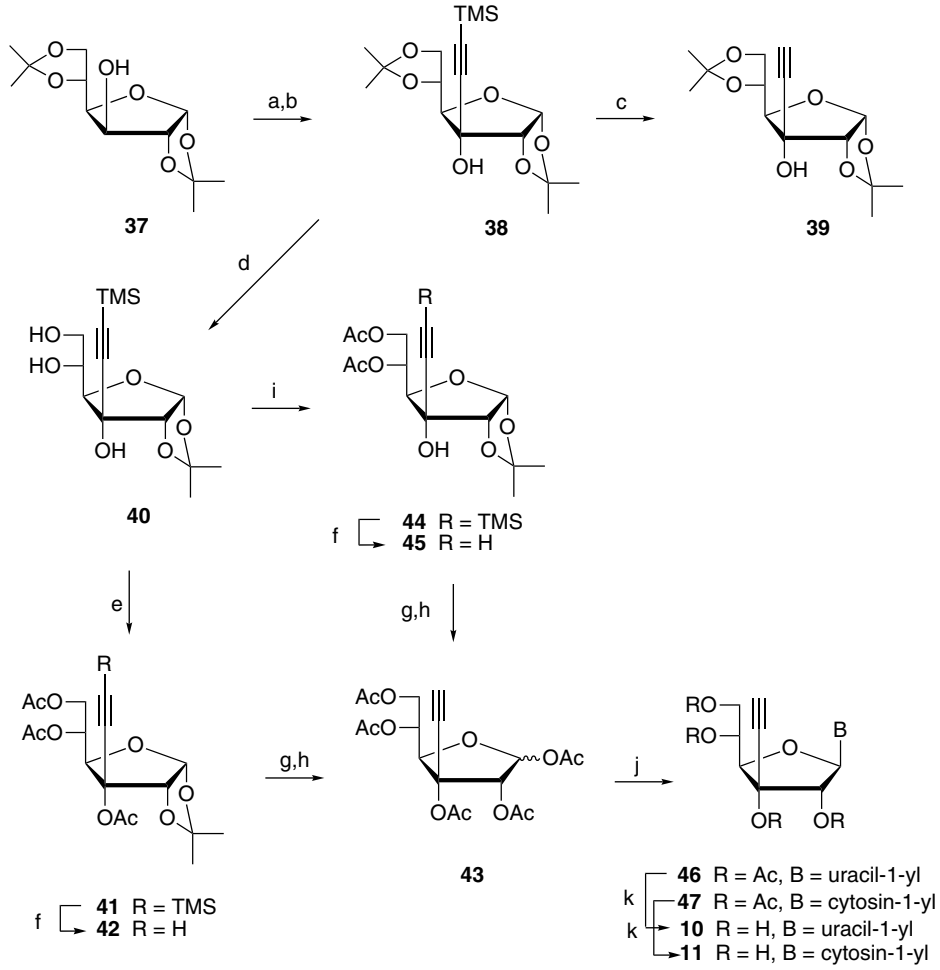


Figure 3. Molecular structures (ORTEP-plots) of 4′-*C*-hydroxymethyl nucleosides **8** and **9**.



Scheme 4. Reagents and conditions: (a) Ac_2O, pyridine, 0 °C to rt; (b) 80% aq TFA, 0 °C; (c) Ac_2O, DMAP, pyridine, 100 °C, 55% (three steps, from **20**); (d) uracil/cytosine, BSA, TMSOTf, 1,2-dichloroethane, reflux, 77% for **35**, 54% for **36**; (e) NH_3/MeOH, rt, 97% for **8**, 75% for **9**.



Scheme 5. Reagents and conditions: (a) PDC, AcOH, 3 Å molecular sieves powder, CH_2Cl_2, 0 °C to rt; (b) TMSC≡CH, *n*-BuLi, THF, −78 °C, 73% (two steps); (c) TBAF, THF, rt, 76%; (d) 80% aq AcOH, rt, 82%; (e) Ac_2O, DMAP, pyridine, rt, 64%; (f) TBAF, AcOH, THF, rt, 82% for **42**, 92% for **45**; (g) 80% aq TFA, 0 °C; (h) Ac_2O, DMAP, pyridine, rt, 61% from **42** (two steps), 82% from **45** (two steps); (i) Ac_2O, pyridine, 0 °C to rt, 70%; (j) uracil/cytosine, BSA, TMSOTf, CH_3CN, reflux, 46% for **46**, 52% for **47**; (k) satd NH_3/MeOH, rt, 69% for **10**, 54% for **11**.

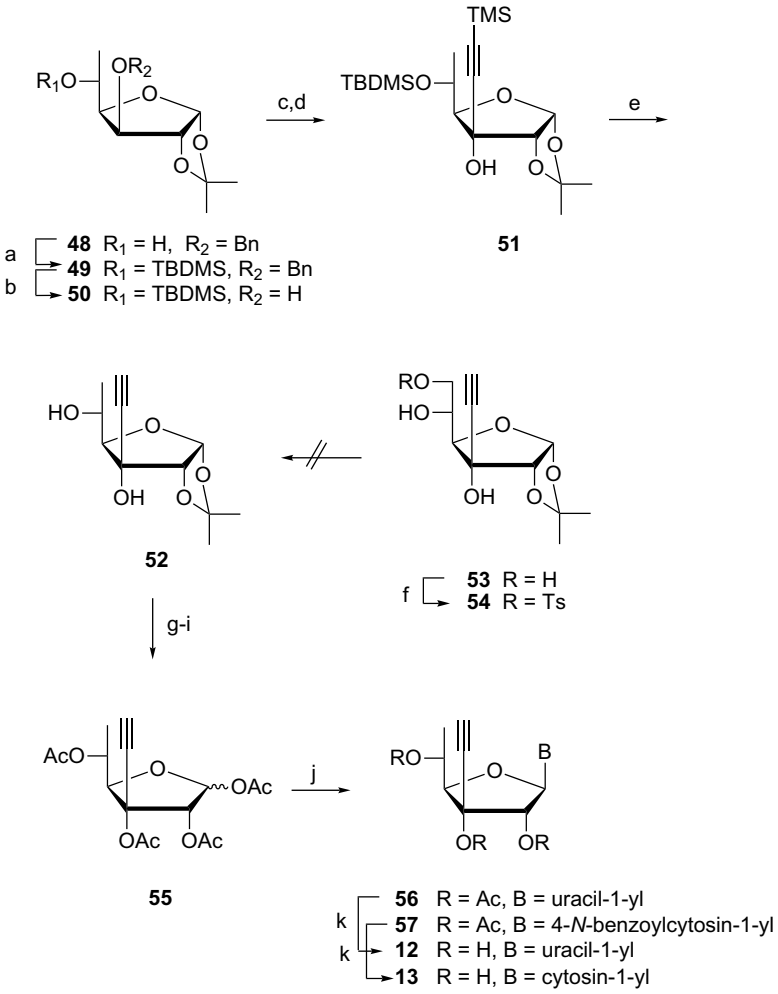
yielding furanose **41** in 64% yield. Desilylation of furanose **41** using a mixture of TBAF and acetic acid gave furanose **42** in 82% yield. In absence of acetic acid, polar byproducts (presumably deacylated products) were formed resulting in lower yields. Cleavage of the remaining isopropylidene group of furanose **42** with trifluoro-

acetic acid was followed by peracetylation to give glycosyl donor **43** in 61% yield over two steps. In an alternative route to glycosyl donor **43**, triol **40** was selectively diacetylated to give alcohol **44** in 70% yield, which on desilylation (using the same conditions as for **41**) gave furanose **45** in 92% yield. Conversion of furanose **45** to glycosyl donor **43** (using the same conditions as for **42**) proceeded in 82% yield over two steps. This alternative route to glycosyl donor **43** was more efficient (53% yield of **43** from **40**) than the initial route (33% yield of **43** from **40**). Glycosylation of donor **43** with persilylated uracil or cytosine stereoselectively afforded protected β-nucleosides **46** and **47** in moderate yield (46% and 52%, respectively) which on deacylation with saturated methanolic ammonia furnished target nucleosides **10** and **11** in 69% and 54% yield, respectively.

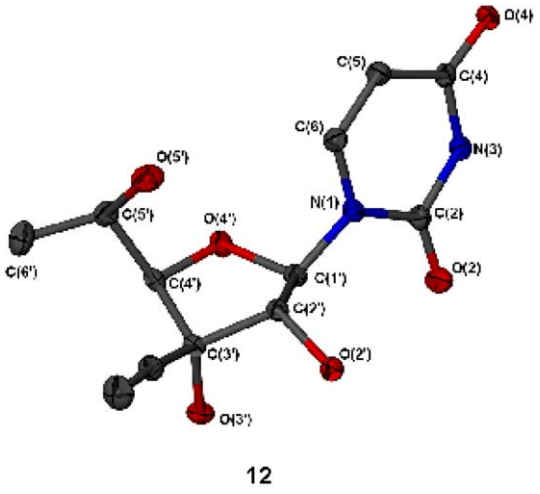
2.6. Synthesis of 5′-*C*-methyl EUrd/ECyd analogs 12 and 13

Retrosynthetic analysis identified diol **52** as the key intermediate in the synthesis of 5′-*C*-methyl EUrd/ECyd analogs **12** and **13** (Scheme 6). Alcohol **48**,[36] obtained from 1,2;5,6-di-*O*-isopropylidene-α-D-glucose in four steps, was protected as a TBDMS ether using standard procedures to give furanose **49** in 91% yield, which on debenzylation by catalytic hydrogenation afforded alcohol **50** in 86% yield. PDC oxidation of the O3-hydroxyl group of **50** gave the 3-ulose intermediate, which was reacted directly with LiC≡CTMS formed in situ to stereoselectively give *allo*-configured furanose **51** in excellent 90% yield over two steps.[37] Standard desilylation of furanose **51** afforded key intermediate **52** in 98% yield.

Efforts were made to shorten the synthetic route to key intermediate **52**. Known triol **53**,[28] easily obtained from 1,2;5,6-di-*O*-isopropylidene-α-D-glucose in three steps, was selectively tosylated in 57% yield to afford furanose **54**, which subsequently was subjected to typical conditions for mild reductive displacement of primary tosylate groups. However, neither treatment of furanose **54** with tetrabutylammonium borohydride[38] in toluene at room temperature or reflux, or sodium borohydride in DMF afforded key intermediate **52**, perhaps due to instability of the alkyne functionality under these conditions.



Scheme 6. Reagents and conditions: (a) TBDMSCl, imidazole, CH_2Cl_2, rt, 91%; (b) H_2, $Pd(OH)_2$/C, EtOAc, 86%; (c) PDC, AcOH, 3 Å molecular sieves powder, CH_2Cl_2, rt; (d) TMSC≡CH, *n*-BuLi, THF, −78 °C, 90% (two steps); (e) TBAF, THF, rt, 98%; (f) TsCl, pyridine, −50 °C to rt, 57%; (g) Ac_2O, DMAP, pyridine, rt; (h) 80% aq TFA, rt; (i) Ac_2O, DMAP, pyridine, rt, 72% (three steps); (j) uracil/4-*N*-benzoylcytosine, BSA, TMSOTf, CH_3N, reflux, 79% for **56**, 75% for **57**; (k) satd NH_3/MeOH, rt, 78% for **12**, 64% for **13**.



12

Figure 4. Molecular structure (ORTEP-plot) of 5′-*C*-methyl nucleoside **12**.

Key intermediate **52** was converted to glycosyl donor **55** by a three-step sequence involving diacetylation, isopropylidene cleavage, and peracetylation (72% yield). Glycosylation of **53** with persilylated uracil or 4-*N*-benzoylcytosine under modified Vorbrüggen conditions,[31] gave β-nucleosides **56** and **57** in 79% and 75% yield, respectively. Deprotection of nucleosides **56** and **57** with saturated methanolic ammonia afforded the target nucleosides **12** and **13** in 78% and 64% yield, respectively.

Single crystal X-ray diffraction study of the 5′-*C*-methyl nucleoside **12** (Fig. 4), verified the relative configuration of the nucleobase and 3′-*C*-ethynyl group. The absolute configuration follows from the stereochemically pure starting materials and the applied synthetic route (Scheme 6).

3. Conformational analysis

Target nucleosides **5**, **7**, **8**, **10**, and **12** (Fig. 1) and ECyd **2** were subjected to Monte Carlo based conformational searches using the AMBER force–field[39] and generalized born/surface area solvation model[40] as implemented in the MACROMODEL V7.2 suite.[41] Values of the pseudorotational phase angle P, maximal puckering amplitude ν_m and glycosidic torsion angle χ (O4′–C1′–N1–C2)[13] of the lowest energy structures are listed in Table 1. $^3J_{H1'-H2'}$ coupling constants were estimated from the observed torsion angles ϕ (H1′–C1′–C2′–H2′) by using the Diez–Altona–Donders equation as implemented within the MESTRE-J program.[42] Very good agreement between predicted $J_{H1'-H2'}$ values and experimental values indicates that solution conformations of nucleosides **2**, **5**, **7**, **8**, **10**, and **12** closely match the lowest energy structures from molecular modeling.

As anticipated, the 3′-*O*,4′-*C*-methylene-linkage of nucleoside **5** restricts the sugar ring in a South conformation ($^2T_1$) similar to ECyd **2**. However, a flatter ring pucker ($\Delta\nu_m = -7°$) than in ECyd is observed. Also unsurprisingly, LNA-type EUrd-analog **7** is locked in a North conformation with an extreme pucker ($P = 24°$, $\nu_m = 55°$). Introduction of a hydroxymethyl or methyl group at C-5′ as in nucleosides **10** and **12**, does not seem to influence the sugar conformation, whereas introduction of a C4′-hydroxymethyl group as in **8** results in a more pronounced South conformation ($P = 181°$, $^2T_3$), which also is reflected experimentally in a larger observed value of $J_{H1'-H2'}$ for **8** as compared to ECyd, **10** and **12**. Single crystal X-ray diffraction studies of the 4′-*C*-hydroxymethyl nucleosides **8** and **9** (Fig. 3) and 5′-*C*-methyl nucleoside **12** (Fig. 4) show that the furanose ring also adopts a South conformation in solid state ($P = 168°$, 169°, and 170° for **8**, **9**, and **12**, respectively).

4. Biological evaluation

Reference nucleosides **1**–**2**, target nucleosides **5**–**13** and acylated derivatives **35**, **36**, **46**, **47**, **56**, and **57** were evaluated for antiviral activity against HIV-1 in MT-4 cells as previously described.[43] All compounds were inactive against HIV-1 at the highest tested concentration of 100 μM. EUrd and ECyd displayed significant cytotoxicity (CD_{50} = 0.03 and 0.02 μM, respectively)[44] whereas LNA-type EUrd analog **7** displayed marginal cytotoxicity (CD_{50} = >30 μM). All other compounds were nontoxic toward MT-4 cells. EUrd, ECyd, and nucleosides **5**–**13** were evaluated against human adenocarcinoma breast cancer (MCF-7) and prostate cancer (PC-3) cell lines as previously described.[45] As expected, EUrd and ECyd displayed very potent activities in vitro with IC_{50} values[46] of 2.5 and 2.2 nM, respectively (MCF-7) and 1.7 and 0.15 nM, respectively (PC-3), whereas nucleosides **5**–**13** were inactive at the highest tested concentration of 25 μM.

Although the branched nucleosides **8**–**13** adopt furanose conformations similar to those of EUrd and ECyd in

Table 1. Pseudorotational parameters, torsion angles and predicted $J_{H1'-H2'}$ values of lowest energy structures from molecular modeling and observed experimental $J_{H1'-H2'}$ values

Compounds	P (°)	ν_m (°)	$\chi_{O4'-C1'-N1-C2}$ (°)	$\phi_{H1'-C1'-C2'-H2'}$ (°)	$J_{pre,H1'-H2'}$ (Hz)	$J_{exp,H1'-H2'}$ (Hz)
5	139	32	−163	161	9	7.7
7	24	55	−176	86	1	≈0
8	181	35	−164	157	8	8.2
10	142	39	−164	168	9	7.0
12	144	39	−164	168	9	7.0
ECyd	138	39	−162	168	9	6.6[a]

[a] Data from Ref. 1.

solution, the substituents at the C-4′ or C-5′-position may impose unfavorable steric clashes leading to (1) decreased recognition by UCK or nucleoside kinases, resulting in insufficient phosphorylation or (2) lack of recognition of triphosphates by RNA polymerase. The lack of anticancer activity of conformationally restricted nucleosides **5**–**7** may, in addition to the aforementioned reasons, arise from antipodal conformational requirements for furanose conformation of UCK, nucleotide kinases and RNA polymerase.

5. Experimental

5.1. General experimental section

All solvents and reagents were obtained from commercial suppliers and used without further purification unless stated otherwise. Reference compounds EUrd and ECyd were obtained via a previously published route.[45] All solvents used for chromatography were of technical grade and used without further purification except CH_2Cl_2, which was distilled prior to use. Petroleum ether of the distillation range 60–80 °C was used. Solvents for use in reactions were of analytical grade. Anhydrous DMF and pyridine were used directly as obtained from commercial suppliers. Acetonitrile was dried through storage over activated 3 Å molecular sieves. Dichloromethane, 1,2-dichloroethane and toluene for use in anhydrous reactions were dried through storage over activated 4 Å molecular sieves. THF was dried either by distillation from sodium/benzophenone or by storing over activated 4 Å molecular sieves. Water content of anhydrous solvents was checked by Karl–Fischer apparatus. Reactions were conducted under an atmosphere of argon when anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with fluorescence indicator (SiO_2-60, F-254), which were visualized (a) under UV light, (b) by dipping in 5% concd sulfuric acid in absolute ethanol (v/v) followed by heating, or (c) by dipping in a solution of molybdato–phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% concd sulfuric acid in water (v/v) followed by heating. Dry column vacuum chromatography[47] and silica gel column chromatography using moderate pressure (pressure ball) were performed with Silica gel 60 (particle size 0.040–0.063 mm, Merck). Evaporation of solvents was carried out under reduced pressure with a temperature not exceeding 50 °C unless stated otherwise. After column chromatography, appropriate fractions were pooled, evaporated, and dried at high vacuum for at least 12 h to give obtained products in high purity (>95%), unless stated otherwise. In absence of elemental analysis, ^{1}H NMR and/or ^{13}C NMR ascertained sample purity. No corrections in yields were made for solvent of crystallization. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane or deuterated solvent as the internal standard (δ_H: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.50 ppm; δ_C: $CDCl_3$ 77.00 ppm, DMSO-d_6 39.43 ppm). Exchangeable (ex) protons were detected by disappearance of peaks on D_2O addition. Assignments of NMR spectra were based on 2D spectra (HETCOR, COSY) and follow standard carbohydrate/nucleoside nomenclature. The carbon atom of C4 substituents is numbered C-5′ in furanose derivatives and C-5″ in nucleoside derivatives. Similar conventions apply for the corresponding hydrogen atoms. Assignments of C-5/C-5′ and H-5/H-5′ in furanose derivatives and C-5′/C-5″ and H-5′/H-5″ in monocyclic nucleoside derivatives may be interchanged. Quaternary carbons were not assigned in ^{13}C NMR. Traces of solvents in NMR spectra were identified by reference to published data.[48] MALDI-HRMS were recorded in positive ion mode on a IonSpec Fourier transform mass spectrometer. UV spectra were recorded at room temperature on a Shimadzu UV-160A spectrophotometer in the range 230–500 nm, using a quartz cell with a 1 cm path length. The pH was adjusted by addition of concentrated aqueous HCl or NaOH. The concentrations of the aqueous solutions of compounds were adjusted to give absorption peaks in the linear range. Elemental analyses were obtained from the Microanalytical Department, University of Copenhagen.

5.2. Synthesis of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (14) from diacetone-α-D-glucose without purification of intermediates

A suspension of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (40.29 g, 0.15 mol) in anhydrous THF (100 mL) was added dropwise over 30 min to an ice-cold suspension of NaH (60% suspension in mineral oil, 9.60 g, 0.24 mol) in anhydrous THF (30 mL). After ended addition, benzyl bromide (22.4 mL, 0.19 mol), and tetrabutylammonium iodide (4.00 g, 10.8 mmol) were added and the reaction mixture stirred for 6 h at rt. The reaction mixture was cooled to 0 °C and H_2O (75 mL) added. The separated aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic phase evaporated to dryness to give the crude O3-benzylated furanose, which was directly dissolved in 80% aqueous acetic acid (300 mL). After stirring for 22 h, the reaction mixture was washed with petroleum ether (4 × 100 mL) to remove remaining benzyl bromide from the previous step. The aqueous phase was evaporated to dryness and coevaporated with absolute EtOH/toluene (3 × 100 mL, 1:1 v/v). The resulting residue was taken up in CH_2Cl_2 (200 mL), washed with satd aq $NaHCO_3$ (2 × 75 mL) and the organic phase evaporated to dryness to leave the crude diol, which was used in the next step without further purification. To an ice-cold solution of the crude diol in THF/H_2O (500 mL, 1:1 v/v) was added sodium periodate (32.90 g, 0.15 mol) and the reaction mixture stirred at 0 °C for 2 h, whereupon insoluble solids were filtered off and washed with ether (500 mL). Combined filtrates were collected and the phases separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 150 mL) and the combined organic phase evaporated to dryness. The resulting crude aldehyde was used immediately in the next reaction without further purification. To a solution of the crude aldehyde in 1,4-dioxane (150 mL) was added formaldehyde (37% solution in H_2O, 40 mL, 0.54 mol)

and 2 M NaOH (150 mL, 0.30 mol). The reaction mixture was stirred for 42 h at rt whereupon it was diluted with ether (200 mL) and saturated with NaCl (s). The separated aqueous phase was extracted with CH_2Cl_2 (4×100 mL) and the combined organic phase evaporated to dryness and purified by silica gel column chromatography (0–65% EtOAc in petroleum ether, v/v) to afford the known furanose **14**[20] (30.39 g, 64% from 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose) as a white solid material. ^{13}C NMR data were identical to previously reported data.[20]

5.3. 3-*O*-Benzyl-5-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(*tert*-butyldimethylsilyloxymethyl)-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (15)

To a stirred solution of diol **14**[20] (0.89 g, 2.87 mmol) in anhydrous DMF (5 mL) at rt, was added imidazole (1.16 g, 17.0 mmol) and TBDMSCl (1.36 g, 9.02 mmol). After stirring at 35 °C for 20 h, H_2O (10 mL) was added and the aqueous phase extracted with Et_2O (2×40 mL). The combined organic phase was washed with satd aq $NaHCO_3$ (2×25 mL) and brine (25 mL), dried ($MgSO_4$), filtered, and evaporated to dryness. Purification by silica gel column chromatography (0–6% EtOAc in petroleum ether, v/v) afforded furanose **15** (1.10 g, 71%) as a clear oil. $R_f = 0.5$ (10% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 561.3061 ([M+Na]$^+$, $C_{28}H_{50}O_6Si_2{\cdot}Na^+$: Calcd 561.3038); ^1H NMR ($CDCl_3$): δ 7.18–7.22 (m, 5H, Ph), 5.89 (d, 1H, $J = 4.4$ Hz, H-1), 4.65 (dd, 1H, $J = 4.4$ Hz, 2.2 Hz, H-2), 4.59 (d, 1H, $J = 11.5$ Hz, CH_2Ph), 4.40 (d, 1H, $J = 11.5$ Hz, CH_2Ph), 4.00 (d, 1H, $J = 2.2$ Hz, H-3), 3.42–3.69 (m, 4H, H-5, H-5′), 1.43 (s, 3H, $C(CH_3)_2$), 1.25 (s, 3H, $C(CH_3)_2$), 0.75 (s, 9H, $C(CH_3)_3$), 0.74 (s, 9H, $C(CH_3)_3$), −0.10 (s, 6H, $Si(CH_3)_2$), −0.11 (s, 3H, $Si(CH_3)_2$), −0.12 (s, 3H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 137.9, 128.2 (Ph), 127.6 (Ph), 112.9, 105.0 (C-1), 89.6, 86.9 (C-2), 84.4 (C-3), 72.3, 63.6 (C-5), 63.2 (C-5′), 27.8 ($C(CH_3)_2$), 27.3 ($C(CH_3)_2$), 25.9 ($C(CH_3)_3$), 18.4, 18.3, −5.4 ($Si(CH_3)_2$), −5.45 ($Si(CH_3)_2$), −5.47 ($Si(CH_3)_2$), −5.6 ($Si(CH_3)_2$). Anal. Calcd for $C_{28}H_{50}O_6Si_2$: C, 62.41; H, 9.35. Found: C, 62.18; H, 9.23.

5.4. 5-*O*-(*tert*-Butyldimethylsilyl)-4-*C*-(*tert*-butyldimethylsilyloxymethyl)-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (16)

Method A (*from* **15**): To a stirred solution of pentofuranose **15** (26.61 g, 49.4 mmol) in absolute EtOH (50 mL) was added 20% $Pd(OH)_2/C$ (2.76 g). The mixture was evacuated with H_2 several times. After stirring for 18 h at rt, the mixture was filtered through a Celite pad, which was washed with absolute EtOH. The combined filtrate was evaporated and the residue purified by dry column vacuum chromatography (2–5% EtOAc in petroleum ether, v/v) to furnish furanose **16** (16.95 g, 76%) as a white solid material. *Method B* (*from* **17**): TBDMSCl (10.26 g, 68.1 mmol) was added to a stirred mixture of triol **17**[22] (4.06 g, 18.4 mmol) in anhydrous DMF (30 mL) at 0 °C. Subsequently, imidazole (9.66 g, 0.14 mol) was added in three portions over 30 min. After stirring for 72 h at rt, the reaction mixture was concentrated to near dryness, diluted with H_2O (100 mL) and extracted with EtOAc (3×100 mL). The combined organic phase was washed with satd aq $NaHCO_3$ (2×50 mL) and brine (2×50 mL), dried ($MgSO_4$), filtered, and evaporated. Purification of the residue by silica gel column chromatography (20% EtOAc in petroleum ether, v/v) afforded **16** (8.27 g, 71%) as a white solid material. $R_f = 0.3$ (10% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 471.2572 ([M+Na]$^+$, $C_{21}H_{44}O_6Si_2{\cdot}Na^+$: Calcd 471.2569); ^1H NMR ($CDCl_3$): δ 5.94 (d, 1H, $J = 3.8$ Hz, H-1), 4.57 (d, 1H, $J = 3.8$ Hz, H-2), 4.29 (d, 1H, ex, $J = 6.8$ Hz, 3-OH), 4.20 (d, 1H, $J = 6.8$ Hz, H-3), 3.56–4.12 (m, 4H, H-5, H-5′), 1.53 (s, 3H, $C(CH_3)_2$), 1.39 (s, 3H, $C(CH_3)_2$), 0.89–0.90 (m, 18H, $C(CH_3)_3$), 0.05–0.11 (m, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 112.5, 105.1 (C-1), 89.3, 88.7 (C-2), 78.8 (C-3), 65.5 (C-5), 65.3 (C-5′), 27.2 ($C(CH_3)_2$), 26.4 ($C(CH_3)_2$), 25.9 ($C(CH_3)_3$), 25.7 ($C(CH_3)_3$), 18.3, 18.1, −5.4 ($Si(CH_3)_2$), −5.45 ($Si(CH_3)_2$), −5.49 ($Si(CH_3)_2$), −5.7 ($Si(CH_3)_2$). Anal. Calcd for $C_{21}H_{44}O_6Si_2$: C, 56.21; H, 9.88. Found: C, 56.24; H, 9.95.

5.5. Synthesis of 4-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-β-L-*threo*-pentofuranose (17) from diacetone-α-D-glucose without purification of intermediates

1,2;5,6-Di-*O*-isopropylidene-α-D-glucofuranose (80.00 g, 0.31 mol) was dissolved in 67% aqueous acetic acid (1.0 L) and stirred for 15 h at rt, whereupon the reaction mixture was evaporated to dryness and coevaporated with absolute EtOH/toluene (3×300 mL, 1:1 v/v) affording crude triol. To an ice-cold solution of the crude triol in MeOH/H_2O (1.0 L, 5:1 v/v) was added sodium periodate (75.65 g, 0.35 mol). After stirring for 2 h at rt, ethylene glycol (10 mL) was added, and nonsoluble residues filtered off, washed (MeOH) and the combined organic phase evaporated to dryness. The residue was partitioned between EtOAc (300 mL) and brine (100 mL) and the separated aqueous phase extracted with EtOAc (3×200 mL). The combined organic phase was evaporated to dryness affording crude aldehyde, which was directly dissolved in H_2O (400 mL). To this, formaldehyde (37% solution in H_2O, 190 mL, 2.55 mol) and aqueous NaOH (1.0 M, 950 mL, 0.95 mol) were added and the reaction mixture stirred at rt for 72 h. The mixture was neutralized with formic acid, evaporated to dryness, and coevaporated with toluene (3×200 mL). Purification of the residue by silica gel column chromatography (80–100% EtOAc in petroleum ether, then 0–10% MeOH in EtOAc, v/v) afforded **17** (43.03 g, 64% from 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose) as a white solid material. ^{13}C NMR (DMSO-d_6): δ 111.2, 104.0, 90.1, 88.2, 75.7, 61.0, 27.0, 26.6. ^1H NMR (DMSO-d_6) data identical to previously reported data.[22]

5.6. 5-*O*-(*tert*-Butyldimethylsilyl)-4-*C*-(*tert*-butyldimethylsilyloxymethyl)-1,2-*O*-isopropylidene-α-D-*glycero*-pentofuranos-3-ulose (18)

Method A: Dess–Martin periodinane (231.5 mg, 0.55 mmol) was slowly added to furanose **16**

(160.6 mg, 0.36 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C. After allowing the reaction mixture to reach rt, it was stirred for 21 h, whereupon it was evaporated to dryness, resuspended in Et_2O (8 mL), and filtered through a short pad of Na_2SO_4. The pad was washed with Et_2O and the combined filtrate was stirred with $Na_2S_2O_3$ (1 g) in satd aq $NaHCO_3$ (20 mL). The organic phase was separated, dried (Na_2SO_4), filtered and evaporated to afford 3-ulose **18** (155.1 mg, 97%) as a clear oil pure by NMR. *Method B*: To a stirred solution of furanose **16** (8.67 g, 19.3 mmol) in CH_2Cl_2 (20 mL) was first added 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO, 0.30 g, 1.92 mmol) and then [(diacetoxy)iodo]benzene (BAIB, 8.00 g, 24.9 mmol) in three portions over 15 min. The reaction mixture was stirred at rt for 24 h when CH_2Cl_2 (50 mL) and satd aq $Na_2S_2O_3$ (15 mL) were added. The separated aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL), and the combined organic phase was dried ($MgSO_4$), filtered, evaporated to dryness and purified by silica gel column chromatography (0–6% EtOAc in petroleum ether, v/v) to give 3-ulose **18** (7.84 g, 91%) as a clear oil. $R_f = 0.5$ (10% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 469.2434 ($[M+Na]^+$, $C_{21}H_{42}O_6Si_2{\cdot}Na^+$: Calcd 469.2412); ^1H NMR ($CDCl_3$): δ 6.12 (d, 1H, $J = 4.4$ Hz, H-1), 4.32 (d, 1H, $J = 4.4$ Hz, H-2), 3.73–3.84 (m, 4H, H-5, H-5′), 1.49 (s, 3H, $C(CH_3)_2$), 1.41 (s, 3H, $C(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 0.85 (s, 9H, $C(CH_3)_3$), 0.05 (2s, 6H, $Si(CH_3)_2$), 0.02 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 210.2, 114.8, 102.8 (C-1), 90.5, 78.4 (C-2), 66.8 (C-5), 64.9 (C-5′), 28.0 ($C(CH_3)_2$), 27.4 ($C(CH_3)_2$), 26.0 ($C(CH_3)_3$), 26.0 ($C(CH_3)_3$), 18.6, 18.3, −5.2 ($Si(CH_3)_2$), −5.4 ($Si(CH_3)_2$), −5.6 ($Si(CH_3)_2$). Anal. Calcd for $C_{21}H_{42}O_6Si_2$: C, 56.46; H, 9.48. Found: C, 56.43; H, 9.49.

5.7. 5-*O*-(*tert*-Butyldimethylsilyl)-4-*C*-(*tert*-butyldimethylsilyloxymethyl)-1,2-*O*-isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-α-D-*erythro*-pentofuranose (19)

A solution of *n*-butyllithium (2.0 M in cyclohexane, 20.0 mL, 40.0 mmol) was added dropwise over 10 min to trimethylsilylacetylene (6.5 mL, 46.0 mmol) in anhydrous THF (75 mL) at −78 °C. After stirring for 30 min, a solution of 3-ulose **18** (8.20 g, 18.4 mmol) in anhydrous THF (35 mL) was added dropwise over 30 min to the metal acetylide solution. Satd aq NH_4Cl (30 mL) was added 45 min after completed addition, and the mixture was warmed to rt, concentrated to 1/4 volume and extracted with Et_2O (2 × 100 mL). The combined organic phase was washed with brine (2 × 75 mL), dried ($MgSO_4$), filtered, evaporated to dryness and the resulting residue purified by silica gel column chromatography (CH_2Cl_2) to afford furanose **19** (9.01 g, 90%) as a white solid material. $R_f = 0.3$ (5% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 567.2964 ($[M+Na]^+$, $C_{26}H_{52}O_6Si_3{\cdot}Na^+$: Calcd 567.2980); ^1H NMR ($CDCl_3$): δ 5.94 (d, 1H, $J = 4.9$ Hz, H-1), 4.87 (d, 1H, $J = 4.9$ Hz, H-2), 3.76–4.11 (m, 4H, H-5, H-5′), 3.75 (s, 1H, ex, 3-OH), 1.60 (s, 3H, $C(CH_3)_2$), 1.39 (s, 3H, $C(CH_3)_2$), 0.92 (s, 9H, $C(CH_3)_3$), 0.90 (s, 9H, $C(CH_3)_3$), 0.15 (s, 12H, $Si(CH_3)_2$), 0.09 (s, 3H, $Si(CH_3)_3$), 0.08 (s, 3H, $Si(CH_3)_3$), 0.06 (s, 3H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 114.9, 105.4 (C-1), 105.1, 92.9, 89.9, 87.7 (C-2), 73.7, 66.7 (C-5), 63.1 (C-5′), 27.4 ($C(CH_3)_2$), 26.8 ($C(CH_3)_2$), 26.1 ($C(CH_3)_3$), 26.0 ($C(CH_3)_3$), 18.4, 18.3, 0.00 ($Si(CH_3)_3$), −5.3 ($Si(CH_3)_2$), −5.36 ($Si(CH_3)_2$), −5.38 ($Si(CH_3)_2$), −5.5 ($Si(CH_3)_2$). Anal. Calcd for $C_{26}H_{52}O_6Si_3$: C, 57.31; H, 9.62. Found: C, 57.33; H, 9.61.

5.8. 3-*C*-Ethynyl-4-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (20)

To a solution of furanose **19** (4.13 g, 7.58 mmol) in THF (40 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 11.5 mL, 11.5 mmol). After stirring for 3 h at rt, the reaction mixture was evaporated to dryness, coevaporated several times with toluene and purified by dry column vacuum chromatography (80–100% EtOAc in petroleum ether, v/v) to give triol **20** (1.37 g, 74%) as a white solid material. $R_f = 0.2$ (EtOAc); MALDI-HRMS *m/z* 267.0835 ($[M+Na]^+$, $C_{11}H_{16}O_6{\cdot}Na^+$: Calcd 267.0839); ^1H NMR (DMSO-d_6): δ 5.80 (d, 1H, $J = 4.4$ Hz, H-1), 5.64 (s, 1H, ex, 3-OH), 4.67 (d, 1H, $J = 4.4$ Hz, H-2), 4.65 (t, 1H, ex, $J = 5.6$ Hz, 5-OH), 4.25 (t, 1H, ex, $J = 5.6$ Hz, 5′-OH), 3.61–3.71 (m, 4H, H-5, H-5′), 3.53 (s, 1H, HC≡C), 1.49 (s, 3H, CH_3), 1.18 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 113.3, 103.8 (C-1), 90.2, 87.0 (C-2), 84.1, 76.7, 74.0, 63.2 (C-5), 60.4 (C-5′), 27.1 (CH_3), 26.5 (CH_3). Anal. Calcd for $C_{11}H_{16}O_6$: C, 54.09; H, 6.60. Found: C, 53.75; H, 6.73.

5.9. 3-*C*-Ethynyl-1,2-*O*-isopropylidene-4-*C*-(methanesulfonoxymethyl)-5-*O*-methanesulfonyl-α-D-*erythro*-pentofuranose (22)

Triol **20** (3.90 g, 16.0 mmol) was dried by coevaporation with anhydrous pyridine, dissolved in anhydrous pyridine (35 mL) and MsCl (2.5 mL, 32.0 mmol) added dropwise over 5 min at 0 °C. The reaction mixture was warmed to rt and after stirring for 3 h the heterogeneous orange reaction mixture was partitioned between EtOAc (150 mL) and satd aq $NaHCO_3$ (30 mL). The phases were separated and the aqueous phase extracted with EtOAc (4 × 50 mL). The combined organic phase was evaporated to dryness and coevaporated several times with anhydrous EtOH and toluene (1:1 v/v). The residue was absorbed on Kieselguhr and purified by silica gel column chromatography (25–70% EtOAc in petroleum ether, v/v) to afford pentofuranose **22** (4.93 g, 77%) as a white solid material. $R_f = 0.5$ (60% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 423.0395 ($[M+Na]^+$, $C_{13}H_{20}O_{10}S_2{\cdot}Na^+$: Calcd 423.0390); ^1H NMR (DMSO-d_6): δ 6.78 (s, 1H, ex, 3-OH), 5.87 (d, 1H, $J = 3.7$ Hz, H-1), 4.72 (d, 1H, $J = 3.7$ Hz, H-2), 4.40–4.59 (m, 2H, H-5), 4.32 (br s, 2H, H-5′), 3.97 (s, 1H, HC≡C), 3.23 (s, 3H, CH_3SO_2), 3.20 (s, 3H, CH_3SO_2), 1.55 (s, 3H, $C(CH_3)_2$), 1.30 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (DMSO-d_6): δ 113.3, 103.8 (C-1), 85.3 (C-2), 83.8, 81.3, 80.1, 75.5, 68.4 (C-5′), 67.4 (C-5), 36.8 (CH_3SO_2), 36.6 (CH_3SO_2), 25.80 ($C(CH_3)_3$), 25.76 ($C(CH_3)_3$). Anal. Calcd for $C_{13}H_{20}O_{10}S_2$: C, 38.99; H, 5.03; S, 16.02. Found: C, 39.29; H, 5.07; S, 15.62.

5.10. 1,2,3-Tri-*O*-acetyl-3-*C*-ethynyl-4-*C*-(methanesulfonoxy)methyl-5-*O*-methanesulfonyl-α,β-D-*erythro*-pentofuranose (23)

Bis-sulfonic ester **22** (3.18 g, 7.96 mmol) was dissolved in ice-cold 80% aqueous TFA (40 mL). The reaction mixture was allowed to warm up to rt and after stirring for 90 min analytical TLC showed full conversion to two polar products (R_f = 0.2 and 0.3, respectively, 80% EtOAc in petroleum ether, v/v). The reaction mixture was evaporated to dryness and the resulting residue coevaporated with toluene (2 × 25 mL) to afford crude anomeric triol as a yellow oil (3.2 g), which was used in the next step without further purification. To a suspension of crude triol in anhydrous CH_2Cl_2 (20 mL), was added Ac_2O (3.8 mL, 39.8 mmol) followed by dropwise addition of TMSOTf (140 μL, 0.80 mmol) over 10 min at 0 °C. After stirring for 70 min at 0 °C, MeOH (5 mL) was added and the reaction mixture evaporated to dryness. The residue was taken up in CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ (2 × 40 mL). The combined organic phase was evaporated to dryness, and the resulting residue purified by silica gel column chromatography (0–5% MeOH in CH_2Cl_2, v/v) to afford an anomeric mixture (≈1:3 by ^{1}H NMR) of glycosyl donor **23** (3.39 g, 88% over two steps) as a white solid material. Data for anomeric mixture: R_f = 0.7 (80% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 509.0389 ([M+Na]$^+$, $C_{16}H_{22}O_{13}S_2{\cdot}Na^+$: Calcd 509.0394); ^{13}C NMR ($CDCl_3$): δ 168.9, 168.7, 168.2, 168.1, 167.8, 167.7, 97.9, 93.5, 86.0, 84.2, 80.30, 80.28, 79.3, 77.4, 77.3, 75.9, 75.5, 74.5, 68.8, 68.0, 66.1, 65.6, 38.1, 38.03, 37.99, 37.8, 21.1, 20.8, 20.6, 20.3. Anal. Calcd for $C_{16}H_{22}O_{13}S_2{\cdot}1/2\ H_2O$: C, 38.79; H, 4.68; S, 12.94. Found: C, 38.62; H, 4.28; S, 12.58.

5.11. 1-[2,3-Di-*O*-acetyl-3-*C*-ethynyl-4-*C*-(methanesulfonoxymethyl)-5-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl]uracil (24)

Uracil (0.91 g, 8.15 mmol) was dried by coevaporation with anhydrous 1,2-dichloroethane (2 × 25 mL), and resuspended in anhydrous 1,2-dichloroethane (20 mL). To this was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA, 3.4 mL, 13.6 mmol) and the reaction mixture was refluxed for 1 h. After cooling the homogenous solution to rt, glycosyl donor **23** (2.20 g, 4.53 mmol) dissolved in anhydrous 1,2-dichloroethane (20 mL) and TMSOTf (2.5 mL, 13.6 mmol) were added. After refluxing the reaction mixture for 26 h, satd aq $NaHCO_3$ (30 mL) was added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (4 × 30 mL), the combined organic phases evaporated to dryness and the residue purified by silica gel column chromatography (0–4% MeOH in $CHCl_3$, v/v) to afford protected nucleoside **24** (1.80 g, 74%) as a white foam, which was used in the next step without further purification. R_f = 0.6 (10% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 561.0430 ([M+Na]$^+$, $C_{18}H_{22}N_2O_{13}S_2{\cdot}Na^+$: Calcd 561.0456); ^{1}H NMR (DMSO-d_6): δ 11.57 (s, 1H, ex, NH), 7.74 (d, 1H, *J* = 8.1 Hz, H-6), 6.14 (d, 1H, *J* = 5.1 Hz, H-1′), 5.75–5.79 (m, 2H, H-2′, H-5), 4.61–4.69 (2d, 2H, *J* = 10.6 Hz, H-5′), 4.56–4.60 (d, 1H, *J* = 11.0 Hz, H-5″), 4.44–4.49 (d, 1H, *J* = 11.0 Hz, H-5″), 4.27 (s, 1H, HC≡C), 3.30 (s, 6H, CH_3SO_2), 2.17 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO); ^{13}C NMR (DMSO-d_6): δ 168.2, 167.4, 162.5, 150.0, 139.5 (C-6), 103.2 (C-5), 86.4 (C-1′), 84.0, 83.7, 76.2 (C-2′), 75.9, 75.5, 67.8 (C-5′), 65.5 (C-5″), 36.8 (CH_3SO_2), 36.7 (CH_3SO_2), 20.5 (CH_3CO), 20.1 (CH_3CO). A trace amount of $CHCl_3$ was identified.

5.12. 1-[2,3-Di-*O*-acetyl-3-*C*-ethynyl-4-*C*-(methanesulfonoxymethyl)-5-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl]-4-*N*-benzoylcytosine (25)

4-*N*-Benzoylcytosine (0.58 g, 2.68 mmol) was dried by coevaporation with 1,2-dichloroethane (2 × 15 mL), and resuspended in 1,2-dichloroethane (10 mL). BSA (1.3 mL, 5.35 mmol) was added and the heterogeneous solution refluxed for 60 min. After cooling to rt, glycosyl donor **23** (0.87 g, 1.78 mmol) dissolved in anhydrous 1,2-dichloroethane (10 mL) and TMSOTf (1.0 mL, 5.35 mmol) were added and the reaction mixture refluxed for 24 h. Then satd aq $NaHCO_3$ (20 mL) and H_2O (50 mL) were added, and the phases separated, and the aqueous phase extracted with CH_2Cl_2 (4 × 30 mL). The combined organic phase was evaporated to dryness and the resulting residue purified by silica gel column chromatography (0–4% MeOH in CH_2Cl_2, v/v) to afford protected nucleoside **25** (0.85 g, 74%) as off-white foam, which was used in the next step without further purification. R_f = 0.6 (10% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 664.0852 ([M+Na]$^+$, $C_{25}H_{27}N_3O_{13}S_2{\cdot}Na^+$: Calcd 664.0878); ^{1}H NMR (DMSO-d_6): δ 11.39 (br s, 1H, ex, NH), 8.24 (d, 1H, *J* = 7.7 Hz, H-6), 8.00 (d, 2H, *J* = 8.1 Hz, Ph), 7.60–7.64 (t, 1H, *J* = 7.5 Hz, Ph), 7.44–7.55 (m, 3H, H-5, Ph), 6.18 (d, 1H, *J* = 4.0 Hz, H-1′), 5.82 (d, 1H, *J* = 4.0 Hz, H-2′), 4.76 (br s, 2H, H-5′), 4.60–4.65 (d, 1H, *J* = 11.0 Hz, H-5″), 4.50–4.54 (d, 1H, *J* = 11.0 Hz, H-5″), 4.29 (s, 1H, HC≡C), 3.33 (s, 3H, CH_3SO_2), 3.31–3.32 (overlap with H_2O, CH_3SO_2), 2.15 (s, 3H, CH_3CO), 2.11 (s, 3H, CH_3CO); ^{13}C NMR (DMSO-d_6): δ 167.6, 167.4, 163.7, 153.9, 144.6 (C-6), 132.9, 132.8, 128.42, 128.36, 96.9 (C-5), 88.7 (C-1′), 84.8, 84.3, 77.4 (C-2′), 75.8, 75.6, 67.9 (C-5″), 65.5 (C-5′), 36.9 (CH_3SO_2), 20.4 (CH_3CO), 20.1 (CH_3CO). A trace impurity of EtOAc was identified.

5.13. (1*S*,3*R*,4*R*,5*S*)-5-Ethynyl-4-hydroxy-1-hydroxymethyl-3-(uracil-1-yl)-2,6-dioxabicyclo[3.2.0]heptane (5)

Protected nucleoside **24** (0.78 g, 1.45 mmol) was dissolved in satd methanolic ammonia (80 mL) and stirred in a sealed flask for 23 h whereupon analytic TLC showed full conversion to a slightly more polar product (R_f = 0.4; 10% MeOH in CH_2Cl_2, v/v). The reaction mixture was evaporated to dryness and the residue coevaporated several times with anhydrous EtOH to give a crude bicyclic nucleoside (0.8 g), which was tentatively assigned as (1*R*,3*R*,4*R*,5*S*)-5-ethynyl-4-hydroxy-1-(methanesulfonoxy)methyl-3-(uracil-1-yl)-2,6-dioxabicyclo[3.2.0]heptane (MALDI-HRMS *m/z* 381.0358 ([M+Na]$^+$, $C_{13}H_{14}N_2O_8S{\cdot}Na^+$: Calcd 381.0363), and

used in the next step without further purification. To a stirred solution of crude mesylate in anhydrous DMF (20 mL) was added sodium benzoate (0.42 g, 2.89 mmol). After heating at 110 °C for 19 h, analytical TLC showed full conversion to a less polar product (R_f = 0.8; 10% MeOH in CH_2Cl_2, v/v). Solids were filtered off, washed (EtOAc), and the combined organic filtrates evaporated to near dryness. The residue was partitioned between H_2O (25 mL) and CH_2Cl_2 (25 mL), the phases separated and the aqueous phase extracted with CH_2Cl_2 (4 × 25 mL). The combined organic phase was evaporated to dryness and the residue purified on a short silica plug (0–5% MeOH in CH_2Cl_2, v/v) to give a crude nucleoside (0.8 g) tentatively assigned as (1*R*,3*R*,4*R*,5*S*)-1-(benzoyloxy)methyl-5-ethynyl-4-hydroxy-3-(uracil-1-yl)-2,6-dioxabicyclo[3.2.0]heptane (MALDI-HRMS *m/z* 407.0850 ($[M+Na]^+$, $C_{19}H_{16}N_2O_7{\cdot}Na^+$: Calcd 407.0869), which was dissolved in satd methanolic ammonia (40 mL). After stirring in a sealed flask for 46 h, the reaction mixture was evaporated to dryness and coevaporated with anhydrous EtOH (20 mL). The resulting residue was adsorbed on Kieselguhr and purified by silica gel column chromatography with (0–12% MeOH in CH_2Cl_2, v/v) to afford bicyclic nucleoside **5** (0.25 g, 62% from **24**, over three steps) as an off-white solid material. R_f = 0.3 (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 258 nm, λ_{max} H_2O, 258 nm, λ_{max} pH 11, 262 nm; MALDI-HRMS *m/z* 303.0571 ($[M+Na]^+$, $C_{12}H_{12}N_2O_6{\cdot}Na^+$: Calcd 303.0588); ^{1}H NMR (DMSO-d_6): δ 11.32 (br s, 1H, ex, NH), 7.79 (d, 1H, *J* = 8.1 Hz, H-6), 6.19 (d, 1H, *J* = 7.7 Hz, H-1′), 6.02 (d, 1H, ex, *J* = 7.0 Hz, 2′-OH), 5.72 (d, 1H, *J* = 8.1 Hz, H-5), 5.01 (br t, 1H, ex, *J* = 5.5 Hz, 5′-OH), 4.72 (d, 1H, *J* = 8.1 Hz, H-5″), 4.31 (d, 1H, *J* = 8.1 Hz, H-5″), 4.06 (br t, 1H, H-2′), 4.02 (s, 1H, HC≡C), 3.72 (br s, 2H, H-5′); ^{13}C NMR (DMSO-d_6): δ 162.8, 150.8, 141.0 (C-6), 102.6 (C-5), 86.0, 85.3 (C-1′), 84.7, 82.0, 79.2, 77.2 (C-2′), 75.9 (C-5″), 60.8 (C-5′).

5.14. (1*S*,3*R*,4*R*,5*S*)-3-(Cytosin-1-yl)-5-ethynyl-4-hydroxy-1-hydroxymethyl-2,6-dioxabicyclo[3.2.0]heptane (6)

Protected nucleoside **25** (164.8 mg, 0.26 mmol) was dissolved in satd methanolic ammonia (15 mL) and after stirring in a sealed flask at rt for 42 h, analytical TLC showed full conversion to a single product with lower mobility (R_f = 0.2, 10% MeOH in CH_2Cl_2, v/v). The reaction mixture was evaporated to dryness and coevaporated several times with anhydrous EtOH to give a crude nucleoside, which tentatively was assigned as (1*R*,3*R*,4*R*,5*S*)-3-(cytosin-1-yl)-5-ethynyl-4-hydroxy-1-(methanesulfonoxy)methyl-2,6-dioxabicyclo[3.2.0]heptane (MALDI-HRMS *m/z* 380.0519 ($[M+Na]^+$, $C_{13}H_{15}N_3O_7S{\cdot}Na^+$: Calcd 380.0523). To a solution of crude bicyclic mesylate (0.15 g) in anhydrous DMF (5 mL), was added sodium benzoate (74 mg, 0.51 mmol) and the reaction mixture was heated at 110 °C for 14 h. At this time analytical TLC showed complete conversion into a product with higher mobility (R_f = 0.7, 20% MeOH in CH_2Cl_2, v/v). The dark reaction mixture was evaporated to dryness and filtered through a short silica pad (0–20% MeOH in CH_2Cl_2, v/v) to afford a crude nucleoside which was tentatively assigned as (1*R*,3*R*,4*R*,5*S*)-1-(benzoyloxy)methyl-3-(cytosin-1-yl)-5-ethynyl-4-hydroxy-2,6-dioxabicyclo[3.2.0]heptane (MALDI-HRMS *m/z* 406.1010 ($[M+Na]^+$, $C_{19}H_{17}N_3O_6{\cdot}Na^+$: Calcd 406.1001). Crude bicyclic benzoate (174 mg) was dissolved in satd methanolic ammonia (10 mL) and after stirring in a sealed flask at rt for 18 h, the reaction mixture was evaporated to near dryness, and was coevaporated several times with anhydrous EtOH. The resulting residue was taken up in H_2O (15 mL) and washed with CH_2Cl_2 (2 × 15 mL) and the aqueous phase evaporated to dryness. The resulting crude powder was purified by recrystallization from *i*-PrOH and minimal H_2O to give bicyclic nucleoside **6** (18.7 mg, 26% yield from **25**, over three steps) as white needles. R_f = 0.2 (20% MeOH in CH_2Cl_2, v/v); mp (*i*-PrOH/H_2O) > 220 °C; UV λ_{max} pH 1, 277 nm, λ_{max} H_2O, 238, 268 nm, λ_{max} pH 11, 272 nm; MALDI-HRMS *m/z* 302.0755 ($[M+Na]^+$, $C_{12}H_{13}N_3O_5{\cdot}Na^+$: Calcd 302.0747); ^{1}H NMR (DMSO-d_6) 7.68 (d, 1H, *J* = 7.7 Hz, H-6), 7.29 (s, 1H, ex, NH), 7.26 (s, 1H, ex, NH), 6.26 (d, 1H, *J* = 8.1 Hz, H-1′), 5.87 (d, 1H, ex, *J* = 7.6 Hz, 2′-OH), 5.78 (d, 1H, *J* = 7.3 Hz, H-5), 4.93 (t, 1H, ex, *J* = 5.6 Hz, 5′-OH), 4.70 (d, 1H, *J* = 7.9 Hz, H-5″), 4.29 (d, 1H, *J* = 7.9 Hz, H-5″), 4.05 (m, 1H, H-2′), 3.99 (s, 1H, HC≡C), 3.70 (d, 2H, *J* = 5.6 Hz, H-5′); ^{13}C NMR (DMSO-d_6): δ 165.4, 155.3, 142.0 (C-6), 94.9 (C-5), 86.4 (C-1′), 85.5, 85.0, 81.8, 79.6, 77.4 (C-2′), 75.9 (C-5″), 60.8 (C-5′).

5.15. 3-*O*-Benzyl-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(4,4′-dimethoxytrityloxy)methyl-3-*C*-ethynyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (26)

Triol **20** (4.50 g, 18.4 mmol) was dried by coevaporation with anhydrous pyridine (2 × 25 mL), dissolved in anhydrous pyridine (50 mL), and 4,4′-dimethoxytrityl chloride (15.61 g, 46.1 mmol) and DMAP (2.25 g, 18.4 mmol) added. After stirring the reaction mixture at rt for 44 h, additional 4,4′-dimethoxytrityl chloride (3.10 g, 9.15 mmol) and DMAP (0.45 g, 3.68 mmol) were added. After stirring for further 23 h, MeOH (20 mL) was added and the reaction mixture was evaporated to almost dryness, coevaporated with toluene (75 mL) and the residue taken up in EtOAc (250 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 100 mL) and brine (100 mL) and evaporated to almost dryness and coevaporated with toluene (2 × 100 mL). The resulting residue was purified by silica gel column chromatography (0–39.5% EtOAc and 0.5% pyridine in petroleum ether, v/v/v) to afford a crude light yellow solid material, which was tentatively assigned as 5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(4,4′-dimethoxytrityloxy)methyl-3-*C*-ethynyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (R_f = 0.4, 50% EtOAc in petroleum ether, v/v; MALDI-HRMS *m/z* 871.3470 ($[M+Na]^+$, $C_{53}H_{52}O_{10}{\cdot}Na^+$: Calcd 871.3453). Crude DMT-protected furanose (12.8 g), was coevaporated with toluene (50 mL), dissolved in anhydrous THF (110 mL) and added over 45 min at 0 °C to a suspension of sodium hydride in anhydrous THF (30 mL). After stirring the reaction mixture for 10 min, benzyl bromide (1.9 mL,

15.7 mmol) and tetrabutylammonium iodide (0.55 g, 1.50 mmol) were added. After stirring for 16 h at rt, crushed ice (75 mL) was added, the reaction mixture diluted with EtOAc (50 mL), the phases separated and the organic phase washed with brine (2 × 75 mL). The organic phase was evaporated to dryness affording crude furanose **26** as a slightly red foam (16.2 g), which was used in the next step without further purification. An aliquot was purified by silica gel column chromatography (0–29.5% EtOAc and 0.5% pyridine in petroleum ether, v/v/v). R_f = 0.2 (20% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 961.3921 ([M+Na]$^+$, $C_{60}H_{58}O_{10}$·Na$^+$: Calcd 961.3922); ^{1}H NMR ($CDCl_3$): δ 6.62–7.42 (m, 31H, Ar), 6.07 (d, 1H, J = 4.4 Hz, H-1), 4.93 (d, 1H, J = 4.4 Hz, H-2), 4.79–4.85 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.65–4.70 (d, 1H, J = 11.4 Hz, CH_2Ph), 3.85–3.89 (d, 1H, J = 8.8 Hz, H-5), 3.56–3.79 (m, 15H, H-5, 2 × H-5′, 4 × CH_3O), 2.14 (s, 1H, HC≡C), 1.30 (s, 3H, C(CH_3)$_2$), 1.16 (s, 3H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$): δ 158.40, 158.36, 158.29, 158.28, 145.1, 144.7, 138.8, 136.4, 136.2, 136.1, 135.9, 130.8, 130.63, 130.58, 129.2, 129.0, 128.6, 128.4, 128.1, 127.8, 127.6, 127.1, 127.0, 126.6, 126.4, 125.4, 114.4, 113.09, 113.05, 112.9, 105.5, 91.0, 87.6, 87.0, 86.4, 81.9, 80.4, 78.2, 68.5, 65.8, 63.8, 55.3, 55.2, 27.2, 26.6.

5.16. 3-*O*-Benzyl-3-*C*-ethynyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (27)

Crude furanose **26** (16.2 g) was suspended in 80% aqueous acetic acid (200 mL) and stirred at rt for 23 h, whereupon the reaction mixture was evaporated to near dryness, and coevaporated with toluene (100 mL). The residue was purified by silica gel column chromatography (0–50% EtOAc in petroleum ether, v/v) to give diol **27** (3.38 g, 55% from **20**, over three steps) as a yellow solid material. R_f = 0.1 (10% AcOH, 18% EtOAc in petroleum ether, v/v/v); MALDI-HRMS *m/z* 357.1305 ([M+Na]$^+$, $C_{18}H_{22}O_6$·Na$^+$: Calcd 357.1309); ^{1}H NMR (DMSO-d_6): δ 7.23–7.38 (m, 5H, Ph), 5.85 (d, 1H, J = 4.0 Hz, H-1), 4.80–4.85 (m, 2H, H-2, CH_2Ph), 4.69 (t, 1H, ex, J = 5.5 Hz, OH), 4.60–4.65 (d, 1H, J = 11.3 Hz, CH_2Ph), 4.05 (dd, 1H, ex, J = 6.6 Hz, 5.1 Hz, OH), 3.87–3.94 (m, 2H, HC≡C, H-5), 3.70–3.78 (m, 2H, H-5′), 3.61 (dd, 1H, J = 10.6 Hz, 5.9 Hz, H-5), 1.49 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 138.3, 128.0 (Ph), 127.2 (Ph), 127.0 (Ph), 112.9, 103.4 (C-1), 89.1, 85.3 (C-2), 81.8, 81.5, 79.7, 67.7 (CH_2Ph), 62.3 (C-5′), 59.5 (C-5), 26.4 (C(CH_3)$_3$), 26.2 (C(CH_3)$_3$). Anal. Calcd for $C_{18}H_{22}O_6$: C, 64.66; H, 6.63. Found: C, 64.61; H, 6.62.

5.17. 1,2-Di-*O*-acetyl-3-*O*-benzyl-3-*C*-ethynyl-4-*C*-(methanesulfonoxy)methyl-5-*O*-methanesulfonyl-α,β-D-*erythro*-pentofuranose (29)

Furanose **28** (3.16 g, 9.45 mmol) was dried by coevaporation with anhydrous pyridine (2 × 25 mL) and dissolved in anhydrous pyridine (25 mL). MsCl (2.2 mL, 28.4 mmol) was added dropwise over 5 min and the mixture stirred for 20 h at rt. Analytical TLC showed complete conversion to one product with high mobility (R_f = 0.6, 90% EtOAc in petroleum ether, v/v) whereupon crushed ice (20 mL) was added. The reaction mixture was evaporated to near dryness, coevaporated with toluene (75 mL) and the residue taken up in EtOAc (150 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 50 mL), and then evaporated to dryness and coevaporated with toluene (2 × 75 mL) to afford a crude residue tentatively assigned as 3-*O*-benzyl-3-*C*-ethynyl-1,2-*O*-isopropylidene-4-*C*-(methanesulfonoxy)-methyl-5-*O*-methanesulfonyl-α-D-*erythro*-pentofuranose (MALDI-HRMS *m/z* 513.0866 ([M+Na]$^+$, $C_{18}H_{22}O_6$·Na$^+$: Calcd 513.0860), which was directly dissolved in ice-cold 80% aqueous TFA (50 mL) and stirred at 0 °C for 2 h. At this time analytical TLC showed full conversion to one compound of lower mobility (R_f = 0.4, 90% EtOAc in petroleum ether, v/v). Solvents were evaporated off and the residue coevaporated with toluene (2 × 50 mL) to afford a crude residue, which was tentatively assigned as 3-*O*-benzyl-3-*C*-ethynyl-4-*C*-(methanesulfonoxy)methyl-5-*O*-methanesulfonyl-α,β-D-*erythro*-pentofuranose (MALDI-HRMS *m/z* 473.0531 ([M+Na]$^+$, $C_{17}H_{22}O_{10}S_2$·Na$^+$: Calcd 473.0547) and used in the next step without further purification. The crude diol was dried by coevaporation with anhydrous pyridine (25 mL) and dissolved in anhydrous pyridine (25 mL). To this was added Ac_2O (3.58 mL, 37.8 mmol) and the reaction mixture was stirred at rt for 20 h whereupon crushed ice (20 mL) was added. The reaction mixture was evaporated to dryness, coevaporated with toluene (50 mL) and the residue taken up in EtOAc (150 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 50 mL), and the organic phase evaporated to near dryness and subsequently coevaporated with toluene (2 × 50 mL). The resulting residue was purified by silica gel column chromatography (30–40% EtOAc in petroleum ether, v/v) to give an anomeric mixture (ratio ≈9:10 by ^{1}H NMR) of glycosyl donor **29** (4.50 g, 89% over three steps) as a yellow foam. Data for anomeric mixture: R_f = 0.6 (90% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 557.0732 ([M+Na]$^+$, $C_{21}H_{26}O_{12}S_2$·Na$^+$: Calcd 557.0758); ^{13}C NMR ($CDCl_3$): δ 169.3, 169.03, 169.00, 168.9, 137.2, 136.7, 128.6, 128.5, 128.2, 128.1, 127.8, 127.7, 98.2, 93.5, 86.0, 85.5, 82.2, 81.4, 80.4, 79.5, 79.4, 76.5, 76.2, 76.0, 70.3, 70.0, 68.2, 68.1, 67.11, 67.07, 37.84, 37.81, 37.7, 37.6, 21.1, 20.9, 20.7, 20.5. Anal. Calcd for $C_{21}H_{26}O_{12}S_2$·1/16 H_2O: C, 47.09; H, 4.92. Found: C, 46.77; H, 4.94.

5.18. 1-[2–*O*-Acetyl-3-*O*-benzyl-3-*C*-ethynyl-4-*C*-(methanesulfonoxymethyl)-5-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl]uracil (30)

Glycosyl donor **29** (2.50 g, 4.68 mmol) and uracil (1.05 g, 9.35 mmol) were coevaporated with anhydrous acetonitrile (50 mL) and suspended in anhydrous acetonitrile (50 mL). To this was added BSA (4.6 mL, 18.7 mmol) and the solution was refluxed until becoming homogenous (<1 h). After cooling the solution to rt, TMSOTf (1.9 mL, 10.3 mmol) was added and the reaction mixture heated at 50 °C for 15 h. Since analytical TLC revealed the reaction only to occur sluggishly a second portion of TMSOTf (1.0 mL, 5.53 mmol) was added after cooling the reaction mixture to rt. After stirring at 50 °C for further 24 h, starting material was fully

converted and the reaction mixture was poured into ice-cold satd aq $NaHCO_3$ (20 mL). The solvents were evaporated and the residue taken up in EtOAc (200 mL) and H_2O (100 mL). The phases were separated, and the aqueous phase extracted with EtOAc (2 × 100 mL). The combined organic phase was evaporated and the resulting residue purified by silica gel column chromatography (0–2% MeOH in CH_2Cl_2, v/v) to afford the nucleoside **30** (2.41 g, 88%) as a white solid material. R_f = 0.5 (5% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 609.0835 ($[M+Na]^+$, $C_{23}H_{26}N_2O_{12}S_2{\cdot}Na^+$: Calcd 609.0819); ^{1}H NMR (DMSO-d_6): δ 11.50 (s, 1H, ex, NH), 7.82 (d, 1H, *J* = 8.1 Hz, H-6), 7.32–7.47 (m, 5H, Ph), 6.10 (d, 1H, *J* = 7.7 Hz, H-1′), 5.89 (d, 1H, *J* = 7.7 Hz, H-2′), 5.74 (d, 1H, *J* = 8.1 Hz, H-5), 4.85–4.93 (2d, 2H, H-5′/H-5″/CH_2Ph), 4.71–4.76 (d, 1H, *J* = 10.5 Hz, H-5′/H-5″/CH_2Ph), 4.59–4.64 (d, 1H, *J* = 10.5 Hz, H-5′/H-5″/CH_2Ph), 4.43–4.48 (d, 1H, *J* = 10.3 Hz, H-5′/H-5″/CH_2Ph), 4.32–4.37 (m, 2H, HC≡C, H-5′/H-5″/CH_2Ph), 3.31 (s, 3H, CH_3SO_2), 3.17 (s, 3H, CH_3SO_2), 2.12 (s, 3H, CH_3CO); ^{13}C NMR (DMSO-d_6): δ 169.2, 162.6, 150.5, 140.3 (C-6), 137.4, 128.3 (Ph), 127.82 (Ph), 127.79 (Ph), 102.7 (C-5), 84.7, 84.6, 84.5, 78.1, 77.2 (C-2′), 74.7, 69.9, 68.4, 65.9, 36.7 (CH_3SO_2), 36.6 (CH_3SO_2), 20.2 (CH_3CO). Anal. Calcd for $C_{23}H_{26}N_2O_{12}S_2{\cdot}1/16\ H_2O$: C, 47.00; H, 4.48; N, 4.77; S, 10.91. Found: C, 46.67; H, 4.24; N, 4.76; S, 10.70.

5.19. (1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-7-ethynyl-1-(methanesulfonoxy)methyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (31)

To a stirred solution of nucleoside **30** (0.50 g, 0.85 mmol) in 1,4-dioxane–H_2O (3 mL, 2:1, v/v) was added 2 M aqueous NaOH (3 mL, 6 mmol). Shortly after addition (5 min), analytical TLC (10% MeOH in CH_2Cl_2, v/v) revealed approximately 50% conversion to the O2′-deacetylated nucleoside (vide infra) having greater polarity (R_f = 0.4, 10% MeOH in $CHCl_3$, v/v) than starting material **30**. After stirring the reaction mixture for further 3 h at rt, the O2′-deacetylated nucleoside had fully disappeared to give a product having identical mobility as the starting material **30**. Hereupon the reaction mixture was neutralized with satd aq NH_4Cl and diluted with EtOAc (25 mL) and H_2O (25 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 25 mL). The combined organic phase was evaporated and the resulting residue purified by silica gel column chromatography (0–5% MeOH in CH_2Cl_2, v/v) to afford LNA derivative **31** (0.36 g, 94%) as a white solid material. R_f = 0.6 (10% MeOH in $CHCl_3$, v/v); MALDI-HRMS *m/z* 471.0820 ($[M+Na]^+$, $C_{20}H_{20}N_2O_8S{\cdot}Na^+$: Calcd 471.0833); ^{1}H NMR (DMSO-d_6): δ 11.44 (s, 1H, ex, NH), 7.75 (d, 1H, *J* = 8.1 Hz, H-6), 7.29–7.37 (m, 5H, Ph), 5.56–5.60 (m, 2H, H-1′, H-5), 4.99 (s, 1H, H-2′), 4.75–4.79 (d, 1H, *J* = 11.7 Hz, H-5′/H-5″/CH_2Ph), 4.69–4.73 (d, 1H, *J* = 11.0 Hz, H-5′/H-5″/CH_2Ph), 4.59–4.64 (m, 2H, H-5′/H-5″/CH_2Ph), 4.15–4.18 (d, 1H, *J* = 8.4 Hz, H-5′/H-5″/CH_2Ph), 4.10 (s, 1H, HC≡C), 4.00–4.04 (d, 1H, *J* = 8.4 Hz, H-5′/H-5″/CH_2Ph), 3.29 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 163.3, 149.9, 140.7 (C-6), 137.0, 128.2 (Ph), 127.7 (Ph), 127.6 (Ph), 99.4 (C-5), 88.1, 88.0, 84.7, 79.2 (C-2′), 77.8, 75.3, 72.7, 68.5, 66.0, 36.9 (CH_3).

5.20. (1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-7-ethynyl-1-(hydroxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (32)

To a solution of LNA derivative **31** (0.33 g, 0.74 mmol) in anhydrous DMF (10 mL) was added sodium benzoate (213 mg, 1.48 mmol) and the mixture was stirred at 110 °C for 48 h, whereupon analytical TLC revealed approximately 40% conversion to a less polar product (R_f = 0.6, EtOAc). Additional sodium benzoate (250 mg, 1.73 mmol) was added and heating increased to 140 °C. After stirring for additional 24 h, reaction mixture was cooled to rt and unsoluble residues were filtered off and washed (EtOAc). The combined filtrates were evaporated to near dryness and diluted with EtOAc (25 mL) and brine (25 mL) and the phases separated. Extraction of the aqueous phase with EtOAc (2 × 25 mL) was complicated by formation of emulsions. The combined organic phase was evaporated to dryness to afford the crude benzoate (0.43 g, MALDI-HRMS *m/z* 497.1325 ($[M+Na]^+$, $C_{26}H_{22}N_2O_7{\cdot}Na^+$: Calcd 497.1325), which was immediately dissolved in satd methanolic ammonia (10 mL) and stirred in a sealed container at rt for 48 h. The reaction mixture was evaporated to dryness, coevaporated with absolute EtOH (2 × 10 mL) and the resulting residue adsorbed on silica gel and purified by silica gel column chromatography (50–90% EtOAc in petroleum ether, v/v) to afford nucleoside **32** (170 mg, 62%, over two steps) as a white solid material. R_f = 0.3 (EtOAc); MALDI-HRMS *m/z* 393.1061 ($[M+Na]^+$, $C_{19}H_{18}N_2O_6{\cdot}Na^+$: Calcd 393.1057); ^{1}H NMR (DMSO-d_6): δ 11.40 (s, 1H, ex, NH), 7.71 (d, 1H, *J* = 8.2 Hz, H-6), 7.28–7.38 (m, 5H, Ph), 5.58 (d, 1H, *J* = 8.2 Hz, H-5), 5.52 (s, 1H, H-1′), 5.14 (t, 1H, ex, *J* = 5.9 Hz, 5′–OH), 4.88 (s, 1H, H-2′), 4.65–4.70 (d, 1H, *J* = 11.0 Hz, CH_2Ph), 4.55–4.60 (d, 1H, *J* = 11.0 Hz, CH_2Ph), 4.09–4.13 (d, 1H, *J* = 8.1 Hz, H-5″), 3.78–3.99 (m, 4H, HC≡C, H-5′, H-5″); ^{13}C NMR (DMSO-d_6): δ 163.5, 150.1, 141.0, 137.5, 128.4, 127.8, 127.7, 99.8, 91.3, 87.8, 83.9, 79.4, 77.7, 76.5, 73.5, 68.4, 57.4.

5.21. (1*S*,3*R*,4*R*,7*S*)-7-Ethynyl-7-hydroxy-1-(hydroxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (7)

Nucleoside **32** (92.3 mg, 0.25 mmol) was dried by coevaporation with anhydrous 1,2-dichloroethane (2 × 10 mL), dissolved in anhydrous CH_2Cl_2 (5 mL) and the solution cooled to −78 °C. To this was added BCl_3 (1 M in hexane, 3.0 mL, 3.0 mmol) over 30 min. After complete addition, the reaction mixture was allowed to warm to rt and stirred for 48 h, when additional BCl_3 (1 M in hexane, 1.0 mL, 1.0 mmol) was added. After stirring the reaction mixture for further 24 h, H_2O (1 mL) was added. The reaction mixture was evaporated to near dryness, coevaporated with absolute EtOH (2 × 10 mL) and the resulting residue adsorbed on silica gel and purified by silica gel column chromatography (0–7% MeOH in $CHCl_3$, v/v) to afford nucleoside **32** (43.8 mg, 63%) as a white solid material. R_f = 0.4 (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1,

263 nm, λ_{max} H_2O, 263 nm, λ_{max} pH 11, 263 nm; MALDI-HRMS *m/z* 303.0589 ($[M+Na]^+$, $C_{12}H_{12}N_2O_6{\cdot}Na^+$: Calcd 303.0588); ^1H NMR (DMSO-d_6): δ 11.34 (br s, 1H, ex, NH), 7.64 (d, 1H, $J = 8.2$ Hz, H-6), 6.67 (s, 1H, ex, 3′-OH), 5.55 (dd, 1H, $J = 8.2$, 2.2 Hz, H-5), 5.37 (s, 1H, H-1′), 5.04 (t, 1H, ex, $J = 5.7$ Hz, 5′-OH), 4.39 (s, 1H, H-2′), 4.00–4.04 (d, 1H, $J = 8.1$ Hz, H-5′), 3.80–3.90 (m, 3H, H-5′, H-5″), 3.65 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6): δ 163.3, 150.0, 140.7 (C-6), 99.2 (C-5), 91.2, 87.5 (C-1′), 81.8 (C-2′), 81.1, 79.7, 72.7 (C-5′), 71.2, 57.3 (C-5″). $CHCl_3$ was identified as a trace impurity.

5.22. 4-*C*-(Acetoxymethyl)-5-*O*-acetyl-3-*C*-ethynyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (33)

To a solution of triol **20** (4.41 g, 18.1 mmol) in anhydrous pyridine (150 mL) was added acetic anhydride (5.0 mL, 52.9 mmol) at 0 °C. After stirring for 60 h at rt, crushed ice (50 mL) was added and the mixture evaporated to dryness. The residue was taken up in EtOAc (100 mL), and the organic phase washed with satd aq $NaHCO_3$ (2×50 mL) and brine (30 mL). The organic phase was evaporated to dryness and coevaporated several times with toluene, affording alcohol **33** (5.96 g) as a brown oil, which was used in the next step without purification. Purification of an aliquot by silica gel column chromatography (6% MeOH in CH_2Cl_2, v/v) afforded a pure sample of furanose **33** as a pale yellow oil. $R_f = 0.7$ (12% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 351.1055 ($[M+Na]^+$, $C_{15}H_{20}O_8{\cdot}Na^+$: Calcd 351.1050); ^1H NMR (DMSO-d_6): δ 6.38 (s, 1H, ex, 3–OH), 5.81 (d, 1H, $J = 4.4$ Hz, H-1), 4.68 (d, 1H, $J = 4.4$ Hz, H-2), 4.29–4.39 (m, 2H, H-5), 4.19 (s, 2H, H-5′), 3.78 (s, 1H, HC≡C), 2.01 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.48 (s, 3H, $C(CH_3)_2$), 1.28 (s, 3H, $C(CH_3)_2$); ^{13}C NMR (DMSO-d_6): δ 169.9, 169.8, 113.0, 103.7 (C-1), 85.7 (C-2), 85.0, 82.2, 78.6, 75.0, 64.3 (C-5), 62.3 (C-5′), 26.1 ($C(CH_3)_2$), 26.0 ($C(CH_3)_2$), 20.52 (CH_3CO), 20.47 (CH_3CO).

5.23. 4-*C*-(Acetoxymethyl)-1,2,3,5-tetra-*O*-acetyl-3-*C*-ethynyl-α,β-D-*erythro*-pentofuranose (34)

Crude alcohol **33** (5.96 g) was dissolved in ice-cold 80% aqueous TFA (55 mL). After stirring for 30 min at room temperature analytical TLC showed full conversion to a polar product ($R_f = 0.7$, 7% MeOH in CH_2Cl_2, v/v). The brown reaction mixture was evaporated to dryness and coevaporated several times with toluene and then anhydrous pyridine to give a residue, which was directly dissolved in anhydrous pyridine (200 mL). To this was added acetic anhydride (12.0 mL, 0.13 mol) and DMAP (0.33 g, 2.72 mmol) and after heating at 100 °C for 12 h, the dark reaction mixture was cooled to rt and crushed ice (20 mL) added. The mixture was evaporated to dryness and the residue coevaporated several times with toluene. The residue was taken up in CH_2Cl_2 (200 mL) and washed with satd aq $NaHCO_3$ (2×50 mL) and brine (50 mL). The organic phase was evaporated to near dryness, coevaporated several times with toluene, and purified by dry column vacuum chromatography (60% EtOAc in petroleum ether, v/v) to afford an anomeric mixture (ratio ~1:3 by ^1H NMR) of furanose **34** (4.08 g, 55% from **20**) as a white solid material. $R_f = 0.7$ (7% MeOH in CH_2Cl_2, v/v); MALDI-HRMS *m/z* 437.1053 ($[M+Na]^+$, $C_{18}H_{22}O_{11}{\cdot}Na^+$: Calcd 437.1054); ^{13}C NMR (DMSO-d_6 main isomer): δ 169.6, 169.5, 168.5, 168.2, 167.6, 97.4, 86.7, 81.6, 78.3, 77.0, 76.2, 63.5, 61.4, 20.7, 20.5, 20.4, 20.2, 20.2. Anal. Calcd for $C_{18}H_{22}O_{11}$ (anomeric mixture): C, 52.17; H, 5.35. Found: C, 52.10; H, 5.31.

5.24. 1-[4-*C*-(Acetoxymethyl)-2,3,5-tri-*O*-acetyl-3-*C*-ethynyl-β-D-*erythro*-pentofuranosyl]uracil (35)

To a suspension of uracil (97.4 mg, 0.87 mmol) in anhydrous 1,2-dichloroethane (8 mL) was added BSA (0.32 mL, 1.30 mmol) and the suspension was heated under reflux for 1 h. The resulting homogenous solution was cooled to rt whereupon a solution of glycosyl donor **34** (200.0 mg, 0.48 mmol) in anhydrous 1,2-dichloroethane (6 mL) was added. TMSOTf (0.17 mL, 0.91 mmol) was added and the mixture heated under reflux for 5 h. After cooling to rt, the mixture was diluted with $CHCl_3$ (15 mL), and satd aq $NaHCO_3$ (5 mL) was added. The separated aqueous phase was extracted with $CHCl_3$ (2×15 mL), and the combined organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–5% MeOH in CH_2Cl_2, v/v) to give nucleoside **35** (186.6 mg, 77%) as an off-white solid material. $R_f = 0.7$ (12% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 259 nm, λ_{max} H_2O, 259 nm, λ_{max} pH 11, 262 nm; MALDI-HRMS *m/z* 489.1099 ($[M+Na]^+$, $C_{20}H_{22}N_2O_{11}{\cdot}Na^+$: Calcd 489.1116); ^1H NMR ($CDCl_3$): δ 8.91 (br s, 1H, NH), 7.60 (d, 1H, $J = 8.2$ Hz, H-6), 6.23 (d, 1H, $J = 4.4$ Hz, H-1′), 5.81 (d, 1H, $J = 8.2$ Hz, H-5), 5.73 (d, 1H, $J = 4.4$ Hz, H-2′), 4.42–4.66 (m, 4H, H-5′, H-5″), 2.93 (s, 1H, HC≡C), 2.14 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.10 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.1, 169.7, 168.3, 167.3, 162.3, 149.8, 138.8 (C-6), 103.8 (C-5), 86.8 (C-1′), 85.6, 80.0, 77.7 (C-2′), 76.8, 76.5, 63.9 (C-5′), 60.7 (C-5″), 20.7 (CH_3), 20.4 (CH_3). Anal. Calcd for $C_{20}H_{22}N_2O_{11}{\cdot}2\,H_2O$: C, 47.81; H, 4.41; N, 5.58. Found: C, 47.69; H, 4.42, N, 5.43.

5.25. 1-[4-*C*-(Acetoxymethyl)-2,3,5-tri-*O*-acetyl-3-*C*-ethynyl-β-D-*erythro*-pentofuranosyl]cytosine (36)

A suspension of cytosine (241.1 mg, 2.17 mmol) and BSA (0.81 mL, 3.28 mmol) in anhydrous 1,2-dichloroethane (20 mL) was heated under reflux for 1 h. The homogenous solution was allowed to cool to rt whereupon a solution of glycosyl donor **34** (0.50 g, 1.21 mmol) in anhydrous 1,2-dichloroethane (15 mL) was added. TMSOTf (0.41 mL, 2.27 mmol) was added dropwise and the mixture heated under reflux for 48 h. After cooling to rt, the mixture was diluted with $CHCl_3$ (15 mL) and satd aq $NaHCO_3$ (10 mL). The separated aqueous phase was extracted with $CHCl_3$ (2×30 mL) and the combined organic phase was dried ($MgSO_4$), filtered and evaporated. The resulting residue was purified by silica gel column chromatography (0–7% MeOH in CH_2Cl_2, v/v) to afford nucleoside **36** (0.31 g, 54%) as a white solid material. $R_f = 0.4$ (12% MeOH in CH_2Cl_2,

v/v); UV λ_{max} pH 1, 277 nm, λ_{max} H_2O, 269 nm, λ_{max} pH 11, 273 nm; MALDI-HRMS *m/z* 488.1252 ($[M+Na]^+$, $C_{20}H_{23}N_3O_{10}{\cdot}Na^+$: Calcd 488.1276); ^1H NMR ($CDCl_3$): δ 7.63 (d, 1H, J = 7.7 Hz, H-6), 6.29 (d, 1H, J = 4.1 Hz, H-1′), 5.89 (d, 1H, J = 7.7 Hz, H-5), 5.76 (d, 1H, J = 4.1 Hz, H-2′), 4.40–4.70 (m, 4H, H-5′, H-5″), 2.98 (s, 1H, HC≡C), 2.08–2.11 (m, 12H, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.1, 169.9, 168.1, 167.4, 165.8, 155.4, 140.0 (C-6), 96.2 (C-5), 87.8 (C-1′), 85.5, 80.4, 78.3 (C-2′), 76.9, 76.6, 64.0 (C-5′), 60.9 (C-5″), 20.8 (CH_3), 20.5 (CH_3). Anal. Calcd for $C_{20}H_{23}N_3O_{10}{\cdot}1/2\ H_2O$: C, 50.63; H, 5.10; N, 8.86. Found: C, 50.83; H, 4.91; N, 8.83.

5.26. 1-[3-*C*-Ethynyl-4-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]uracil (8)

Protected nucleoside **35** (0.36 g, 0.76 mmol) was dissolved in satd methanolic ammonia (6 mL) and methanol (14 mL) and the reaction mixture stirred for 21 h in a sealed flask. The reaction mixture was evaporated to dryness and coevaporated with absolute EtOH several times to afford nucleoside **8** (0.22 g, 97%) as a white solid material pure by NMR. An analytically pure sample of **8** was obtained by dissolving a sample of **8** in a minimal amount of methanol. Several hours after addition of a few drops of toluene, white crystals of **8** were obtained and isolated by filtration. R_f = 0.3 (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 260 nm, λ_{max} H_2O, 261 nm, λ_{max} pH 11, 263 nm; MALDI-HRMS *m/z* 321.0691 ($[M+Na]^+$, $C_{12}H_{14}N_2O_7Na^+$: Calcd 321.0693); ^1H NMR (DMSO-d_6): δ 11.31 (br s, 1H, ex, NH), 8.14 (d, 1H, J = 8.2 Hz, H-6), 5.86 (d, 1H, J = 8.2 Hz, H-1′), 5.79 (d, 1H, ex, J = 7.1 Hz, 2′–OH), 5.71 (s, 1H, ex, 3′–OH), 5.69 (d, 1H, J = 8.2 Hz, H-5), 5.16 (t, 1H, J = 4.1 Hz, ex, 5′–OH), 4.58 (t, 1H, ex, J = 5.8 Hz, 5″-OH), 4.40–4.46 (m, 1H, H-2′), 3.67–3.84 (m, 2H, H-5′), 3.50–3.55 (m, 2H, H-5″), 3.46 (s, 1H, C≡CH); ^{13}C NMR (DMSO-d_6): δ 162.9, 151.0, 141.0 (C-6), 102.1 (C-5), 88.8, 84.8 (C-1′), 83.0, 77.5 (C-2′), 76.3, 73.6, 63.3, 61.7 (C-5′, C-5″). Anal. Calcd for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39. Found: C, 48.38; H, 4.66; N, 9.41.

5.27. 1-[3-*C*-Ethynyl-4-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]cytosine (9)

Protected nucleoside **36** (245.2 mg, 0.53 mmol) was dissolved in satd methanolic ammonia (15 mL). After stirring for 12 h at rt in a sealed flask, the reaction mixture was evaporated to dryness and coevaporated several times with absolute EtOH to give an off-white solid material, which was purified by crystallization from aqueous EtOH to give **9** (89.9 mg, 57%) as pale yellow crystals. Repeating the crystallization procedure on the mother liquor furnished a second crop of crystals (27.7 mg, 75% combined yield). R_f = 0.2 (40% MeOH in CH_2Cl_2, v/v); mp (H_2O/EtOH) >220 °C; UV λ_{max} pH 1, 278 nm, λ_{max} H_2O, 270 nm, λ_{max} pH 11, 273 nm; MALDI-HRMS *m/z* 320.0841 ($[M+Na]^+$, $C_{12}H_{15}N_3O_6{\cdot}Na^+$: Calcd 320.0853); ^1H NMR (DMSO-d_6): δ 7.98 (d, 1H, J = 7.1 Hz, H-6), 7.20 (br s, 1H, ex, NH_2), 7.16 (br s, 1H, ex, NH_2), 5.87 (d, 1H, J = 8.2 Hz, H-1′), 5.73 (d, 1H, J = 7.1 Hz, H-5), 5.61–5.62 (m, 2H, 2ex, 2′–OH, 3′–OH), 5.05 (t, 1H, ex, J = 5.7 Hz, 5′-OH), 4.52 (t, 1H, ex, J = 5.7 Hz, 5″-OH), 4.38–4.43 (m, 1H, H-2′), 3.65–3.80 (m, 2H, H-5′), 3.47–3.57 (m, 2H, H-5″), 3.43 (s, 1H, HC≡C); ^{13}C NMR (DMSO-d_6): δ 165.3, 155.7, 142.1 (C-6), 94.3 (C-5), 88.2, 86.2 (C-1′), 83.3, 77.9 (C-2′), 76.0, 73.7, 63.4 (C-5′), 61.7 (C-5″). Anal. Calcd for $C_{12}H_{15}N_3O_6$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.40; H, 5.01; N, 13.93.

5.28. 1,2;5,6-Di-*O*-isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-α-D-*allo*-hexofuranose (38)

1,2;5,6-Di-*O*-isopropylidene-α-D-*gluco*-furanose **37** (15.00 g, 57.6 mmol) was dried by coevaporation with toluene (2 × 75 mL) and resuspended in anhydrous CH_2Cl_2 (300 mL). To this, PDC (28.04 g, 74.5 mmol) and freshly activated 3 Å molecular sieve powder (53 g) were added. The suspension was cooled to 0 °C and glacial AcOH (5.9 mL, 0.10 mol) added dropwise over 10 min. After stirring the heterogeneous mixture at rt for 16 h, the mixture was evaporated to dryness and coevaporated with toluene (2 × 75 mL). The dark residue was suspended in HPLC grade EtOAc (400 mL), and solid material filtered off on a 5 cm silica pad, which was washed with EtOAc (1.5 L). The solvent was evaporated off and the crude ketone dried by coevaporation with toluene (75 mL) and used in the next step without further purification. The crude ketone (11.73 g) in anhydrous THF (80 mL), was added dropwise over 40 min at −78 °C to a solution of *n*-butyllithium (33.1 mL, 2.0 M in cyclohexane, 66.2 mmol) and (trimethylsilyl)acetylene (10.7 mL, 75.6 mmol) in anhydrous THF (80 mL). After ended addition, the reaction mixture was stirred for additional 15 min whereupon satd aq NH_4Cl (25 mL) was added and solvents were evaporated off. The residue was taken up in H_2O (50 mL) and the aqueous phase extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phase was dried ($MgSO_4$), and evaporated affording furanose **38** (15.06 g, 73% over two steps) as an off-white solid material, which was pure by NMR. Recrystallization of a small sample from anhydrous EtOH afforded an analytically pure sample as white crystals. R_f = 0.6 (3% MeOH in CH_2Cl_2, v/v); mp (abs EtOH) 114–115 °C; MALDI-HRMS *m/z* 379.1562 ($[M+Na^+]$, $C_{17}H_{28}O_6Si{\cdot}Na^+$: Calcd 379.1547); ^1H NMR ($CDCl_3$): δ 5.81 (d, 1H, J = 3.3 Hz, H-1), 4.56 (d, 1H, J = 3.3 Hz, H-2), 4.40 (m, 1H, H-5), 4.13 (dd, 1H, J = 8.2 Hz, 6.2 Hz, H-6), 4.01 (dd, 1H, J = 8.2 Hz, 5.5 Hz, H-6), 3.87 (d, 1H, J = 7.1 Hz, H-4), 3.02 (s, 1H, ex, 3-OH), 1.58 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$), 1.36 (s, 6H, $C(CH_3)_2$), 0.18 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 113.6, 109.5, 104.2 (C-1), 101.6, 94.2, 83.9 (C-2), 81.4 (C-4), 76.0, 74.7 (C-5), 66.8 (C-6), 26.8 ($C(CH_3)_2$), 26.60 ($C(CH_3)_2$), 26.59 ($C(CH_3)_2$), 25.1 ($C(CH_3)_2$), −0.3 ($Si(CH_3)_3$). Anal. Calcd for $C_{17}H_{28}O_6Si$: C, 57.28; H, 7.92. Found: C, 57.23; H, 7.96.

5.29. 3-*C*-Ethynyl-1,2;5,6-di-*O*-isopropylidene-α-D-*allo*-hexofuranose (39)

To a solution of alcohol **38** (18.72 g, 52.5 mmol) in THF (100 mL) was added tetrabutylammonium fluoride (1 M

solution in THF, 52.5 mL, 52.5 mmol) and reaction mixture stirred for 45 min at rt, whereupon it was evaporated to dryness. The residue was taken up in EtOAc (250 mL), which was washed with brine (3 × 50 mL), dried (Na_2SO_4), evaporated to dryness and purified by silica gel column chromatography (0–40% EtOAc in petroleum ether, v/v) to give furanose **39** (11.30 g, 76%) as a white solid material. ^{1}H NMR ($CDCl_3$) data are identical with previously published data.[35]

5.30. 1,2-*O*-Isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-α-D-*allo*-hexofuranose (40)

Furanose **38** (6.57 g, 18.4 mmol) was dissolved in 80% aqueous AcOH (75 mL) and stirred for 24 h at rt, whereupon the reaction mixture was evaporated to dryness and coevaporated several times with anhydrous EtOH–toluene (1:1 v/v). The resulting residue was purified by crystallization from H_2O affording triol **40** (4.80 g, 82%) as a white powder. R_f = 0.2 (70% EtOAc in petroleum ether, v/v); mp (H_2O) 106–111 °C; MALDI-HRMS *m*/*z* 339.1223 ([M+Na]$^+$, $C_{14}H_{24}O_6Si{\cdot}Na^+$: Calcd 339.1234); ^{1}H NMR (DMSO-d_6): δ 5.67 (d, 1H, *J* = 3.8 Hz, H-1), 5.46 (s, 1H, ex, 3-OH), 4.40–4.47 (m, 3H, 2ex, H-2, 5-OH, 6-OH), 3.71–3.76 (m, 2H, H-4, H-5), 3.59 (dd, 1H, *J* = 11.2 Hz, 4.9 Hz, H-6), 3.35 (dd, 1H, *J* = 11.2 Hz, 5.5 Hz, H-6), 1.43 (s, 3H, $C(CH_3)_2$), 1.27 (s, 3H, $C(CH_3)_2$), 0.16 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR (DMSO-d_6): δ 112.1, 105.0, 103.1 (C-1), 91.3, 84.1 (C-2), 79.5 (C-4), 75.8, 71.4 (C-5), 63.4 (C-6), 26.6 ($C(CH_3)_2$), 26.5 ($C(CH_3)_2$), −0.2 ($Si(CH_3)_3$). Anal. Calcd for $C_{14}H_{24}O_6Si$: C, 53.14; H, 7.65. Found: C, 52.82; H, 7.56.

5.31. 3,5,6-Tri-*O*-acetyl-1,2-*O*-isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-α-D-*allo*-hexofuranose (41)

Furanose **40** (0.40 g, 1.28 mmol) was dried by coevaporation with anhydrous pyridine (15 mL), and dissolved in anhydrous pyridine (10 mL). Ac_2O (1.2 mL, 12.7 mmol) and DMAP (23.4 mg, 0.19 mmol) were added and the reaction mixture stirred at rt for 3 h, whereupon crushed ice (3 mL) was added. The reaction mixture was evaporated to dryness, coevaporated with toluene (10 mL) and the residue taken up in EtOAc (20 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 8 mL), and the aqueous phase back-extracted with EtOAc (10 mL). The combined organic phase was evaporated to dryness, coevaporated with toluene (2 × 15 mL) and the resulting residue purified by silica gel column chromatography (30–50% EtOAc in petroleum ether, v/v) to afford **41** (0.36 g, 64%), as an amorphous white solid material. R_f = 0.6 (70% EtOAc in petroleum ether, v/v); MALDI-HRMS *m*/*z* 465.1554 ([M+Na]$^+$, $C_{20}H_{30}O_9Si{\cdot}Na^+$: Calcd 465.1551); ^{1}H NMR ($CDCl_3$): δ 5.84 (d, 1H, *J* = 3.7 Hz, H-1), 5.37 (ddd, 1H, *J* = 8.4, 5.3, 2.3 Hz, H-5), 5.11 (d, 1H, *J* = 3.7 Hz, H-2), 4.59 (dd, 1H, *J* = 12.4 Hz, 2.3 Hz, H-6), 4.26 (d, 1H, *J* = 8.4 Hz, H-4), 4.19 (dd, 1H, *J* = 12.4 Hz, 5.3 Hz, H-6), 2.10 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.51 (s, 3H, $C(CH_3)_2$), 1.32 (s, 3H, $C(CH_3)_2$), 0.16 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 170.6, 169.1, 168.1, 113.2, 104.2 (C-1), 97.5, 95.7, 82.6 (C-2), 78.5, 78.2 (C-4), 70.2 (C-5), 63.3 (C-6), 26.8 ($C(CH_3)_3$), 26.6 ($C(CH_3)_3$), 20.9 (CH_3CO), 20.8 (CH_3CO), −0.5 ($Si(CH_3)_3$). Anal. Calcd for $C_{20}H_{30}O_9Si$: C, 54.28; H, 6.83. Found: C, 54.21; H, 6.91.

5.32. 3,5,6-Tri-*O*-acetyl-1,2-*O*-isopropylidene-3-*C*-ethynyl-α-D-*allo*-hexofuranose (42)

To a solution of furanose **41** (0.89 g, 2.00 mmol) in THF (20 mL), was sequentially added glacial AcOH (130 μL, 2.27 mmol) and TBAF (1.0 M solution in THF, 2.0 mL, 2.00 mmol). After stirring for 30 min at rt, the reaction mixture was evaporated to dryness, and the residue partitioned between EtOAc (80 mL) and brine (20 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 × 40 mL). The combined organic phase was dried ($MgSO_4$), evaporated to dryness, and the resulting residue adsorbed on Kieselguhr and purified by silica gel column chromatography (30% EtOAc in petroleum ether, v/v) to afford peracetylated furanose **42** (0.61 g, 82%) as white solid material. R_f = 0.5 (70% EtOAc in petroleum ether, v/v); MALDI-HRMS *m*/*z* 393.1156 ([M+Na]$^+$, $C_{17}H_{22}O_9{\cdot}Na^+$: Calcd 393.1147); ^{1}H NMR ($CDCl_3$): δ 5.85 (d, 1H, *J* = 3.7 Hz, H-1), 5.39–5.46 (m, 1H, H-5), 5.14 (d, 1H, *J* = 3.7 Hz, H-2), 4.59 (dd, 1H, *J* = 12.3 Hz, 2.2 Hz, H-6), 4.28 (d, 1H, *J* = 8.4 Hz, H-4), 4.18 (dd, 1H, *J* = 12.3 Hz, 5.5 Hz, H-6), 2.68 (s, 1H, HC≡C), 2.10 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 1.53 (s, 3H, $C(CH_3)_2$), 1.33 (s, 3H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 170.7, 169.5, 168.4, 113.6, 104.2 (C-1), 82.7 (C-2), 78.4, 78.3, 77.9 (C-4), 77.1, 70.1 (C-5), 63.4 (C-6), 27.0 ($C(CH_3)_3$), 26.7 ($C(CH_3)_3$), 21.0 (CH_3CO), 20.92 (CH_3CO), 20.90 (CH_3CO). Anal. Calcd for $C_{17}H_{22}O_9$: C, 55.13; H, 5.99. Found: C, 55.42; H, 6.06.

5.33. 1,2,3,5,6-Penta-*O*-acetyl-3-*C*-ethynyl-α,β-D-*allo*-hexofuranose (43)

Method A: Triacetylated furanose **42** (0.58 g, 1.56 mmol) was dissolved in ice-cold 80% aqueous TFA (5 mL) and stirred for 2.5 h at 0 °C, whereupon the reaction mixture was evaporated to dryness and coevaporated with toluene (10 mL) and anhydrous pyridine (2 × 10 mL). The crude anomeric diol was dissolved in anhydrous pyridine (10 mL), and Ac_2O (1.7 mL, 18.0 mmol) and DMAP (10.7 mg, 0.09 mmol) were added. After stirring at rt for 18 h, crushed ice (5 mL) was added and the reaction mixture was diluted with EtOAc (30 mL) and washed with satd aq $NaHCO_3$ (2 × 10 mL). The combined aqueous phase was back-extracted with EtOAc (15 mL) and the combined organic phase evaporated to dryness and coevaporated with toluene (2 × 30 mL). The resulting residue purified by silica gel column chromatography (20–45% EtOAc in petroleum ether, v/v) to afford an anomeric mixture (~1:1.5 by ^{1}H NMR) of glycosyl donor **43** (0.39 g, 61% over two steps) as an amorphous white solid material. *Method B*: Diacetylated furanose **45** (2.00 g, 6.10 mmol) was dissolved in ice-cold 80% aqueous TFA (15 mL) and stirred at 0 °C for 2 h, whereupon the reaction mixture was evaporated to dryness, coevaporated with toluene (20 mL) and anhydrous

pyridine (2 × 20 mL). The crude anomeric triol was dissolved in anhydrous pyridine (30 mL) and Ac_2O (6.0 mL, 63.4 mmol) and DMAP (37.3 mg, 0.30 mmol) were added. The reaction mixture was stirred for 18 h at rt, when crushed ice (20 mL) was added, and the reaction mixture diluted with EtOAc (100 mL). The organic phase was washed with satd aq solution $NaHCO_3$ (2 × 40 mL), and the aqueous phase back extracted with EtOAc (50 mL). The combined organic phase was evaporated to dryness, coevaporated with toluene (2 × 100 mL), and the resulting residue adsorbed on Kieselguhr and purified by silica gel column chromatography (20–55% EtOAc in petroleum ether, v/v) to afford an anomeric mixture (~1:3 by ^{1}H NMR) of glycosyl donor **43** (2.01 g, 82% over two steps) as an amorphous white solid material. Physical data for major anomer in both methods: R_f = 0.5 (70% EtOAc in petroleum ether, v/v); ^{1}H NMR ($CDCl_3$): δ 6.08 (s, 1H, H-1), 5.74 (s, 1H, H-2), 5.35 (ddd, 1H, J = 9.2, 4.5, 2.6 Hz, H-5), 4.65 (dd, 1H, J = 12.5 Hz, 2.6 Hz, H-6), 4.48 (d, 1H, J = 9.2 Hz, H-4), 4.15 (dd, 1H, J = 12.5 Hz, 4.5 Hz, H-6), 2.75 (s, 1H, HC≡C), 2.07–2.12 (5s, 5 × 3H, 5 × CH_3CO; ^{13}C NMR ($CDCl_3$): δ 170.7, 169.6, 169.0, 168.5, 168.3, 98.5, 82.8, 78.7, 78.5, 76.7, 76.3, 71.1, 62.9, 21.1, 21.0, 20.94, 20.89, 20.6. Physical data for pure fractions of minor anomer in both methods: R_f = 0.4 (70% EtOAc in petroleum ether, v/v); ^{1}H NMR ($CDCl_3$): δ 6.47 (d, 1H, J = 4.4 Hz, H-1), 5.76 (d, 1H, J = 4.4 Hz, H-2), 5.41 (ddd, 1H, J = 7.9, 5.5, 2.7 Hz, H-5), 4.61 (d, 1H, J = 12.5 Hz, 2.7 Hz, H-6), 4.47 (d, 1H, J = 7.9 Hz, H-4), 4.19 (dd, 1H, J = 12.5 Hz, 5.5 Hz, H-6), 2.77 (s, 1H, HC≡C), 2.07–2.13 (5s, 5 × 3H, 5 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.7, 169.4, 168.8, 168.5, 168.0, 93.6, 80.7, 78.8, 76.4, 76.2, 74.6, 70.2, 62.7, 21.1, 20.94, 20.89, 20.6, 20.3. Physical data for anomeric mixture: MALDI-HRMS *m/z* 437.1054 ([M+Na]$^+$, $C_{18}H_{22}O_{11}$·Na$^+$: Calcd 437.1060). Anal. Calcd for $C_{18}H_{22}O_{11}$: C, 52.17; H, 5.35. Found: C, 52.18; H, 5.40.

5.34. 5,6-Di-*O*-acetyl-1,2-*O*-isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-α-D-*allo*-hexofuranose (44)

Furanose **40** (3.48 g, 11.0 mmol) was dried by coevaporation with anhydrous pyridine (50 mL), dissolved in anhydrous pyridine (50 mL) and cooled to 0 °C whereupon Ac_2O (2.6 mL, 27.5 mmol) was added slowly over 10 min. The reaction mixture was allowed to warm up to rt and was further stirred for 24 h, whereupon crushed ice (20 mL) was added. The reaction mixture was evaporated to dryness, coevaporated with toluene (35 mL), and the residue taken up in EtOAc (150 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 60 mL), evaporated to dryness, coevaporated with toluene (2 × 100 mL) and the resulting residue absorbed on Kieselguhr and purified by silica gel column chromatography (0–50% EtOAc in petroleum ether, v/v) to afford furanose **44** (3.08 g, 70%) as a white solid material along with peracetylated furanose **41** (1.10 g, 23%) as a colorless oil. R_f = 0.6 (70% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 423.1456 ([M+Na]$^+$, $C_{18}H_{28}O_8Si$·Na$^+$: Calcd 423.1446); ^{1}H NMR ($CDCl_3$): δ 5.85 (d, 1H, J = 3.7 Hz, H-1), 5.29 (ddd, 1H, J = 8.4, 5.1 Hz, 2.4 Hz, H-5), 4.55–4.62 (m, 2H, H-6, H-2), 4.18 (dd, 1H, J = 12.3 Hz, 5.1 Hz, H-6), 4.07 (d, 1H, J = 8.4 Hz, H-4), 2.94 (s, 1H, ex, 3-OH), 2.08 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.58 (s, 3H, $C(CH_3)_2$), 1.38 (s, 3H, $C(CH_3)_2$), 0.18 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 170.6, 169.5, 113.6, 103.9 (C-1), 100.3, 94.7, 84.1 (C-2), 79.6 (C-4), 75.8, 70.9 (C-5), 63.1 (C-6), 26.8 ($C(CH_3)_3$), 26.7 ($C(CH_3)_3$), 20.9 (CH_3CO), 20.7 (CH_3CO), −0.5 ($Si(CH_3)_3$). Anal. Calcd for $C_{18}H_{28}O_8Si$: C, 53.98; H, 7.05. Found: C, 54.15; H, 7.05.

5.35. 5,6-Di-*O*-acetyl-3-*C*-ethynyl-1,2–*O*-isopropylidene-α-D-*allo*-hexofuranose (45)

To a solution of furanose **44** (2.98 g, 7.44 mmol) in THF (60 mL) was added glacial AcOH (0.47 mL, 8.21 mmol) and then TBAF (1 M solution in THF, 7.4 mL, 7.4 mmol), and the reaction mixture was stirred for 2 h at rt, whereupon it was evaporated to dryness and partitioned between EtOAc (100 mL) and brine (20 mL). The phases were separated and the aqueous phase extracted with EtOAc (2 × 50 mL). The combined organic phase was dried ($MgSO_4$), evaporated to dryness, and the resulting crude yellow oil absorbed on Kieselguhr and purified by silica gel column chromatography (40% EtOAc in petroleum ether, v/v) to afford **45** (2.24 g, 92%) as white solid material. R_f = 0.4 (70% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 351.1050 ([M+Na]$^+$, $C_{15}H_{20}O_8$·Na$^+$: Calcd 351.1050); ^{1}H NMR ($CDCl_3$): δ 5.85 (d, 1H, J = 3.7 Hz, H-1), 5.35 (ddd, 1H, J = 8.4, 5.2, 2.4 Hz, H-5), 4.56–4.61 (m, 2H, H-2, H-6), 4.15 (dd, 1H, J = 12.2 Hz, 5.2 Hz, H-6), 4.07 (d, 1H, J = 8.4 Hz, H-4), 3.00 (s, 1H, ex, 3-OH), 2.65 (s, 1H, HC≡C), 2.09 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 1.58 (s, 3H, $C(CH_3)_2$), 1.38 (s, 3H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 170.8, 169.9, 113.9, 103.9 (C-1), 84.2 (C-2), 79.7, 79.3 (C-4), 77.5, 75.9, 70.7 (C-5), 63.2 (C-6), 26.9 ($C(CH_3)_3$), 26.8 ($C(CH_3)_3$), 21.0 (CH_3CO), 20.9 (CH_3CO). Anal. Calcd for $C_{15}H_{20}O_8$: C, 54.87; H, 6.14. Found: C, 54.86; H, 6.16.

5.36. 1-[2,3,5,6-Tetra-*O*-acetyl-3-*C*-ethynyl-β-D-*allo*-hexofuranosyl]uracil (46)

Glycosyl donor **43** (0.96 g, 2.33 mmol) and uracil (0.33 g, 2.91 mmol) were dried by coevaporation with anhydrous CH_3CN (20 mL), resuspended in anhydrous CH_3CN (25 mL), BSA (1.7 mL, 7.00 mmol) added and refluxed for 1 h until a homogenous solution was obtained. The solution was cooled to rt, TMSOTf (0.57 mL, 3.14 mmol) added and the reaction mixture refluxed for 14 h whereupon it was poured into satd aq $NaHCO_3$ (25 mL). The phases were separated and the aqueous phase extracted with EtOAc (3 × 50 mL). The combined organic phase was evaporated to dryness and the resulting residue purified by silica gel column chromatography (0–3% MeOH in CH_2Cl_2, v/v) to afford nucleoside **46** (0.50 g, 46%) as a white solid material. R_f = 0.5 (10% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 259 nm, λ_{max} H_2O, 259 nm, λ_{max} pH 11, 263 nm; MALDI-HRMS *m/z* 489.1124 ([M+Na]$^+$, $C_{20}H_{22}N_2O_{11}$·Na$^+$: Calcd 489.1116);^{1}H NMR ($CDCl_3$): δ 8.94 (br s, 1H, ex,

NH), 7.52 (d, 1H, $J = 8.1$ Hz, H-6), 6.06 (d, 1H, $J = 3.7$ Hz, H-1′), 5.83 (d, 1H, $J = 8.1$ Hz, H-5), 5.58 (d, 1H, $J = 3.7$ Hz, H-2′), 5.52 (ddd, 1H, $J = 8.4$, 4.8, 2.4 Hz, H-5′), 4.65 (dd, 1H, $J = 12.3$ Hz, 2.4 Hz, H-6′), 4.27 (d, 1H, $J = 8.4$ Hz, H-4′), 4.12 (dd, 1H, $J = 12.3$ Hz, 4.8 Hz, H-6′), 2.89 (s, 1H, HC≡C), 2.09–2.14 (4s, 12H, $4 \times CH_3CO$); ^{13}C NMR ($CDCl_3$): δ 170.7, 169.4, 168.3, 168.0, 162.4, 150.0, 139.2 (C-6), 104.1 (C-5), 88.1 (C-1′), 79.7, 79.6, 77.5 (C-2′), 77.0, 75.3, 69.8 (C-5′), 62.7 (C-6′), 20.92 (CH_3), 20.86 (CH_3), 20.7 (CH_3), 20.4 (CH_3). Anal. Calcd for $C_{20}H_{22}N_2O_{11}$: C, 51.50; H, 4.75; N, 6.01. Found: C, 51.22; H, 4.81; N, 5.74.

5.37. 1-[2,3,5,6-Tetra-*O*-acetyl-3-*C*-ethynyl-β-D-*allo*-hexofuranosyl]cytosine (47)

Glycosyl donor **43** (0.80 g, 1.94 mmol) and cytosine (0.27 g, 2.42 mmol) were dried by coevaporation with anhydrous CH_3CN (20 mL), resuspended in CH_3CN (20 mL), BSA (1.4 mL, 5.82 mmol) added and refluxed for 1 h when a clear homogenous solution was obtained. After cooling to rt, TMSOTf (0.47 mL, 2.60 mmol) was added and the reaction mixture refluxed for 40 h. At this time analytical TLC showed approx 50% conversion and the reaction mixture was therefore cooled to rt and a second portion of TMSOTf (0.25 mL, 1.38 mmol) added. After stirring for further 40 h, the reaction mixture was poured into satd aq $NaHCO_3$ (20 mL), phases separated and the aqueous phase extracted with EtOAc (4×30 mL). The combined organic phase was evaporated to dryness and the residue purified by silica gel column chromatography (0–12% MeOH in CH_2Cl_2, v/v) to afford nucleoside **47** (0.47 g, 52%) as a white solid material. $R_f = 0.5$ (10% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 277 nm, λ_{max} H_2O, 234, 269 nm, λ_{max} pH 11, 272 nm; MALDI-HRMS *m/z* 488.1256 ($[M+Na]^+$, $C_{20}H_{23}N_3O_{10}{\cdot}Na^+$: Calcd 488.1276); ^{1}H NMR ($CDCl_3$): δ 7.54 (d, 1H, $J = 7.2$ Hz, H-6), 6.19 (d, 1H, $J = 3.7$ Hz, H-1′), 5.87 (d, 1H, $J = 7.2$ Hz, H-5), 5.69 (d, 1H, $J = 3.7$ Hz, H-2′), 5.49–5.56 (m, 1H, H-5′), 4.67 (dd, 1H, $J = 12.3$ Hz, 2.2 Hz, H-6′), 4.32 (d, 1H, $J = 8.8$ Hz, H-4′), 4.14 (dd, 1H, $J = 12.3$ Hz, 4.8 Hz, H-6′), 2.89 (s, 1H, HC≡C), 2.04–2.14 (m, 12H, $4 \times CH_3CO$); ^{13}C NMR ($CDCl_3$): δ 170.6, 169.3, 167.85, 167.78, 165.9, 155.4, 140.2 (C-6), 96.4 (C-5), 89.1 (C-1′), 80.1, 79.5, 77.4 (C-2′), 76.6, 75.4, 69.9 (C-5′), 62.6 (C-6′), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.4 (CH_3).

5.38. 1-[3-*C*-Ethynyl-β-D-*allo*-hexofuranosyl]uracil (10)

Protected nucleoside **46** (248 mg, 0.53 mmol) was dissolved in satd methanolic ammonia (15 mL) and stirred in a sealed container for 72 h, whereupon the reaction mixture was evaporated to dryness and taken up in H_2O (15 mL). The aqueous phase was washed with CH_2Cl_2 (2×20 mL) and ether (2×20 mL), evaporated to dryness and the resulting residue coevaporated with anhydrous EtOH and purified by silica gel column chromatography (0–15% MeOH in CH_2Cl_2, v/v) to afford target nucleoside **10** (110 mg, 69%) as a white solid material. $R_f = 0.2$ (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 262 nm, λ_{max} H_2O, 262 nm, λ_{max} pH 11, 263 nm; MALDI-HRMS *m/z* 321.0700 ($[M+Na]^+$, $C_{12}H_{14}N_2O_7{\cdot}Na^+$: Calcd 321.0693); ^{1}H NMR (DMSO-d_6): δ 11.35 (br s, 1H, ex, NH), 7.83 (d, 1H, $J = 8.1$ Hz, H-6), 5.84 (d, 1H, ex, $J = 6.2$ Hz, 2′-OH), 5.72–5.75 (m, 2H, 1ex, H-1′, 3′-OH), 5.66 (d, 1H, $J = 8.1$ Hz, H-5), 4.99 (d, 1H, ex, $J = 4.8$ Hz, 5′-OH), 4.59 (t, 1H, ex, $J = 5.5$ Hz, 6′-OH), 4.16 (br t, 1H, H-2′), 3.89 (d, 1H, $J = 4.4$ Hz, H-4′), 3.63–3.79 (m, 2H, H-5′, H-6′), 3.40–3.56 (m, 2H, H-6′, HC≡C); Selected data ^{1}H NMR (DMSO-d_6 + one drop D_2O): δ 5.72 (d, 1H, $J = 7.0$ Hz, H-1′), 4.15 (d, 1H, $J = 7.0$ Hz, H-2′); ^{13}C NMR (DMSO-d_6): δ 162.9, 150.8, 140.7 (C-6), 102.0 (C-5), 85.8 (C-1′/C-4′), 85.7 (C-1′/C-4′), 83.0, 78.1 (C-2′), 77.8, 72.7, 72.2, 62.4 (C-6′). Anal. Calcd for $C_{12}H_{14}N_2O_7{\cdot}11/16$ MeOH: C, 47.68; H, 5.07; N, 8.77. Found: C, 47.30; H, 4.84; N, 8.64.

5.39. 1-[3-*C*-Ethynyl-β-D-*allo*-hexofuranosyl]cytosine (11)

Protected nucleoside **47** (172 mg, 0.37 mmol) was dissolved in satd methanolic ammonia (15 mL) and stirred in a sealed container for 21 h, whereupon the reaction mixture was evaporated to dryness and coevaporated with absolute EtOH (2×10 mL) and *o*-xylene (3×5 mL). The residue was taken up in MeOH (15 mL) and washed with *n*-hexane (10 mL). Methanol was evaporated off and the resulting residue purified by silica gel column chromatography (0–36% MeOH in CH_2Cl_2, v/v) to afford target nucleoside **11** (59 mg, 54%) as a white solid material. $R_f = 0.2$ (40% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 278 nm, λ_{max} H_2O, 269 nm, λ_{max} pH 11, 271 nm; MALDI-HRMS *m/z* 320.0853 ($[M+Na]^+$, $C_{12}H_{15}N_3O_6{\cdot}Na^+$: Calcd 320.0853); ^{1}H NMR (DMSO-d_6): δ 7.70 (d, 1H, $J = 7.3$ Hz, H-6), 7.25 (br s, 1H, ex, NH), 7.21 (br s, 1H, ex, NH), 5.71–5.74 (m, 3H, 1ex, H-5, H-1′, 2′-OH), 5.57 (s, 1H, ex, 3′-OH), 4.87 (d, 1H, ex, $J = 4.8$ Hz, 5′-OH), 4.54 (br t, 1H, ex, 6′-OH), 4.11 (br t, 1H, H-2′), 3.83 (d, 1H, H-4′), 3.76 (m, 1H, H-5′), 3.62–3.70 (m, 1H, H-6′), 3.41–3.54 (m, 2H, C≡CH, H-6′); Selected data ^{1}H NMR (DMSO-d_6 + one drop D_2O): δ 5.72 (d, 1H, $J = 5.9$ Hz, H-1′), 4.09 (d, 1H, $J = 5.9$ Hz, H-2′); ^{13}C NMR (DMSO-d_6): δ 165.5, 155.4, 141.6 (C-6), 94.2 (C-5), 87.5 (C-1′), 84.9 (C-4′), 83.5, 78.7 (C-2′), 77.7, 72.52, 72.49, 62.6 (C-6′).

5.40. 3-*O*-Benzyl-5-*O*-(*tert*-butyldimethylsilyl)-6-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*-hexofuranose (49)

To a solution of 6-deoxyfuranose **48**[36] (2.97 g, 10.1 mmol) in anhydrous CH_2Cl_2 (100 mL) was added TBDMSCl (6.14 g, 40.7 mmol) and imidazole (2.75 g, 40.4 mmol) and the reaction mixture was stirred for 24 h at rt. Solid residues were filtered off and washed with CH_2Cl_2. The combined organic phase was washed with H_2O (50 mL) and brine (2×50 mL), and evaporated to dryness. The resulting residue was purified by silica gel column chromatography with (50% Et_2O in petroleum ether, v/v) to afford furanose **49** (3.76 g, 91%) as a clear oil. $R_f = 0.8$ (50% EtOAc in petroleum ether, v/v); MALDI-HRMS *m/z* 431.2224 ($[M+Na]^+$, $C_{22}H_{36}O_5Si{\cdot}Na^+$: Calcd 431.2221); ^{1}H NMR ($CDCl_3$):

δ 7.18–7.29 (m, 5H, Ph), 5.79 (d, 1H, J = 4.0 Hz, H-1), 4.48–4.60 (m, 3H, H-2, CH_2Ph), 4.10–4.19 (m, 1H, H-5), 3.95 (d, 1H, J = 2.8 Hz, H-3), 3.83 (dd, 1H, J = 8.6, 2.8 Hz, H-4), 1.45 (s, 3H, $C(CH_3)_2$), 1.21–1.24 (m, 6H, $C(CH_3)_2$, H-6), 0.79 (s, 9H, $C(CH_3)_3$), −0.02 (s, 3H, $Si(CH_3)_2$), −0.08 (s, 3H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 137.9, 128.3 (Ph), 127.6 (Ph), 127.1 (Ph), 111.5, 104.9 (C-1), 84.8 (C-4), 81.6 (C-3), 81.5, 71.5, 65.2 (C-5), 26.8 ($C(CH_3)_2$), 26.2 ($C(CH_3)_2$), 25.9 ($C(CH_3)_3$), 21.7 (C-6), 17.9, −3.7 ($Si(CH_3)_2$), −4.7 ($Si(CH_3)_2$). Anal. Calcd for $C_{22}H_{36}O_5Si$: C, 64.67; H, 8.88. Found: C, 64.55; H, 8.89.

5.41. 5-*O*-(*tert*-Butyldimethylsilyl)-6-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*-hexofuranose (50)

Furanose **49** (3.60 g, 8.81 mmol) was dried by coevaporation with toluene (30 mL) and dissolved in EtOAc (85 mL). To this was added 10% $Pd(OH)_2$/C (0.37 g) and the reaction mixture was stirred under an H_2 atmosphere (balloon) for 19 h at rt. The mixture was then filtered through a Celite pad, which was washed with EtOAc. The organic phase was evaporated off, and the resulting residue purified by silica gel column chromatography (20% Et_2O in petroleum ether, v/v) to afford alcohol **50** (2.40 g, 86%) as a clear oil. R_f = 0.7 (50% EtOAc in petroleum ether, v/v); MALDI-HRMS m/z 341.1764 ($[M+Na]^+$, $C_{15}H_{30}O_5Si{\cdot}Na^+$: Calcd 341.1755); ^{1}H NMR (DMSO-d_6): δ 5.76 (d, 1H, J = 3.7 Hz, H-1), 5.11 (d, 1H, ex, J = 4.4 Hz, 3-OH), 4.37 (d, 1H, J = 3.7 Hz, H-2), 4.01 (m, 1H, H-5), 3.90–3.93 (m, 1H, H-3), 3.64 (dd, 1H, J = 8.3, 2.9 Hz, H-4), 1.37 (s, 3H, $C(CH_3)_2$), 1.22 (s, 3H, $C(CH_3)_2$), 1.15 (d, 3H, J = 6.2 Hz, H-6), 0.85 (s, 9H, $C(CH_3)_3$, 0.05 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (DMSO-d_6): δ 110.4, 104.1 (C-1), 84.8 (C-2), 84.2 (C-4), 72.6 (C-3), 64.7 (C-5), 26.6 ($C(CH_3)_2$), 26.0 ($C(CH_3)_2$), 25.6 ($C(CH_3)_3$), 21.4 (C-6), 17.5, −4.6 ($Si(CH_3)_2$), −5.0 ($Si(CH_3)_2$). Anal. Calcd for $C_{15}H_{30}O_5Si$: C, 56.57; H, 9.49. Found: C, 56.62; H, 9.53.

5.42. 5-*O*-(*tert*-Butyldimethylsilyl)-6-deoxy-1,2-*O*-isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-α-D-*allo*-hexofuranose (51)

Alcohol **50** (7.83 g, 24.6 mmol) was dried by coevaporation with toluene (50 mL), and dissolved in anhydrous CH_2Cl_2 (250 mL). To this, freshly activated 3 Å molecular sieves powder (21.2 g), glacial AcOH (2.5 mL, 43.7 mmol) and PDC (9.23 g, 24.5 mmol) were added and the reaction mixture stirred at rt for 22 h. The mixture was evaporated to dryness, coevaporated with toluene (100 mL) and resuspended in EtOAc. Solid residues were filtered off using a 2 cm silica pad, which was thoroughly washed with additional EtOAc. Evaporation of the organic phase afforded the crude ketone, which was dried by coevaporation with toluene and used in the next step without further purification. Crude ketone in anhydrous THF (20 mL) was added to a solution of *n*-butyllithium (20.0 mL, 2.0 M in hexanes, 40.0 mmol) and (trimethylsilyl)acetylene (4.5 mL, 0.03 mol) in anhydrous THF (40 mL) at −78 °C. After ended addition (20 min), the mixture was stirred for 20 min, when satd aq NH_4Cl (12 mL) was added. The solution was allowed to warm up to rt, diluted with H_2O (10 mL) phases separated and the aqueous phase extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phase was evaporated to dryness and the resulting residue purified by silica gel column chromatography (50% EtOAc in petroleum ether, v/v) to afford the furanose **51** (9.17 g, 90%) as a clear oil. R_f = 0.7 (50% EtOAc in petroleum ether, v/v); MALDI-HRMS m/z 437.2144 ($[M+Na]^+$, $C_{20}H_{38}O_5Si_2{\cdot}Na^+$: Calcd 437.2150); ^{1}H NMR (DMSO-d_6): δ 5.67 (d, 1H, J = 3.7 Hz, H-1), 5.50 (s, 1H, ex, 3-OH), 4.39 (d, 1H, J = 3.7 Hz, H-2), 4.00–4.08 (m, 1H, H-5), 3.61 (d, 1H, J = 6.2 Hz, H-4), 1.44 (s, 3H, $C(CH_3)_2$), 1.27 (s, 3H, $C(CH_3)_2$), 1.19 (d, 3H, J = 6.2 Hz, H-6), 0.87 (s, 9H, $C(CH_3)_3$, 0.16 (s, 9H, $Si(CH_3)_3$), 0.11 (s, 3H, $Si(CH_3)_2$), 0.08 (s, 3H, $Si(CH_3)_2$); ^{13}C NMR (DMSO-d_6): δ 111.9, 105.3, 102.7 (C-1), 91.3, 84.5 (C-2), 83.8 (C-4), 75.4, 67.6 (C-5), 26.53 ($C(CH_3)_2$), 26.49 ($C(CH_3)_2$), 25.7 ($C(CH_3)_3$), 21.0 (C-6), 17.7, 0.4 ($Si(CH_3)_3$), −4.2 ($Si(CH_3)_2$), −4.7 ($Si(CH_3)_2$). Anal. Calcd for $C_{20}H_{38}O_5Si_2$: C, 57.93; H, 9.24. Found: C, 57.98; H, 9.34.

5.43. 6-Deoxy-3-*C*-ethynyl-1,2-*O*-isopropylidene-α-D-*allo*-hexofuranose (52)

To a solution of furanose **51** (9.17 g, 22.1 mmol) in THF (220 mL) was added TBAF (44 mL, 1 M solution in THF, 44.0 mmol) and the reaction mixture was stirred for 3 h at rt, whereupon it was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (70% EtOAc in petroleum ether, v/v) to afford diol **52** (4.97 g, 98%) as a white solid material. R_f = 0.7 (EtOAc); MALDI-HRMS m/z 251.0891 ($[M+Na]^+$, $C_{11}H_{16}O_5{\cdot}Na^+$: Calcd 251.0890); ^{1}H NMR (DMSO-d_6): δ 5.68 (d, 1H, J = 3.5 Hz, H-1), 5.54 (s, 1H, ex, 3-OH), 4.49 (d, 1H, ex, J = 5.1 Hz, 5–OH), 4.43 (d, 1H, J = 3.5 Hz, H-2), 3.82–3.92 (m, 1H, H-5), 3.54–3.57 (m, 2H, H-4, HC≡C), 1.45 (s, 3H, $C(CH_3)_2$), 1.27 (s, 3H, $C(CH_3)_2$), 1.14 (d, 3H, J = 6.2 Hz, H-6); ^{13}C NMR (DMSO-d_6): δ 111.9, 102.8 (C-1), 84.2 (C-2), 83.2 (C-4), 82.8, 78.2, 75.2, 65.6 (C-5), 26.6 ($C(CH_3)_2$), 26.4 ($C(CH_3)_2$), 20.7 (C-6). Anal. Calcd for $C_{11}H_{16}O_5$: C, 57.88; H, 7.07. Found: C, 57.88; H, 7.06.

5.44. 3-*C*-Ethynyl-1,2-*O*-isopropylidene-6-*O*-toluenesulfonyl-α-D-*allo*-hexofuranose (54)

Triol **53**[28] (3.29 g, 13.4 mmol) was dried by coevaporation with anhydrous pyridine (3 × 50 mL) and dissolved in anhydrous pyridine (60 mL). To this, a solution of TsCl (3.87 g, 20.3 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise at −50 °C. After ended addition, the reaction mixture was allowed to warm up to rt and stirred for 18 h whereupon the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (30–50% EtOAc in petroleum ether, v/v) to afford diol **54** (3.04 g, 57%) as a white solid material, which was used in the next step without further purification. R_f = 0.6 (EtOAc); MALDI-HRMS m/z 421.0938 ($[M+Na]^+$,

$C_{18}H_{22}O_8S{\cdot}Na^+$: Calcd 421.0928); ^{1}H NMR (DMSO-d_6): δ 7.77 (d, 2H, J = 8.1 Hz), 7.47 (d, 2H, J = 8.1 Hz), 5.77 (s, 1H), 5.65 (d, 1H, J = 3.5 Hz), 5.31 (d, 1H, J = 5.9 Hz), 4.42 (d, 1H, J = 3.5 Hz), 4.16 (d, 1H, J = 8.4 Hz), 3.85–3.97 (m, 2H), 3.75 (d, 1H, J = 6.6 Hz), 3.58 (s, 1H), 2.42 (s, 3H), 1.42 (s, 3H), 1.27 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 144.7, 132.1, 130.0, 127.5, 112.2, 102.9, 84.0, 82.1, 79.6, 78.6, 75.1, 72.7, 67.7, 26.5, 26.4, 21.0. EtOAc was identified as a trace impurity.

5.45. 1,2,3,5-Tetra-*O*-acetyl-6-deoxy-3-*C*-ethynyl-α,β-D-*allo*-hexofuranose (55)

Diol **52** (1.25 g, 5.48 mmol) was dried by coevaporation with anhydrous pyridine (20 mL) and dissolved in anhydrous pyridine (55 mL). To this Ac_2O (2.0 mL, 21.1 mmol) and DMAP (75.6 mg, 0.62 mmol) were added and the reaction mixture was stirred for 18 h at rt whereupon analytical TLC showed clean conversion to a less polar product (R_f = 0.4, 50% EtOAc in petroleum ether, v/v). Subsequently, crushed ice (10 mL) was added and the mixture evaporated to dryness, coevaporated with toluene (20 mL) and the residue taken up in EtOAc (25 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 10 mL), evaporated to dryness and coevaporated with toluene (20 mL) to leave a residue, which was directly dissolved in 80% aqueous TFA (55 mL). The reaction mixture was stirred at rt for 3 h, whereupon analytical TLC showed full conversion to two polar compounds (both R_f = 0.1, 50% EtOAc in petroleum ether, v/v). The reaction mixture was evaporated to dryness, coevaporated with toluene (3 × 20 mL) and anhydrous pyridine (20 mL) and directly dissolved in anhydrous pyridine (55 mL). To this Ac_2O (2.0 mL, 21.1 mmol) and DMAP (67.4 mg, 0.55 mmol) were added and the reaction mixture was stirred at rt for 94 h, whereupon crushed ice (10 mL) was added and the reaction mixture evaporated to dryness. The residue was taken up in EtOAc (50 mL) and the organic phase washed with satd aq $NaHCO_3$ (2 × 20 mL). The organic phase was evaporated to dryness and purified by silica gel column chromatography (50% EtOAc in petroleum ether, v/v) affording an anomeric mixture (1:1 by ^{1}H NMR) of glycosyl donor **55** (1.41 g, 72%, over three steps) as a white solid material. Physical data for anomeric mixture: R_f = 0.8 (EtOAc); MALDI-HRMS m/z 379.0985 ([M+Na]$^+$, $C_{16}H_{20}O_9{\cdot}Na^+$: Calcd 379.1000); ^{13}C NMR ($CDCl_3$): δ 169.5, 169.4, 168.9, 168.8, 168.5, 168.3, 168.1, 168.0, 98.1, 93.3, 86.4, 84.5, 78.6, 78.2, 77.9, 76.6, 76.3, 76.2, 75.8, 74.8, 70.1, 69.4, 21.0, 20.9, 20.8, 20.72, 20.67, 20.4, 20.1, 17.3, 16.5.

5.46. 1-[2,3,5-Tri-*O*-acetyl-6-deoxy-3-*C*-ethynyl-β-D-*allo*-hexofuranosyl]uracil (56)

Glycosyl donor **55** (0.72 g, 2.02 mmol) was dried by coevaporation with anhydrous CH_3CN (2 × 20 mL) and suspended in anhydrous CH_3CN (6 mL). To this was added uracil (0.46 g, 4.10 mmol) and BSA (1.5 mL, 6.07 mmol) and the solution refluxed until a homogenous solution was formed (<1 h). After cooling to rt, TMSOTf (0.91 mL, 5.06 mmol) was added and the reaction mixture refluxed for 20 h, whereupon satd aq $NaHCO_3$ (10 mL) was added, and the mixture evaporated to dryness. The residue was partitioned between CH_2Cl_2 (25 mL) and brine (25 mL) and after separation of the phases, the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was evaporated to dryness and purified by silica gel column chromatography (0–1% MeOH in CH_2Cl_2, v/v) to afford protected nucleoside **56** (0.65 g, 79%) as a white solid material. R_f = 0.6 (10% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 259 nm, λ_{max} H_2O, 259 nm, $_{max}$ pH 11, 263 nm; MALDI-HRMS m/z 431.1065 ([M+Na]$^+$, $C_{18}H_{20}N_2O_9{\cdot}Na^+$: Calcd 431.1061); ^{1}H NMR ($CDCl_3$): δ 8.94 (s, 1H, ex, NH), 7.51 (d, 1H, J = 8.1 Hz, H-6), 6.04 (d, 1H, J = 4.0 Hz, H-1′), 5.81 (dd, 1H, J = 8.1, 2.2 Hz, H-5), 5.55 (d, 1H, J = 4.0 Hz, H-2′), 5.29–5.38 (m, 1H, H-5′), 4.09 (d, 1H, J = 7.7 Hz, H-4′), 2.86 (s, 1H, HC≡C), 2.15 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 1.31 (d, 3H, J = 6.2 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 169.4, 168.3, 168.0, 162.4, 149.9, 139.1 (C-6), 103.8 (C-5), 87.6 (C-1′), 83.6 (C-4′), 79.1, 77.6, 77.2, 75.0, 68.9 (C-5′), 21.0 (CH_3CO), 20.6 (CH_3CO), 20.3 (CH_3CO), 17.3 (C-6′). Anal. Calcd for $C_{18}H_{20}N_2O_9{\cdot}1/16\ CH_2Cl_2$: C, 52.32; H, 4.89; N, 6.76. Found: C, 52.24; H, 4.82; N, 6.52.

5.47. 1-[2,3,5-Tri-*O*-acetyl-6-deoxy-3-*C*-ethynyl-β-D-*allo*-hexofuranosyl]-4-*N*-benzoylcytosine (57)

Glycosyl donor **55** (0.63 g, 1.77 mmol) and 4-*N*-benzoylcytosine (0.75 g, 3.48 mmol) were dried by coevaporation with anhydrous CH_3CN (2 × 20 mL) and suspended in anhydrous CH_3CN (20 mL). To this BSA (1.3 mL, 5.26 mmol) was added and the mixture refluxed until it became homogenous (<1 h). After cooling to rt, TMSOTf (0.80 mL, 4.43 mmol) was added and the reaction mixture refluxed for 21 h, whereupon satd aq $NaHCO_3$ (10 mL) was added. The mixture was further diluted with H_2O (20 mL) and EtOAc (20 mL), the phases separated and the aqueous phase extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was evaporated to dryness and the resulting residue purified by silica gel column chromatography (1% MeOH in CH_2Cl_2, v/v) to afford protected nucleoside **57** (0.68 g, 75%) as a white solid material. R_f = 0.7 (10% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 257, 315 nm, λ_{max} H_2O, 261, 301 nm, λ_{max} pH 11, 316 nm; MALDI-HRMS m/z 534.1476 ([M+Na]$^+$, $C_{25}H_{25}N_3O_9{\cdot}Na^+$: Calcd 534.1483); ^{1}H NMR ($CDCl_3$): δ 8.97 (s, 1H, ex, NH), 7.89–7.94 (m, 3H, H-6, Ph/H-5), 7.47–7.62 (m, 4H, Ph/H-5), 6.21 (d, 1H, J = 3.5 Hz, H-1′), 5.68 (d, 1H, J = 3.5 Hz, H-2′), 5.34–5.43 (m, 1H, H-5′), 4.20 (d, 1H, J = 7.3 Hz, H-4′), 2.83 (s, 1H, HC≡C), 2.11 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.46 (d, 3H, J = 6.2 Hz, H-6′); ^{13}C NMR ($CDCl_3$): δ 169.4, 167.9, 167.8, 162.5, 143.7 (C-6), 133.2 (Ph), 129.0 (Ph), 127.6 (Ph), 97.5 (C-5), 88.5 (C-1′), 84.1 (C-4′), 79.4, 77.9 (C-2′), 77.0, 75.1, 69.0 (C-5′), 21.0 (CH_3CO), 20.6 (CH_3CO), 20.4 (CH_3CO), 17.3 (C-6′). Anal. Calcd for $C_{25}H_{25}N_3O_9{\cdot}5/16$ EtOH: C, 58.53; H, 5.15; N, 7.99. Found: C, 58.19; H, 4.85; N, 8.16.

5.48. 1-[6-Deoxy-3-*C*-ethynyl-β-D-*allo*-hexofuranosyl]uracil (12)

Protected nucleoside **56** (0.48 g, 1.18 mmol) was dissolved in satd methanolic ammonia (12 mL) and stirred at rt in a sealed container for 71 h whereupon the reaction mixture was evaporated to dryness and coevaporated with anhydrous EtOH (2 × 10 mL). The resulting residue was adsorbed on silica gel and purified by silica gel column chromatography (10% MeOH in CH_2Cl_2, v/v) to afford target nucleoside **12** (0.26 g, 78%) as a white solid material. Crystals for use in single crystal X-ray diffraction studies were obtained by recrystallization from MeOH. $R_f = 0.8$ (EtOAc); UV_{max} pH 1, 261 nm, λ_{max} H_2O, 262 nm, λ_{max} pH 11, 262 nm; MALDI-HRMS *m/z* 305.0757 ($[M+Na]^+$, $C_{12}H_{14}N_2O_6{\cdot}Na^+$: Calcd 305.0744); ^1H NMR (DMSO-d_6): δ 11.35 (s, 1H, ex, NH), 7.79 (d, 1H, $J = 8.1$ Hz, H-6), 5.86 (d, 1H, ex, $J = 6.2$ Hz, 2′-OH), 5.78 (s, 1H, ex, 3′-OH), 5.72 (d, 1H, $J = 7.0$ Hz, H-1′), 5.67 (d, 1H, $J = 8.1$ Hz, H-5), 4.80 (d, 1H, ex, $J = 4.8$ Hz, 5′-OH), 4.11 (br t, 1H, H-2′), 3.89–3.94 (m, 1H, H-5′), 3.68 (d, 1H, $J = 4.4$ Hz, H-4′), 3.55 (s, 1H, HC≡C), 1.23 (d, 3H, $J = 6.6$ Hz, H-6′); ^{13}C NMR (DMSO-d_6): δ 162.9, 150.8, 140.6 (C-6), 102.0 (C-5), 89.0 (C-4′), 85.6 (C-1′), 83.2, 77.9, 77.8, 71.9, 66.8 (C-5′), 19.5 (C-6′).

5.49. 1-[6-Deoxy-3-*C*-ethynyl-β-D-*allo*-hexofuranosyl]cytosine (13)

Protected nucleoside **57** (0.30 g, 0.59 mmol) was dissolved in satd methanolic ammonia (6 mL) and stirred at rt for 49 h, whereupon the reaction mixture was evaporated to dryness and coevaporated with anhydrous EtOH (2 × 10 mL). The resulting residue was adsorbed on silica gel and purified by silica gel column chromatography (0–10% MeOH in CH_2Cl_2, v/v) to afford target nucleoside **13** (106 mg, 64%) as a white solid material. $R_f = 0.2$ (20% MeOH in CH_2Cl_2, v/v); UV λ_{max} pH 1, 279 nm, λ_{max} H_2O, 269 nm, λ_{max} pH 11, 271 nm; MALDI-HRMS *m/z* 304.0893 ($[M+Na]^+$, $C_{12}H_{15}N_3O_5{\cdot}Na^+$: Calcd 304.0904); ^1H NMR (DMSO-d_6): δ 7.66 (d, 1H, $J = 7.3$ Hz, H-6), 7.24 (br s, 1H, ex, NH), 7.18 (br s, 1H, ex, NH), 5.72–5.78 (m, 3H, 1ex, H-5, H-1′, 2′-OH), 5.63 (s, 1H, ex, 3′-OH), 4.74 (d, 1H, ex, $J = 5.1$ Hz, 5′-OH), 4.05 (br t, 1H, H-2′), 3.90–3.95 (m, 1H, H-5′), 3.65 (d, 1H, $J = 5.1$ Hz, H-4′), 3.52 (s, 1H, HC≡C), 1.21 (d, 3H, $J = 6.2$ Hz, H-6′). Selected ^1H NMR signals (DMSO-d_6 + one drop D_2O): δ 5.73 (d, 1H, $J = 6.3$ Hz, H-1′), 4.04 (d, 1H, $J = 6.3$ Hz, H-2′); ^{13}C NMR (DMSO-d_6): δ 165.5, 155.2, 141.5 (C-6), 94.4 (C-5), 88.4 (C-4′), 87.3 (C-1′), 83.7, 78.5 (C-2′), 77.7, 72.2, 66.7 (C-5′), 19.5 (C-6′). Anal. Calcd for $C_{12}H_{15}N_3O_5{\cdot}1/2\ H_2O$: C, 49.65; H, 5.56; N, 14.48. Found: C, 49.49; H, 5.46; N, 14.08.

5.50. Molecular modeling

All calculations were performed using the MACROMODEL V7.2 suite[41] of software programs running on 1400 MHz Athlon PC's under the Linux Mandrake 8.0 operating system. Conformational searches using the mixed Monte Carlo[49] Low Mode[50,51] method were performed after initial minimization of nucleosides using the all-atom AMBER force field[39] and GB/SA model.[40] Non-bonded interactions were treated without cut-off's. Throughout conformational searches, the generated structures were first minimized with the truncated Newton conjugate gradient method till a convergence criterion of 0.005 kJ/(Å mol) was reached and then refined using a full matrix Newton Raphson method using the same convergence criterion. Searches were regarded as complete when all conformations within 15 kJ/mol of the global energy minimum were found at least 10 times. Unique conformations were determined by superimposition of all heavy (nonhydrogen) atoms and regarded as duplicate if the interatomic distance in RMS superimpositions was below 0.25 Å.

5.51. Crystallography methods

Reflection intensities of **8** and **9** were collected on a Siemens/Bruker SMART 1K CCD diffractometer with graphite monochromated Mo K_α radiation, $\lambda = 0.71069$ Å. Reflection intensities of **12** were collected on a Bruker–Nonius X8APEX-II CCD diffractometer. Data collection, integration of frame data and conversion to intensities were performed using the programs SMART, SAINT, and SADABS.[52,53] Structure solution, refinement and analysis, and production of crystallographic illustrations were carried out using the programs SIR97,[54] SHELXL,[55] and X-SEED.[56] In no case could the absolute configuration be established from the X-ray analysis.

5.52. Crystallographic data of 8

$C_{12}H_{14}N_2O_7$, $M = 298.25$, monoclinic, $a = 6.4587(8)$ Å, $b = 6.4659(8)$ Å, $c = 14.7700(17)$ Å, $\beta = 97.401(2)°$, $V = 611.68(13)$ Å^3, $P2_1$ (no. 4), $Z = 2$, $D_x = 1.619$ g cm^{-3}, $F(000) = 312$, $\mu = 0.135$ mm^{-1}, $T = 120$ K. 7928 reflections were measured to $\theta_{max} = 29.99°$ and were merged ($R_{int} = 0.0325$) to 3090 unique reflections (including Friedel equivalents). The refinement using 202 parameters converged at $R_1 = 0.0365$ (for $F_o > 4\sigma(F_o)$) and $wR_2 = 0.0801$ (for all data).

5.53. Crystallographic data of 9

$C_{12}H_{15}N_3O_6$, $M = 297.27$, tetragonal, $a = 7.8839(14)$ Å, $c = 40.106(6)$ Å, $V = 2492.8(7)$ Å^3, $P4_12_12$ (no. 92), $Z = 8$, $D_x = 1.584$ g cm^{-3}, $F(000) = 1248$, $\mu = 0.129$ mm^{-1}, $T = 180$ K. 6412 reflections were measured to $\theta_{max} = 24.70°$ and were merged ($R_{int} = 0.0704$) to 2044 unique reflections (including Friedel equivalents). The refinement using 208 parameters converged at $R_1 = 0.0477$ (for $F_o > 4\sigma(F_o)$) and $wR_2 = 0.0978$ (for all data).

5.54. Crystallographic data of 12

$C_{12}H_{14}N_2O_6$, $M = 282.25$, monoclinic, $a = 8.1725(8)$ Å, $b = 5.5898(6)$ Å, $c = 13.5508(14)$ Å, $\beta = 91.749(4)$, $V = 618.75(11)$ Å^3, $P2_1$ (no. 4), $Z = 2$, $D_x = 1.515$ g cm^{-3}, $F(000) = 296$, $\mu = 0.123$ mm^{-1}, $T = 180$ K. 8924 reflections were measured to $\theta_{max} = 29.12°$ and were merged ($R_{int} = 0.0264$) to 2777 unique reflections (including

Friedel equivalents). The refinement using 192 parameters converged at $R_1 = 0.0302$ (for $F_o > 4\sigma(F_o)$) and $wR_2 = 0.0808$ (for all data).

5.55. Biological assays

Target nucleosides were evaluated for antiviral activity against HIV-1 in MT-4 cells and anticancer activity against human adenocarcinoma breast cancer (MCF-7) and prostate cancer (PC-3) cell lines as previously described.[43,45]

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Supplementary data

^{1}H NMR spectra of **25**, **31**, and **39** and ^{13}C NMR spectra of **5**–**7**, **11**–**12**, **14**, **17**, **24**, **26**, **32**–**33**, **47**, **54**–**55**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.01.029.

Crystallographic data (excluding structure factors) for **8**, **9** and **12** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 261822–261824. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, (fax: +44-(0)1223-336033 or email: deposit@ccdc.cam.uk).

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