DOI: 10.1002/ejoc.200801285

A Novel Galactosyltransferase Inhibitor with Diamino Sugar as a Pyrophosphate Mimic

Nobuyuki Mitsuhashi[a] and Hideya Yuasa*[a]

Keywords: Bioorganic chemistry / Inhibitors / Glycoconjugates / Enzymes / Chelation

Conjugates of 2,4-diamino sugars and uridine have been synthesized as galactosyltransferase inhibitors. The relationship between inhibitory activity and the chelation abilility of the hinge-like diamino sugar towards a metal ion was studied by NMR spectroscopy. One of the conjugates exhibited a moderate inhibition that ranks between those of UDP and UMP probably through the chelation of the hinge-like diamino sugar to Mn^{II}.

Introduction

The biosyntheses of native oligosaccharides are catalyzed by glycosyltransferases (GTs),[1] which transfer a monosaccharide from a sugar nucleotide to the non-reducing end of a precursory oligosaccharide (Figure 1). Because the biosyntheses of cell walls or crustaceous shell polysaccharides are vital for bacteria and insects, the inhibitors of GTs are potential antibiotics or insecticides.[2] These inhibitors can also be used in the functional studies of the oligosaccharides of glycoproteins or glycolipids. Thus, a number of potential GT inhibitors have been designed and synthesized[3] that in most cases comprise a pyrophosphate derivative and a nucleoside as the essential components, for example, compound **1** (Figure 2).[4] The pyrophosphate group seems indispensable for inhibitory activity because it acts as a strong chelator to the Mn^{II} ion bound within GTs. However, the pyrophosphate group is labile[5] and thus there have been efforts to create stable pyrophosphate mimics. In the simplest case, an alkyl chain was substituted (compound **2**), but this exhibited no inhibitory activity.[6] The use of either malonate or tartrate groups as pyrophosphate mimics has also been unsuccessful.[7] Interestingly, glucose can substitute for the pyrophosphate moiety of uridine diphosphate galactose (UDPGal) (compound **3**), leaving the binding ability of the enzyme galactosyltransferase (GalT) almost the same as the native substrate.[7] However, other efforts to produce a GalT inhibitor by using a sugar to mimic pyrophosphate have failed.[8]

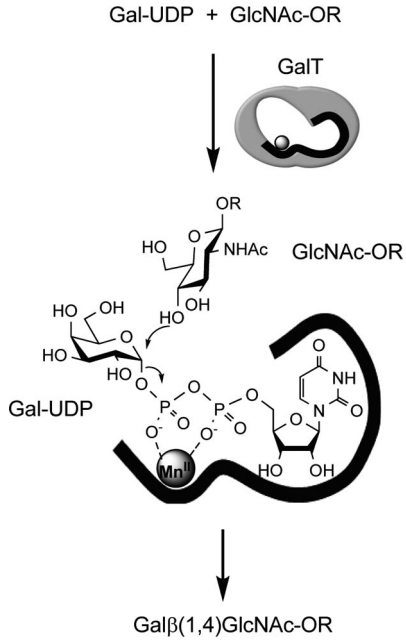
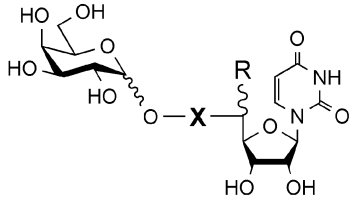


Figure 1. A proposed mechanism for the galactosyl transfer reaction catalyzed by galactosyltransferase (GalT).

We have synthesized 2,4-diaminopentopyranosides (**4**) as hinge molecules that change conformation from $^4C_1$ to $^1C_4$, like a hinge, upon the chelation of metal ions (Table 1).[9] We envisaged that these compounds would serve as pyrophosphate mimics because they can chelate Mn^{II} through the hinge motion[10] and the pyranose structure would be accommodated into the pyrophosphate cleft of GTs, as noted with compound **3**. The hinge-like motion potentially endows the inhibitors with induced-fit abilities. In addition, two different functional groups can be attached to the hinge sugar in a step-by-step manner through a glycosidation reaction at C1 and alkylation at 3-OH. These attractive properties of the hinge molecule prompted us to study the inhi-

[a] Department of Life Sciences, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology,
J2-10, 4259 Nagatsutacho, Midoriku, Yokohama 226-8501, Japan
Fax: +81-45-924-5850
E-mail: hyuasa@bio.titech.ac.jp
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

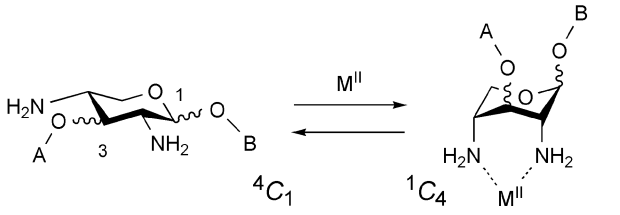


1: X = α-PO(OH)CH_2PO(OH)O-, R = H
2: X = α-$(CH_2)_4$-, R = OH
3: X = β-4-O-Glcβ-1-O-, R = H

Figure 2. Selected examples of synthetic galactosyltransferase (GalT) inhibitors.

bition of GalT activity by four hinge sugar–uridine (U) conjugates **5**–**8**, characterized by single-component-installed hinges for a starting structure and variations of different linkages and stereochemistries at C1 or C3 of the hinge molecules (Table 1). We herein report the synthesis of **5**–**8** and the relationship between the inhibition activities and chelation abilities of these compounds.

Table 1. The structures of reported (**4**) and targeted (**5**–**8**) 2,4-diaminopentopyranosides that change conformation on addition of a metal ion (M^{II}).

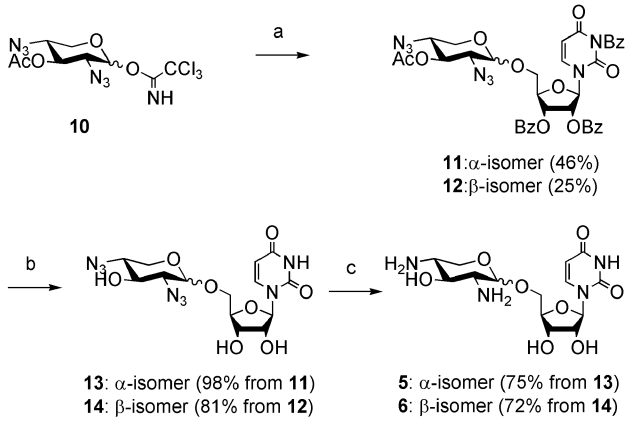


	C1[a]	C3[a]	A	B
4	E→A	E→A	H or sugars	Me or sugars
5	A→E	E→A	H	U
6	E→A	E→A	H	U
7	E→A	E→A	–CH_2-U	Me
8	E→A	A→E	–CH_2-U	Me

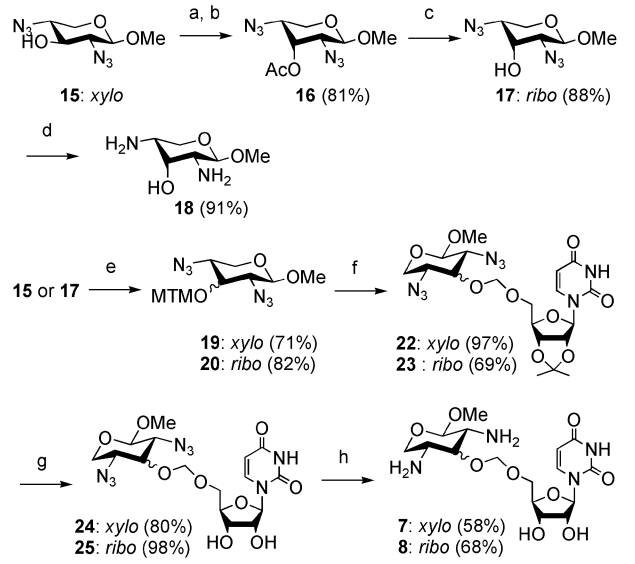
[a] The orientational change of a substituent at C1 or C3 accompanied by the $^4C_1$-to-$^1C_4$ flip. E→A represents the change from an equatorial to an axial orientation.

Results and Discussion

The C1-linked conjugates **5** and **6** were synthesized by glycosylation at 5-OH of 3-*N*-2′,3′-tri-*O*-benzoyl-U[11] (**9**; Scheme 1). The 1-*O*-trichloroacetimidate derivative **10**[9] was used as the glycosyl donor to afford α- and β-glycosides **11** and **12**. Deacylation of **11** and **12** gave compounds **13** and **14**, and then reduction of the azide functionality gave the target compounds **5** and **6**. Methyl 2,4-diazido-β-ribopyranoside (**17**) was synthesized from methyl 2,4-diazido-β-xylopyranoside (**15**) by substitution at C3 to afford acetate **16**, followed by deacetylation at this site (Scheme 2). The azide functions of **17** were reduced to give methyl 2,4-diamino-β-ribopyranoside (**18**), which was used to test the chelation ability of a *ribo* derivative towards metal ions, as shown below. The methylene bridge was incorporated first as the methylthiomethyl (MTM) ether at 3-OH of the pentopyranosides **15** and **17** to give MTM ethers **19** and **20**. The coupling reactions of the MTM ethers **19** and **20** with 2′,3′-*O*-isopropylidene-U (**21**) gave the conjugates **22** and **23**. Deprotection of **22** and **23** gave compounds **24** and **25**, and then reduction of the azide functions afforded the target compounds **7** and **8**.



Scheme 1. Reagents and conditions: a) 3-*N*-2′,3′-Tri-*O*-benzoyl-U (**9**), TMSOTf/CH_2Cl_2, –40 °C; b) 28% aq. NH_3; c) PPh_3, MeOH/THF/H_2O (7.5:6:1).



Scheme 2. Reagents and conditions: a) Tf_2O, 2,6-di-*tert*-butyl-4-methylpyridine/CH_2Cl_2; b) CsOAc/DMF; c) NaOMe/MeOH; d) PPh_3, MeOH/THF/H_2O (7.5:6:1); e) CH_3SCH_2Cl, NaH, Bu_4NI/DMF; f) 2′,3′-*O*-isopropylidene-U (**21**), MeOTf/CH_2Cl_2; g) 80% AcOH, 70 °C; h) PPh_3/28% aq. NH_3/pyridine.

We studied the conformational changes that compounds **5**, **6**, **18**, **7** and **8** underwent upon addition of a metal ion by ^1H NMR spectroscopy. Unfortunately, we were unable to use Mn^{II} because it caused precipitation and its paramagnetism caused fatal signal broadening. We used Zn^{II} instead because the compounds of our study are expected

to show chelation abilities towards Zn^{II} that exceed but parallel those towards Mn^{II}, as demonstrated with a hinge-sugar-based metal-ion sensor.[10]

The effect of the stereochemistry at C1 on the chelation ability was studied with α- and β-*xylo*-1-U **5** and **6**. These pyranosides stayed almost perfectly in the $^4C_1$ conformations (**5**: 100%; **6**: 96%) in solution (Figure 3, A and B).[12] Both anomers underwent slow conformational exchange upon the addition of 2.0 equiv. of Zn^{II} at 30 °C, as indicated by broadened signals. Only the spectrum of β-*xylo*-1-U **6** exhibited sharp signals at 70 °C, with a $^4C_1/^1C_4$ ratio of 61:39 (Figure 3, D), whereas signal-broadening was observed with α-*xylo*-1-U **5** (Figure 3, C). These results suggest that the conformational exchange of α-*xylo*-1-U **5** is considerably slower than that of β-*xylo*-1-U **6**, which indicates that β-*xylo*-1-U **6** is much more flexible and has a better chelating ability than α-*xylo*-1-U **5**.

Compound **18** showed a perfect interchange from $^4C_1$ to $^1C_4$ upon addition of 2.0 equiv. of Zn^{II}, whereas in previous work with the C3 epimer, methyl 2,4-diamino-β-xylopyranoside, partial conformational exchange was observed with $^4C_1/^1C_4$ of around 67:33. These results indicate that the ribopyranoside **18** has greater conformational flexibility and thus superior chelation ability than the xylopyranoside. The extraordinary stability of the $^1C_4$ conformation of **18** is likely due to the anchoring effect of the equatorial 3-OH.

Similar differences in chelation abilities were observed between *xylo*-3-U **7** and *ribo*-3-U **8**, which adopted favourable $^4C_1$ conformations with $^4C_1/^1C_4$ ratios of around 90:10 and 66:34, respectively, in the absence of metal ions (Figure 4, A and B). The addition of 2.0 equiv. of Zn^{II} caused signal-broadening for **7** at 30 °C, which indicates that the pyranoside assumes a slow conformational exchange between the $^4C_1$ and $^1C_4$ structures (Figure 4, C). Upon elevating the temperature to 70 °C, the signals became sharper and the conformational ratio $^4C_1/^1C_4$ changed to 5:95. This high $^1C_4$ population (95%), in comparison with values of 33 and 37% for the corresponding 3-*O*-unsubstituted and 3-*O*-galactosyl derivatives, respectively, was surprising.[9] On the other hand, Zn^{II} addition to **8** also caused drastic changes in the ^1H NMR spectrum but the signals remained unbroadened even at 30 °C and the *J* values indicated 100% $^1C_4$ population (Figure 4, D). In short, the most significant conformational difference between **7** and **8** in the presence of Zn^{II} was observed at 30 °C, which suggests that *ribo*-3-U **8** has kinetically better chelating ability than *xylo*-3-U **7**.

After establishing the relative chelating abilities of the two series of diamino sugar-U conjugates **5**–**8**, we examined the inhibitory activities of these compounds (1 mM) against GalT in comparison with UDP and uridine monophosphate (UMP), known inhibitors of GalT with reported K_i values of 32 and 450 μM, respectively.[13] Umbelliferyl *N*-acetyl-β-D-glucosamine (0.1 mM) and UDPGal (0.1 mM) were used as the glycosyl acceptor and donor. The β-*xylo*-1-U conjugate **6** exhibited a moderate inhibition (45%), which ranks between those of UDP (85%) and UMP (22%), whereas the corresponding α-glycoside **5** showed less inhibition (18%) than UMP. On the other hand, the *ribo*-3-U conjugate **8** barely showed inhibition (10%), whereas the corresponding *xylo* derivative **7** exhibited no inhibition. The trend in the inhibition activities of these compounds, that is, β-*xylo*-1-U **6** > α-*xylo*-1-U **5** and *ribo*-3-U **8** > *xylo*-

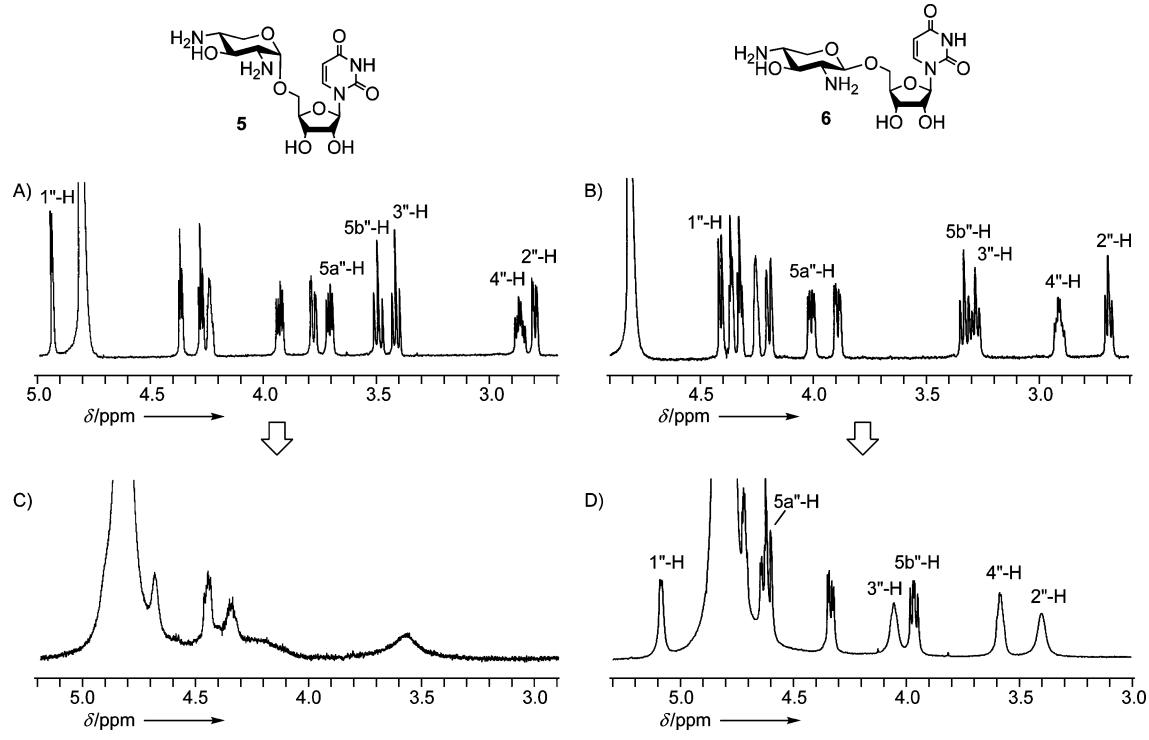


Figure 3. ^1H NMR spectra for compounds **5** and **6** in the absence of metal ions (A and B) and in the presence of 2.0 equiv. of Zn^{II} at 70 °C (C and D).

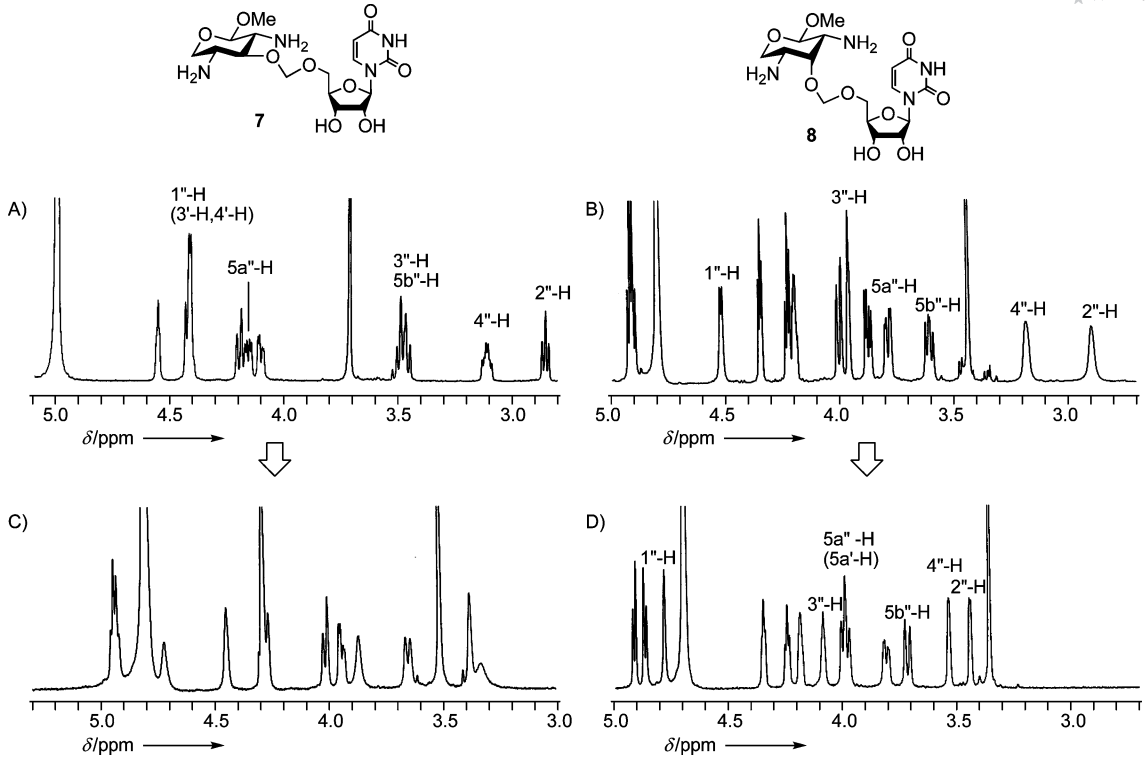


Figure 4. ^{1}H NMR spectra for compounds **7** and **8** in the absence of metal ions (A and B) and in the presence of 2.0 equiv. of Zn^{II} at 30 °C (C and D).

3-U **7**, parallel that of the conformational flexibility upon chelation to Zn^{II}. Therefore, strong chelation towards Mn^{II}, associated with the conformational flexibility of the diamino sugars, might be essential for the inhibitory activity of these pyrophosphate mimics (Figure 5). Further study is required to corroborate the relationship between the inhibition activities and chelation abilities. The poor inhibition results of the conjugate **8** probably originate from the methylene spacer, which restricts the chelation to Mn^{II} (Figure 5, B).

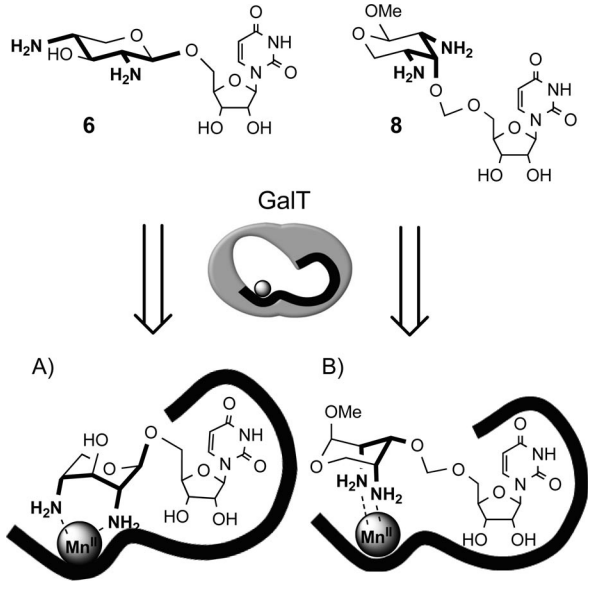


Figure 5. Proposed mechanisms for GalT inhibition for compounds **6** (A) and **8** (B).

Conclusions

We have developed 2,4-diamino sugars as pyrophosphate mimics for GalT inhibitors that are stable in their own right and retain their ability to bind with the enzyme. The inhibition activities appear to depend on conformational flexibility and thus the chelation abilities of the hinge-like molecules to bite Mn^{II} within the structure of GalT. Chelation would necessarily induce orientational switching of the unjoined oxygen atom, O3 for β-*xylo*-1-U **6** or O1 for *ribo*-3-U **8**, awaiting attachment of a glycosyl donor or acceptor unit. When the third component is attached, these compounds may become potential motion-driven inhibitors with induced-fit inhibition enhancements. Further studies are necessary to prove the relationship between the chelation ability of the hinge sugar part of the inhibitors and the inhibition activities.

Experimental Section

General: All solvents and reagents used were reagent grade and, for cases in which further purification was required, standard procedures[14] were followed. Solution transfers for which anhydrous conditions were required were performed under dry argon using syringes. Thin-layer chromatography (TLC) was performed on pre-

coated silica gel Merck 60-F254 plates (Art 5715) and visualized by the quenching of fluorescence and/or by charring after spraying with 1% $CeSO_4$/1.5% $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$/10% H_2SO_4. Column chromatography was performed on Merck Kieselgel 60 (Art 7734), Wako gel C-300 or Kanto Silica gel 60N (spherical, neutral) with the solvent systems specified. Optical rotations were determined with a Horiba SEPA-200 using a 1 dm length cell. ^{1}H NMR spectra were recorded at 600 (Bruker AV-600), 400 (Varian Unity-400) or 270 MHz (JEOL EX-270). Internal tetramethylsilane (δ = 0 ppm) was used as a standard in $CDCl_3$ and solvent peaks were used as standards in [D_6]DMSO (δ = 2.5 ppm) and CD_3OD (δ = 3.3 ppm). Chemical shifts are expressed in ppm referenced to the solvent as an internal standard. The multiplicity of the signals is abbreviated as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, ddd = doublet of doublets of doublets, br. = broad signal, m = multiplet. ^{13}C NMR spectra were recorded at 150 (Bruker AV600) or 67.8 MHz (JEOL JNM-EX-270) and a solvent peak (δ = 77.0 ppm in $CDCl_3$, δ = 49.0 ppm in CD_3OD, or δ = 39.52 ppm in [D_6]DMSO) or acetone (δ = 30.89 ppm in D_2O) was used as a standard. High-resolution mass spectra (HRMS) were recorded with a Mariner Biospectrometry Workstation ESI-TOF MS.

5′-*O*-(3-*O*-Acetyl-2,4-diazido-2,4-dideoxy-α,β-D-xylopyranosyl)-3-*N*-benzoyl-2′,3′-di-*O*-benzoyl-uridine (11 and 12): TMSOTf (1.4 μL, 0.008 mmol) was added to a stirred mixture of 3-*O*-acetyl-2,4-diazido-2,4-dideoxy-xylopyranosyl trichloroacetoimidate (**10**, 32.7 mg, 0.085 mmol), 3-*N*-benzoyl-2′,3′-*O*-dibenzoyluridine (**9**; 57.1 mg, 0.103 mmol) and 4 Å MS in CH_2Cl_2 (1 mL) at –60 °C. After 2 h, the temperature was elevated to –40 °C and the mixture was further stirred. After 3 h from the start of the reaction, triethylamine (8 μL) was added and the mixture was filtered through Celite and the solvents evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc, 3:1–2:1–1:1) to give **11** (30.4 mg, 46%) and **12** (16.3 mg, 25%) each as a syrup.

11: R_f = 0.09 (hexane/EtOAc, 2:1). $[\alpha]_D^{25}$ = –2.7 (c = 1.00, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.00–7.32 (m, 16 H, Ph×3, 6-H), 6.48 (d, $^3J_{H,H}$ = 6.7 Hz, 1 H, 1′-H), 5.96 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, 5-H), 5.76 (dd, $^3J_{H,H}$ = 5.8, $^3J_{H,H}$ = 3.3 Hz, 1 H, 3′-H), 5.63 (dd, $^3J_{H,H}$ = 6.7, $^3J_{H,H}$ = 5.8 Hz, 1 H, 2′-H), 5.49–5.46 (m, 1 H, 3′′-H), 5.03 (d, $^3J_{H,H}$ = 3.5 Hz, 1 H, 1′′-H), 4.52 (m, 1 H, 4′-H), 4.15 (dd, $^3J_{H,H}$ = 2.6, $^2J_{H,H}$ = 11.0 Hz, 1 H, 5a′-H), 3.88–3.84 (m, 2 H, 5b′-H, 5a′′-H), 3.73–3.70 (m, 2 H, 4′′-H, 5b′′-H), 3.64 (dd, $^3J_{H,H}$ = 3.5, $^3J_{H,H}$ = 10.3 Hz, 1 H, 2′′-H), 2.20 (s, 3 H, Ac) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 169.6, 168.3, 165.4, 165.1, 161.7, 149.5, 140.0, 135.0, 133.8, 133.7, 131.2, 130.5, 129.9, 129.7, 129.7, 129.1, 128.6, 128.5, 128.5, 128.4, 128.3, 103.6, 97.8, 86.5, 81.6, 73.5, 71.4, 71.3, 67.5, 62.1, 60.3, 59.3, 20.7 ppm. HRMS (ESI): calcd. for $C_{37}H_{32}N_8O_{12}Na$ [M + Na]$^+$ 803.2038; found 803.2018.

12: R_f = 0.18 (hexane/EtOAc, 2:1). $[\alpha]_D^{25}$ = –71.2 (c = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.03–7.27 (m, 16 H, Ph×3, 6-H), 6.45 (d, $^3J_{H,H}$ = 6.9 Hz, 1 H, 1′-H), 5.94 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, 5-H), 5.82 (dd, $^3J_{H,H}$ = 5.6, $^3J_{H,H}$ = 2.3 Hz, 1 H, 3′-H), 5.71 (dd, $^3J_{H,H}$ = 6.9, $^3J_{H,H}$ = 5.6 Hz, 1 H, 2′-H), 5.03 (t, $^3J_{H,H}$ = 10.1 Hz, 1 H, 3′′-H), 4.58 (m, 1 H, 4′-H), 4.48 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 1′′-H), 4.32 (dd, $^3J_{H,H}$ = 2.0, $^2J_{H,H}$ = 10.8 Hz, 1 H, 5a′-H), 4.15–4.08 (m, 2 H, 5b′-H, 5a′′-H), 3.71–3.65 (m, 1 H, 4′′-H), 3.50 (dd, $^3J_{H,H}$ = 8.1, $^3J_{H,H}$ = 10.1 Hz, 1 H, 2′′-H), 3.30 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.5 Hz, 1 H, 5b′′-H), 2.22 (s, 3 H, Ac) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 169.6, 168.3, 165.4, 165.4, 161.7, 149.4, 139.4, 135.0, 133.8, 133.7, 131.2, 130.4, 129.8, 129.7, 129.0, 128.6, 128.6, 128.4, 128.2, 103.0, 102.4, 86.8, 82.1, 73.9, 72.7, 72.2, 69.4, 64.2, 64.0, 59.2, 20.7 ppm. HRMS (ESI): calcd. for $C_{37}H_{32}N_8NaO_{12}$ [M + Na]$^+$ 803.2038; found 803.2063.

5′-*O*-(2,4-Diazido-2,4-dideoxy-α-D-xylopyranosyl)uridine (13): A 28% NH_3 (1 mL) solution was added to a stirred solution of **11** (76.0 mg, 0.097 mmol) in MeOH (1 mL) at room temp. After 24 h, the solution was evaporated and the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH, 8:1) to give **13** (40.6 mg, 98%) as a foam. R_f = 0.20 ($CHCl_3$/MeOH, 8:1). $[\alpha]_D^{25}$ = 104.5 (c = 1.00, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 8.14 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 5.92 (d, $^3J_{H,H}$ = 4.6 Hz, 1 H, 1′-H), 5.73 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 4.94 (d, $^3J_{H,H}$ = 3.5 Hz, 1 H, 1′′-H), 4.16–4.15 (m, 1 H, 4′-H), 4.13 (t, $^3J_{H,H}$ = 4.6 Hz, 1 H, 3′-H), 4.11 (t, $^3J_{H,H}$ = 4.6 Hz, 1 H, 2′-H), 3.89 (dd, $^3J_{H,H}$ = 2.4, $^2J_{H,H}$ = 11.2 Hz, 1 H, 5a′-H), 3.74 (t, $^3J_{H,H}$ = 9.7 Hz, 1 H, 3′′-H), 3.73–3.69 (m, 2 H, 5a′′-H, 5b′-H), 3.59–3.55 (m, 2 H, 4′′-H, 2′′-H), 3.37 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.3 Hz, 1 H, 5b′′-H) ppm. ^{13}C NMR (67.8 MHz, CD_3OD): δ = 166.3, 152.4, 142.9, 102.7, 98.9, 90.3, 84.0, 75.9, 73.6, 71.0, 67.6, 65.7, 63.4, 61.2 ppm. HRMS (ESI): calcd. for $C_{14}H_{19}N_8O_8$ [M + H]$^+$ 427.1326; found 427.1335.

5′-*O*-(2,4-Diazido-2,4-dideoxy-β-D-xylopyranosyl)uridine (14): A 28% NH_3 (1 mL) solution was added to a stirred solution of **12** (52.4 mg, 0.067 mmol) in MeOH (1 mL) at room temp. After 24 h, the solution was evaporated and the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH, 8:1) to give **14** (23.2 mg, 81%) as a foam. R_f = 0.20 ($CHCl_3$/MeOH, 8:1). $[\alpha]_D^{25}$ = –24.0 (c = 0.68, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 7.97 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 5.90 (d, $^3J_{H,H}$ = 4.6 Hz, 1 H, 1′-H), 5.75 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 4.35 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 1′′-H), 4.23 (t, $^3J_{H,H}$ = 4.7 Hz, 1 H, 3′-H), 4.17–4.14 (m, 3 H, 2′-H, 4′-H, 5a′-H), 3.98 (dd, $^3J_{H,H}$ = 5.4, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5a′′-H), 3.81 (dd, $^3J_{H,H}$ = 2.2, $^2J_{H,H}$ = 10.6 Hz, 1 H, 5b′-H), 3.57–3.53 (m, 1 H, 4′′-H), 3.43 (t, $^3J_{H,H}$ = 9.5 Hz, 1 H, 3′′-H), 3.26 (dd, $^3J_{H,H}$ = 8.1, $^3J_{H,H}$ = 9.5 Hz, 1 H, 2′′-H), 3.17 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.3 Hz, 1 H, 5b′′-H) ppm. ^{13}C NMR (67.8 MHz, CD_3OD): δ = 166.3, 152.4, 142.4, 103.6, 102.6, 90.6, 84.5, 75.9, 75.6, 71.3, 69.8, 68.3, 65.1, 62.9 ppm. HRMS (ESI): calcd. for $C_{14}H_{19}N_8O_8$ [M + H]$^+$ 427.1326; found 427.1315.

5′-*O*-(2,4-Diamino-2,4-dideoxy-α-D-xylopyranosyl)uridine (5): Triphenylphosphane (125 mg, 0.477 mmol) was added to a stirred solution of **13** (40.6 mg, 0.095 mmol) in MeOH/THF/H_2O (7.5:6:1, 1 mL) at room temp. After 9 h, the solution was evaporated and partitioned between water and $CHCl_3$. The water layer was evaporated and the residue was purified by chromatography on silica gel (Kanto Co., 60N spherical gel, *i*PrOH/H_2O/28%NH_3, 35:3:1) to give **5** (26.9 mg, 75%) as a white solid. R_f = 0.32 (*i*PrOH/H_2O/28%NH_3, 10:3:1); m.p. 156–158 °C. $[\alpha]_D^{25}$ = 70.2 (c = 0.56, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 7.70 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 6-H), 5.85 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 5-H), 5.84 (d, $^3J_{H,H}$ = 5.0 Hz, 1 H, 1′-H), 4.94 (d, $^3J_{H,H}$ = 3.5 Hz, 1 H, 1′′-H), 4.37 (t, $^3J_{H,H}$ = 5.0 Hz, 1 H, 2′-H), 4.28 (t, $^3J_{H,H}$ = 5.0 Hz, 1 H, 3′-H), 4.25–4.23 (m, 1 H, 4′-H), 3.93 (dd, $^3J_{H,H}$ = 5.3, $^2J_{H,H}$ = 11.4 Hz, 1 H, 5a′-H), 3.78 (dd, $^3J_{H,H}$ = 2.4, $^2J_{H,H}$ = 11.4 Hz, 1 H, 5b′-H), 3.71 (dd, $^3J_{H,H}$ = 5.2, $^2J_{H,H}$ = 11.3 Hz, 1 H, 5a′′-H), 3.50 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.3 Hz, 1 H, 5b′′-H), 3.42 (t, $^3J_{H,H}$ = 9.9 Hz, 1 H, 3′′-H), 2.89–2.84 (m, 1 H, 4′′-H), 2.80 (dd, $^3J_{H,H}$ = 3.5, $^3J_{H,H}$ = 9.9 Hz, 1 H, 2′′-H) ppm. ^{13}C NMR (67.8 MHz, D_2O): δ = 168.4, 153.3, 142.5, 103.0, 99.8, 91.1, 82.6, 74.1, 74.0, 70.0, 67.4, 62.7, 55.7, 52.7 ppm. HRMS (ESI): calcd. for $C_{14}H_{23}N_4O_8$ [M + H]$^+$ 375.1517; found 375.1528.

5′-*O*-(2,4-Diamino-2,4-dideoxy-β-D-xylopyranosyl)uridine (6): Triphenylphosphane (71 mg, 0.271 mmol) was added to a stirred solution of **14** (23.2 mg, 0.054 mmol) in MeOH/THF/H_2O (7.5:6:1,

1 mL) at room temp. After 9 h, the solution was evaporated and partitioned between water and $CHCl_3$. The water layer was evaporated and the residue was purified by chromatography on silica gel (Kanto Co., 60N spherical gel, *i*PrOH/H_2O/28%NH_3, 35:3:1) to give **6** (14.7 mg, 72%) as a white solid. R_f = 0.28 (*i*PrOH/H_2O/28%NH_3, 10:3:1); m.p. 153–155 °C. $[\alpha]_D^{25}$ = –19.2 (*c* = 0.172, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 7.88 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, 6-H), 5.89 (d, $^3J_{H,H}$ = 4.8 Hz, 1 H, 1′-H), 5.87 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, 5-H), 4.41 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 1′′-H), 4.36 (t, $^3J_{H,H}$ = 4.8 Hz, 1 H, 2′-H), 4.32 (t, $^3J_{H,H}$ = 4.8 Hz, 1 H, 3′-H), 4.25 (m, 1 H, 4′-H), 4.19 (d, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5a′-H), 4.01 (dd, $^3J_{H,H}$ = 5.0, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5a′′-H), 3.89 (dd, $^3J_{H,H}$ = 4.1, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5b′-H), 3.32 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.4 Hz, 1 H, 5b′′-H), 3.28 (t, $^3J_{H,H}$ = 9.6 Hz, 1 H, 3′′-H), 2.91 (dt, $^3J_{H,H}$ = 5.0, $^3J_{H,H}$ = 10.1 Hz, 1 H, 4′′-H), 2.68 (t, $^3J_{H,H}$ = 8.9 Hz, 1 H, 2′′-H) ppm. ^{13}C NMR (67.8 MHz, D_2O): δ = 167.6, 152.8, 142.6, 104.3, 102.7, 90.4, 83.2, 75.9, 74.3, 70.0, 69.2, 66.4, 57.5, 52.6 ppm. HRMS (ESI): calcd. for $C_{14}H_{23}N_4O_8$ $[M + H]^+$ 375.1517; found 375.1518.

Compound 6 (16 mM) + 2.0 Equiv. Zn(OAc)$_2$: ^{1}H NMR (600 MHz, 50 mM [D_3]AcONa buffer, pH 7.0, 70 °C): δ = 8.26 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 6.34 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 6.34 (d, $^3J_{H,H}$ = 5.1 Hz, 1 H, 1′-H), 5.08 (d, $^3J_{H,H}$ = 5.0 Hz, 1 H, 1′′-H), 4.75 (t, $^3J_{H,H}$ = 5.5 Hz, 1 H, 3′-H), 4.72–4.67 (m, 1 H, 4′-H), 4.63 (dd, $^3J_{H,H}$ = 4.0, $^2J_{H,H}$ = 12.2 Hz, 1 H, 5a′′-H), 4.60 (dd, $^3J_{H,H}$ = 2.6, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5a′-H), 4.33 (dd, $^3J_{H,H}$ = 4.6, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5b′-H), 4.05 (br. t, $^3J_{H,H}$ = 7.0 Hz, 1 H, 3′′-H), 3.96 (dd, $^3J_{H,H}$ = 8.0, $^2J_{H,H}$ = 12.2 Hz, 1 H, 5b′′-H), 3.58 (br., 1 H, 4′′-H), 3.40 (br., 1 H, 2′′-H), 2.38 (s, 12 H, Ac) ppm.

Methyl 3-*O*-Acetyl-2,4-diazido-2,4-dideoxy-β-D-ribopyranoside (16): 2,6-Di-*tert*-butyl-4-methylpyridine (83 mg, 0.402 mmol) and Tf_2O (68 μL, 0.402 mmol) were added to a stirred solution of methyl 2,4-diazido-2,4-dideoxy-β-D-ribopyranoside (**15**; 28.7 mg, 0.134 mmol) in CH_2Cl_2 (1 mL) at room temp. After 1 h, the solution was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried with $MgSO_4$, filtered and the solvents evaporated. The residue was dissolved in DMF (1 mL) and CsOAc (38.5 mg, 0.201 mmol) was added. After stirring for 2 h at room temp., the mixture was evaporated and the residue was purified by chromatography on silica gel (hexane/EtOAc, 8:1–3:1) to give **16** (27.8 mg, 81%) as crystals. R_f = 0.43 (hexane/EtOAc, 3:1); m.p. 57–58 °C. $[\alpha]_D^{28}$ = –81.3 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.49 (t, $^3J_{H,H}$ = 3.4 Hz, 1 H, 3-H), 4.64 (d, $^3J_{H,H}$ = 4.6 Hz, 1 H, 1-H), 3.93 (dd, $^3J_{H,H}$ = 2.7, $^2J_{H,H}$ = 11.3 Hz, 1 H, 5a-H), 3.78 (m, 1 H, 4-H), 3.74 (dd, $^3J_{H,H}$ = 6.1, $^2J_{H,H}$ = 11.3 Hz, 1 H, 5b-H), 3.67 (dd, $^3J_{H,H}$ = 4.6, $^3J_{H,H}$ = 3.4 Hz, 1 H, 2-H), 3.48 (s, 3 H, OMe), 2.19 (s, 3 H, Ac) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 169.5 (CH_3C=O), 100.1 (C-1), 68.8 (C-3), 61.1 (C-5), 59.7 (C-2), 56.4 (C-4), 56.0 (OMe), 20.5 (CH_3C=O) ppm. HRMS (ESI): calcd. for $C_8H_{12}N_6O_4Na$ $[M + Na]^+$ 279.0818; found 279.0841.

Methyl 2,4-Diazido-2,4-dideoxy-β-D-ribopyranoside (17): A 0.1 M solution of NaOMe (0.12 mL) was added to a stirred solution of **16** (30 mg, 0.12 mmol) in methanol (0.5 mL) . After 30 min, the solution was neutralized through Dowex50(H^+) and the solvents evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc, 3:1) to give **17** (22.0 mg, 88%) as a white solid. R_f = 0.29 (hexane/EtOAc, 3:1); m.p. 71–72 °C. $[\alpha]_D^{28}$ = –69.5 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.70 (d, $^3J_{H,H}$ = 6.4 Hz, 1 H, 1-H), 4.22 (t, $^3J_{H,H}$ = 3.1 Hz, 1 H, 3-H), 3.91 (dd, $^3J_{H,H}$ = 5.0, $^2J_{H,H}$ = 11.8 Hz, 1 H, 5a-H), 3.87 (dd, $^3J_{H,H}$ = 8.0, $^2J_{H,H}$ = 11.8 Hz, 1 H, 5b-H), 3.56–3.52 (m, 1 H, 4-H), 3.53 (s, 3 H, OMe), 3.43 (dd, $^3J_{H,H}$ = 6.4, $^3J_{H,H}$ = 3.1 Hz, 1 H, 2-H), 2.74 (br., 1 H, OH) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 100.3 (C-1), 68.0 (C-3), 62.5 (C-2), 60.9 (C-5), 58.0 (C-4), 56.6 (OMe) ppm. HRMS (ESI): calcd. for $C_6H_{10}N_6O_3Na$ $[M + Na]^+$ 237.0712; found 237.0725.

Methyl 2,4-Diamino-2,4-dideoxy-β-D-ribopyranoside (18): Triphenylphosphane (56.6 mg, 0.216 mmol) was added to a stirred solution of **17** (22.0 mg, 0.103 mmol) in MeOH/THF/H_2O (7.5:6:1, 0.5 mL). After 6 h, the solution was diluted with $CHCl_3$ and extracted with H_2O. The water layer was evaporated and the residue was purified by chromatography on silica gel (Kanto Co., 60N spherical gel, *i*PrOH/H_2O/28%NH_3, 35:3:1) to give **18** (42.4 mg, 91%) as a white solid. R_f = 0.22 (*i*PrOH/H_2O/28%NH_3, 35:3:1); m.p. 64–65 °C. $[\alpha]_D^{28}$ = –89.0 (*c* = 0.67, H_2O). ^{1}H NMR (400 MHz, D_2O): δ = 4.37 (d, $^3J_{H,H}$ = 8.4 Hz, 1 H, 1-H), 3.95 (t, $^3J_{H,H}$ = 2.9 Hz, 1 H, 3-H), 3.74 (dd, $^3J_{H,H}$ = 5.2, $^2J_{H,H}$ = 11.4 Hz, 1 H, 5a-H), 3.53 (s, 3 H, OMe), 3.50 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.0 Hz, 1 H, 5b-H), 2.96 (ddd, $^3J_{H,H}$ = 2.7, $^3J_{H,H}$ = 5.2, $^3J_{H,H}$ = 11.0 Hz, 1 H, 4-H), 2.57 (dd, $^3J_{H,H}$ = 8.4, $^3J_{H,H}$ = 3.0 Hz, 1 H, 2-H) ppm. ^{13}C NMR (67.8 MHz, D_2O): δ = 103.5 (C-1), 71.3 (C-3), 65.3 (C-5), 57.8 (OMe), 54.6 (C-2), 50.0 (C-4) ppm. HRMS (ESI): calcd. for $C_6H_{15}N_2O_3$ $[M + H]^+$ 163.1083; found 163.1081.

18 (16 mM) + 2.0 Equiv. Zn(OAc)$_2$: ^{1}H NMR (400 MHz, 50 mM [D_3]AcONa buffer, pH 7.0, 70 °C): δ = 5.34 (s, 1 H, 1-H), 4.65 (t, $^3J_{H,H}$ = 4.4 Hz, 1 H, 3-H), 4.55 (dd, $^3J_{H,H}$ = 1.7, $^2J_{H,H}$ = 13.0 Hz, 1 H, 5a-H), 4.25 (dd, $^3J_{H,H}$ = 2.0, $^2J_{H,H}$ = 13.0 Hz, 1 H, 5b-H), 3.95 (m, 4 H, 4-H, OMe), 3.89 (d, $^3J_{H,H}$ = 4.4 Hz, 1 H, 2-H), 2.43 (s, 12 H, Ac) ppm.

Methyl 2,4-Diazido-2,4-dideoxy-3-*O*-methylthiomethyl-β-D-xylopyranoside (19): Compound **15** (94.7 mg, 0.442 mmol) and then methylthiomethyl chloride (55 μL) were added to a stirred mixture of 55% NaH (118.6 mg, 2.72 mmol), prewashed three times with hexane, in THF (5 mL). After 9 h, methanol was added and the mixture was evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc, 12:1) to give **19** (85.5 mg, 71%) as a syrup. R_f = 0.55 (hexane/EtOAc, 4:1). $[\alpha]_D^{25}$ = –26.2 (c = 0.508, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$): δ = 4.95 (m, 2 H, SCH_2O), 4.13 (d, $^3J_{H,H}$ = 7.7 Hz, 1 H, 1-H), 4.03 (dd, $^3J_{H,H}$ = 5.1, $^2J_{H,H}$ = 11.9 Hz, 1 H, 5a-H), 3.56 (s, 3 H, OMe), 3.51 (m, 1 H, 4-H), 3.42 (t, $^3J_{H,H}$ = 9.4 Hz, 1 H, 3-H), 3.32 (dd, $^3J_{H,H}$ = 7.7, $^3J_{H,H}$ = 9.4 Hz, 1 H, 2-H), 3.17 (dd, $^3J_{H,H}$ = 10.6, $^2J_{H,H}$ = 11.9 Hz, 1 H, 5b-H), 2.27 (s, 3 H, MeS) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 103.8 (C-1), 77.7 (C-3), 76.6 (SCH_2O), 65.8 (C-2), 63.9 (C-5), 60.9 (C-4), 57.2 (OMe), 14.5 (MeS) ppm. HRMS (ESI): calcd. for $C_8H_{14}N_6NaO_3S$ $[M + Na]^+$ 297.0746; found 297.0744.

Methyl 2,4-Diazido-2,4-dideoxy-3-*O*-methylthiomethyl-β-D-ribopyranoside (20): Compound **17** (317.3 mg, 1.48 mmol) and then methylthiomethyl chloride (150 μL) and Bu_4NI (206.9 mg, 0.4 equiv.) were added to a stirred mixture of 55% NaH (821.1 mg, 18.8 mmol), prewashed three times with hexane, in DMF (10 mL). After 3.5 h, methanol and then $CHCl_3$ were added and the mixture was washed with H_2O. The organic layer was dried with $MgSO_4$ and the solvents evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc, 8:1) to give **20** (331.8 mg, 82%) as crystals. R_f = 0.42 (hexane/EtOAc, 3:1); m.p. 61–63 °C. $[\alpha]_D^{25}$ = –68.8 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.85 (s, 2 H, SCH_2O), 4.66 (d, $^3J_{H,H}$ = 3.6 Hz, 1 H, 1-H), 4.31 (t, $^3J_{H,H}$ = 3.6 Hz, 1 H, 3-H), 3.87 (dd, $^3J_{H,H}$ = 3.5, $^2J_{H,H}$ = 11.8 Hz, 1 H, 5a-H), 3.77 (dd, $^3J_{H,H}$ = 5.9, $^2J_{H,H}$ = 11.8 Hz, 1 H, 5b-H), 3.72–3.69 (m, 1 H, 4-H), 3.62 (t, $^3J_{H,H}$ = 3.6 Hz, 1 H, 2-H), 3.46 (s, 3 H, OMe), 2.25 (s, 3 H, CH_3S) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 100.3 (C-1), 75.1 (CH_2), 72.2 (C-3), 61.2 (C-5), 60.7 (C-2), 57.4 (C-4), 56.1 (OMe), 14.2 (SMe) ppm. HRMS

(ESI): calcd. for $C_8H_{14}N_6NaO_3S$ $[M + Na]^+$ 297.0746; found 297.0748.

2′,3′-*O*-Isopropylidene-5′-[(methyl 2,4-diazido-2,4-dideoxy-β-D-xylopyranosid-3-*O*-yl)methyl]uridine (22): MeOTf (62 μL, 0.546 mmol) was added to a stirred mixture of **19** (101.6 mg, 0.370 mmol), 2′,3′-*O*-isopropylideneuridine (**21**; 142.3 mg, 0.501 mmol) and 4 Å MS (0.8 g) in CH_2Cl_2 (4 mL) at room temp. After 18 h, further MeOTf (30 μL, 0.264 mmol) was added. After 39 h from the start of the reaction, triethylamine (80 μL) was added and the mixture was filtered through Celite and the solvents evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc, 3:1–1:1) to give **22** (184.2 mg, 97%) as a white solid. R_f = 0.19 (hexane/EtOAc, 1:1); m.p. 60–62 °C. $[\alpha]_D^{25}$ = –14.2 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.67 (br., 1 H, NH), 7.47 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 5.84 (d, $^3J_{H,H}$ = 2.3 Hz, 1 H, 1′-H), 5.72 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 4.99 (d, $^2J_{H,H}$ = 6.9 Hz, 1 H, OCH_2O), 4.92 (d, $^2J_{H,H}$ = 6.9 Hz, 1 H, OCH_2O), 4.89 (dd, $^3J_{H,H}$ = 6.3, $^3J_{H,H}$ = 3.7 Hz, 1 H, 3′-H), 4.85 (dd, $^3J_{H,H}$ = 2.3, $^3J_{H,H}$ = 6.3 Hz, 1 H, 2′-H), 4.40 (m, 1 H, 4′-H), 4.12 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H, 1′′-H), 4.04 (dd, $^3J_{H,H}$ = 5.4, $^2J_{H,H}$ = 11.6 Hz, 1 H, 5a′′-H), 3.96 (dd, $^3J_{H,H}$ = 3.2, $^2J_{H,H}$ = 10.7 Hz, 1 H, 5a′-H), 3.89 (dd, $^3J_{H,H}$ = 4.6, $^2J_{H,H}$ = 10.7 Hz, 1 H, 5b′-H), 3.57 (s, 3 H, OMe), 3.55–3.50 (m, 1 H, 4′′-H), 3.30 (dd, $^3J_{H,H}$ = 7.6, $^3J_{H,H}$ = 9.4 Hz, 1 H, 2′′-H), 3.25 (t, $^3J_{H,H}$ = 9.4 Hz, 1 H, 3′′-H), 3.16 (t, $^3J_{H,H}$ = 11.6 Hz, 1 H, 5b′′-H), 1.59 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 163.4, 150.0, 141.5, 114.4, 103.7, 102.2, 96.3, 93.2, 85.6, 84.7, 80.7, 78.2, 68.6, 65.5, 63.8, 61.0, 57.2, 27.1, 25.3 ppm. HRMS (ESI): calcd. for $C_{19}H_{26}N_8NaO_9$ $[M + Na]^+$ 533.1721; found 533.1758.

2′,3′-*O*-Isopropylidene-5′-[(methyl 2,4-diazido-2,4-dideoxy-β-D-ribopyranosid-3-*O*-yl)methyl]uridine (23): MeOTf (12.5 μL, 0.110 mmol) was added to a stirred mixture of **20** (28.3 mg, 0.1032 mmol), 2′,3′-*O*-isopropylideneuridine (**21**; 42.2 mg, 0.148 mmol) and 4 A MS (0.2 g) in CH_2Cl_2 (1 mL) at room temp. After 21 h, further MeOTf (12.5 μL, 0.110 mmol) was added. After 25 h from the start of the reaction, the reaction mixture was heated to reflux. After 5 h at reflux, triethylamine (30 μL) was added and the mixture was filtered through Celite and the solvents evaporated. The residue was purified by chromatography on silica gel (hexane/EtOAc, 3:1–1:1) to give **23** (36.4 mg, 69%) as crystals. R_f = 0.15 (hexane/EtOAc, 1:1); m.p. 149–150 °C. $[\alpha]_D^{25}$ = –41.4 (*c* = 0.432, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.43 (br., 1 H, NH), 7.40 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 5.77 (d, $^3J_{H,H}$ = 2.1 Hz, 1 H, 1′-H), 5.73 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 4.92–4.85 (m, 4 H, 2′-H, 3′-H, CH_2), 4.63 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H, 1′′-H), 4.38–4.34 (m, 1 H, 4′-H), 4.15 (t, $^3J_{H,H}$ = 3.3 Hz, 1 H, 3′′-H), 3.95 (dd, $^3J_{H,H}$ = 3.7, $^2J_{H,H}$ = 10.7 Hz, 1 H, 5a′-H), 3.91 (dd, $^3J_{H,H}$ = 5.2, $^2J_{H,H}$ = 10.7 Hz, 1 H, 5b′-H), 3.87 (dd, $^3J_{H,H}$ = 3.8, $^2J_{H,H}$ = 11.6 Hz, 1 H, 5a′′-H), 3.76 (dd, $^3J_{H,H}$ = 7.1, $^2J_{H,H}$ = 11.6 Hz, 1 H, 5b′′-H), 3.69–3.65 (m, 1 H, 4′′-H), 3.55 (dd, $^3J_{H,H}$ = 5.3, $^3J_{H,H}$ = 3.3 Hz, 1 H, 2′′-H), 3.48 (s, 3 H, OMe), 1.58 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 163.3, 150.0, 141.7, 114.5, 102.3, 100.4, 95.8, 93.7, 85.8, 84.6, 80.87, 73.6, 68.6, 61.3, 61.2, 57.6, 56.3, 27.1, 25.3 ppm. HRMS (ESI): calcd. for $C_{19}H_{26}N_8NaO_9$ $[M + Na]^+$ 533.1721; found 533.1718.

5′-[(Methyl 2,4-diazido-2,4-dideoxy-β-D-xylopyranosid-3-*O*-yl)methyl]uridine (24): A solution of **22** (72.1 mg, 0.141 mmol) in 80% acetic acid (1.5 mL) was stirred for 21 h at 70 °C. After evaporation of the solvent, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH, 20:1–15:1) to give **24** (52.9 mg, 80%) as a foam. R_f = 0.29 ($CHCl_3$/MeOH, 10:1). $[\alpha]_D^{25}$ = –16.0 (*c* = 1.00, MeOH). ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ = 11.34 (br., 1 H, NH), 7.68 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 6-H), 5.77 (d, $^3J_{H,H}$ = 5.4 Hz, 1 H, 1′-H), 5.65 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 5-H), 5.46 (br., 1 H, OH), 5.23 (br., 1 H, OH), 4.91 (s, 2 H, OCH_2O), 4.35 (d, $^3J_{H,H}$ = 7.0 Hz, 1 H, 1′′-H), 4.03 (br., 1 H, 2′-H), 4.00–3.97 (m, 3 H, 3′-H, 4′-H, 5a′′-H), 3.84 (d, $^2J_{H,H}$ = 10.5 Hz, 1 H, 5a′-H), 3.75 (dd, $^3J_{H,H}$ = 2.6, $^2J_{H,H}$ = 10.5 Hz, 1 H, 5b′-H), 3.71–3.67 (m, 1 H, 4′′-H), 3.45–3.40 (m, 5 H, OMe, 2′′-H, 3′′-H), 3.24 (t, $^3J_{H,H}$ = $^2J_{H,H}$ = 11.1 Hz, 1 H, 5b′′-H) ppm. ^{13}C NMR (67.8 MHz, $[D_6]$DMSO): δ = 163.2, 150.8, 140.7, 102.5, 102.0, 95.9, 88.0, 82.8, 77.8, 73.1, 70.2, 68.4, 65.1, 62.9, 60.4, 56.5 ppm. HRMS (ESI): calcd. for $C_{16}H_{22}N_8NaO_9$ $[M + Na]^+$ 493.1408; found 493.1414.

5′-[(Methyl 2,4-diazido-2,4-dideoxy-β-D-ribopyranosid-3-*O*-yl)methyl]uridine (25): A solution of **23** (42.8 mg, 0.084 mmol) in 80% acetic acid (2 mL) was stirred for 11 h at 70 °C. After evaporation of the solvent, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH, 15:1–12:1–10:1) to give **25** (38.7 mg, 98%) as a white solid. R_f = 0.34 ($CHCl_3$/MeOH, 10:1); m.p. 60–62 °C. $[\alpha]_D^{25}$ = –15.5 (*c* = 0.452, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.76 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 6-H), 5.87 (s, 1 H, 1′-H), 5.73 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 5-H), 4.94 (d, $^2J_{H,H}$ = 6.8 Hz, 1 H, OCH_2O), 4.89 (d, $^2J_{H,H}$ = 6.8 Hz, 1 H, OCH_2O), 4.64 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H, 1′′-H), 4.26 (br., 3 H, 2′-H, 3′-H, 4′-H), 4.14 (br., 1 H, 3′′-H), 4.01 (d, $^2J_{H,H}$ = 10.8 Hz, 1 H, 5a′-H), 3.94 (d, $^2J_{H,H}$ = 10.8 Hz, 1 H, 5b′-H), 3.89 (dd, $^3J_{H,H}$ = 3.3, $^2J_{H,H}$ = 11.6 Hz, 1 H, 5a′′-H), 3.78 (dd, $^3J_{H,H}$ = 7.3, $^2J_{H,H}$ = 11.6 Hz, 1 H, 5b′′-H), 3.69–3.68 (m, 1 H, 4′′-H), 3.55 (br., 1 H, 2′′-H), 3.48 (s, 3 H, OMe) ppm. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 164.2, 151.3, 140.4, 102.3, 100.3, 95.9, 89.9, 83.1, 74.9, 73.7, 70.1, 67.7, 61.1, 57.5, 56.3 ppm. HRMS (ESI): calcd. for $C_{16}H_{22}N_8NaO_9$ $[M + Na]^+$ 493.1408; found 493.1372.

5′-[(Methyl 2,4-diamino-2,4-dideoxy-β-D-xylopyranosid-3-*O*-yl)-methyl]uridine (7): Triphenylphosphane (150 mg, 0.572 mmol) was added to a stirred solution of **24** (38.2 mg, 0.081 mmol) in pyridine (1 mL) at 50 °C. After 16 h, a 28% NH_3 solution (1 mL) was added. After 36 h from the start of the reaction, the solution was evaporated and partitioned between water and $CHCl_3$. The water layer was evaporated and the residue was purified by chromatography on silica gel (Kanto Co., 60N spherical gel, *i*PrOH/H_2O/28%NH_3, 35:3:1–25:3:1–15:3:1) to give **7** (19.6 mg, 58%) as a white solid. R_f = 0.30 (*i*PrOH/H_2O/28%NH_3, 10:3:1); m.p. 108–110 °C. $[\alpha]_D^{25}$ = –8.6 (*c* = 0.444, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 7.72 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 5.87 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 5.86 (d, $^3J_{H,H}$ = 3.3 Hz, 1 H, 1′-H), 4.97 (s, 2 H, OCH_2O), 4.36 (t, $^3J_{H,H}$ = 3.3 Hz, 1 H, 2′-H), 4.23–4.21 (m, 3 H, 3′-H, 4′-H, 1′′-H), 4.00 (d, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5a′-H), 3.96 (dd, $^3J_{H,H}$ = 4.9, $^2J_{H,H}$ = 11.9 Hz, 1 H, 5a′′-H), 3.90 (dd, $^3J_{H,H}$ = 3.8, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5b′-H), 3.51 (s, 3 H, OMe), 3.33–3.25 (m, 2 H, 3′′-H, 5b′′-H), 2.91 (dt, $^3J_{H,H}$ = 4.9, $^3J_{H,H}$ = 9.8 Hz, 1 H, 4′′-H), 2.65 (t, $^3J_{H,H}$ = 8.7 Hz, 1 H, 2′′-H) ppm. ^{13}C NMR (67.8 MHz, D_2O): δ = 169.9, 154.4, 142.1, 105.6, 103.1, 98.3, 90.6, 87.2, 82.7, 74.0, 70.2, 68.9, 66.6, 57.9, 56.5, 51.8 ppm. HRMS (ESI): calcd. for $C_{16}H_{27}N_4O_9$ $[M + H]^+$ 419.1779; found 419.1780.

Compound 7 (16 mM) + 2.0 Equiv. Zn(OAc)$_2$: ^{1}H NMR (600 MHz, 50 mM $[D_3]$AcONa buffer, pH 7.0, 30 °C): δ = 7.79 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H, 6-H), 5.95 (m, 2 H, 5-H, 1′-H), 4.95 (d, $^2J_{H,H}$ = 7.0 Hz, 1 H, OCH_2O), 4.92 (d, $^2J_{H,H}$ = 7.0 Hz, 1 H, OCH_2O), 4.72 (br., 1 H, 1′′-H), 4.44 (br., 1 H, 2′-H), 4.30–4.26 (m, 3 H, 3′-H, 4′-H, 5a′′-H), 4.01 (d, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5a′-H), 3.94 (dd, $^3J_{H,H}$ = 4.2, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5b′-H), 3.87 (br., 1 H, 3′′-H), 3.65 (d, $^2J_{H,H}$ = 12.5 Hz, 1 H, 5b′′-H), 3.51 (s, 3 H, OMe), 3.37 (br., 1 H, 4′′-H), 3.33 (br., 1 H, 2′′-H), 1.98 (s, 12 H, Ac) ppm.

Compound 7 (16 mM) + 2.0 Equiv. Zn(OAc)$_2$: ^{1}H NMR (600 MHz, 50 mM $[D_3]$AcONa buffer, pH 7.0, 70 °C): δ = 8.15 (d, $^3J_{H,H}$ =

8.0 Hz, 1 H, 6-H), 6.36 (d, $^3J_{H,H}$ = 8.0 Hz, 1 H, 5-H), 6.32 (d, $^3J_{H,H}$ = 4.4 Hz, 1 H, 1′-H), 5.35 (d, $^2J_{H,H}$ = 7.2 Hz, 1 H, OCH_2O), 5.33 (d, $^2J_{H,H}$ = 7.2 Hz, 1 H, OCH_2O), 5.08 (br. s, 1 H, 1′′-H), 4.84 (t, $^3J_{H,H}$ = 4.4 Hz, 1 H, 2′-H), 4.69–4.64 (m, 3 H, 3′-H, 4′-H, 5a′′-H), 4.41 (d, $^2J_{H,H}$ = 11.4 Hz, 1 H, 5a′-H), 4.33 (dd, $^3J_{H,H}$ = 4.9, $^2J_{H,H}$ = 11.4 Hz, 1 H, 5b′-H), 4.21 (br. t, $^3J_{H,H}$ = 3.2 Hz, 1 H, 3′′-H), 4.03 (dd, $^3J_{H,H}$ = 3.4, $^2J_{H,H}$ = 12.8 Hz, 1 H, 5b′-H), 3.92 (s, 3 H, OMe), 3.75 (br., 1 H, 4′′-H), 3.68 (br., 1 H, 2′′-H), 2.39 (s, 12 H, Ac) ppm.

5′-[(Methyl 2,4-diamino-2,4-dideoxy-β-D-ribopyranosid-3-*O*-yl)-methyl]uridine (8): Triphenylphosphane (312.5 mg, 1.19 mmol) was added to a stirred solution of **25** (76.7 mg, 0.163 mmol) in pyridine (2 mL) at 50 °C. After 16 h, a 28% NH_3 solution (2 mL) was added. After 21 h from the start of the reaction, the solution was evaporated and partitioned between water and $CHCl_3$. The water layer was evaporated and the residue was purified by chromatography on silica gel (Kanto Co., 60N spherical gel, *i*PrOH/H_2O/28%NH_3, 35:3:1–25:3:1–15:3:1) to give **8** (46.6 mg, 68%) as a white solid. R_f = 0.18 (*i*PrOH/H_2O/28%NH_3, 10:3:1); m.p. 104–105 °C. $[a]_D^{25}$ = –23.1 (*c* = 0.54, H_2O). ^{1}H NMR (600 MHz, D_2O): δ = 7.74 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 5.87 (d, $^3J_{H,H}$ = 5.0 Hz, 1 H, 1′-H), 5.85 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 4.93 (d, $^2J_{H,H}$ = 7.1 Hz, 1 H, OCH_2O), 4.90 (d, $^2J_{H,H}$ = 7.1 Hz, 1 H, OCH_2O), 4.52 (d, $^3J_{H,H}$ = 5.8 Hz, 1 H, 1′′-H), 4.35 (t, $^3J_{H,H}$ = 5.0 Hz, 1 H, 2′-H), 4.23 (t, $^3J_{H,H}$ = 5.0 Hz, 1 H, 3′-H), 4.21–4.19 (m, 1 H, 4′-H), 4.00 (dd, $^3J_{H,H}$ = 2.2, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5a′-H), 3.96 (br., 1 H, 3′′-H), 3.88 (dd, $^3J_{H,H}$ = 5.0, $^2J_{H,H}$ = 11.5 Hz, 1 H, 5b′-H), 3.79 (dd, $^3J_{H,H}$ = 3.5, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5a′′-H), 3.61 (dd, $^3J_{H,H}$ = 8.3, $^2J_{H,H}$ = 11.7 Hz, 1 H, 5b′′-H), 3.44 (s, 3 H, OMe), 3.18 (br., 1 H, 4′′-H), 2.90 (br., 1 H, 2′′-H) ppm. ^{13}C NMR (67.8 MHz, D_2O): δ = 168.9, 153.7, 142.4, 103.0, 97.4, 90.7, 82.9, 78.1, 74.0, 70.2, 68.6, 64.3, 57.1, 53.8, 49.8 ppm. HRMS (ESI): calcd. for $C_{16}H_{27}N_4O_9$ [M + H]$^+$ 419.1779; found 419.1741.

Compound 8 (16 mM) + 2.0 Equiv. $Zn(OAc)_2$: ^{1}H NMR (600 MHz, 50 mM [D_3]AcONa buffer, pH 7.0, 30 °C): δ = 7.76 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, 6-H), 5.87 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H, 1′-H), 5.66 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, 5-H), 4.92 (d, $^2J_{H,H}$ = 7.1 Hz, 1 H, OCH_2O), 4.87 (d, $^2J_{H,H}$ = 7.1 Hz, 1 H, OCH_2O), 4.79 (s, 1 H, 1′′-H), 4.35 (t, $^3J_{H,H}$ = 5.3 Hz, 1 H, 2′-H), 4.25 (t, $^3J_{H,H}$ = 5.3 Hz, 1 H, 3′-H), 4.19 (br., 1 H, 4′-H), 4.09 (br. s, 1 H, 3′′-H), 4.01–3.97 (m, 2 H, 5a′-H, 5a′′-H), 3.81 (dd, $^3J_{H,H}$ = 3.3, $^2J_{H,H}$ = 11.2 Hz, 1 H, 5b′-H), 3.72 (d, $^2J_{H,H}$ = 13.0 Hz, 1 H, 5b′′-H), 3.54 (br. s, 1 H, 4′′-H), 3.44 (d, $^3J_{H,H}$ = 3.3 Hz, 1 H, 2′′-H), 3.36 (s, 3 H, OMe), 1.89 (s, 12 H, Ac) ppm.

Compound 8 (16 mM) + 2.0 Equiv. $Zn(OAc)_2$: ^{1}H NMR (600 MHz, 50 mM [D_3]AcONa buffer, pH 7.0, 70 °C): δ = 8.19 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 6-H), 6.35 (d, $^3J_{H,H}$ = 8.1 Hz, 1 H, 5-H), 6.34 (d, $^3J_{H,H}$ = 5.4 Hz, 1 H, 1′-H), 5.41 (d, $^2J_{H,H}$ = 7.0 Hz, 1 H, OCH_2O), 5.38 (d, $^2J_{H,H}$ = 7.0 Hz, 1 H, OCH_2O), 5.29 (s, 1 H, 1′′-H), 4.84 (t, $^3J_{H,H}$ = 5.4 Hz, 1 H, 2′-H), 4.71 (t, $^3J_{H,H}$ = 5.4 Hz, 1 H, 3′-H), 4.67 (m, 1 H, 4′-H), 4.57 (t, $^3J_{H,H}$ = 4.4 Hz, 1 H, 3′′-H), 4.48 (m, 2 H, 5a′-H, 5a′′-H), 4.32 (dd, $^3J_{H,H}$ = 4.7, $^2J_{H,H}$ = 11.6 Hz, 1 H, 5b′-H), 4.23 (d, $^2J_{H,H}$ = 13.0 Hz, 1 H, 5b′′-H), 4.03 (m, 1 H, 4′′-H), 3.96 (d, $^3J_{H,H}$ = 4.2 Hz, 1 H, 2′′-H), 3.87 (s, 3 H, OMe), 2.39 (s, 12 H, Ac) ppm.

Supporting Information (see also the footnote on the first page of this article): Details of assay methods and ^{1}H and ^{13}C NMR spectra of the compounds.

Acknowledgments

This work was supported by a grant from the Global COE Program and a Grant-in-Aid for Scientific Research (No. 19655056) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

[1] C.-H. Wong, R. L. Halcomb, Y. Ichikawa, T. Kajimoto, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 521–546.
[2] Y. Shiro, K. Kato, M. Fujii, Y. Ida, H. Akita, *Tetrahedron* **2006**, *62*, 8687–8695.
[3] P. Compain, O. R. Martin, *Bioorg. Med. Chem.* **2001**, *9*, 3077–3092.
[4] M. M. Vaghefi, R. J. Bernacki, W. J. Hennen, R. K. Robins, *J. Med. Chem.* **1987**, *30*, 1391–1399.
[5] M. E. Colvin, E. Evleth, Y. Akacem, *J. Am. Chem. Soc.* **1995**, *117*, 4357–4362.
[6] S. Murata, S. Ichikawa, A. Matsuda, *Tetrahedron* **2005**, *61*, 5837–5842.
[7] R. Wang, D. H. Steensma, Y. Takaoka, J. W. Yun, T. Kajimoto, C.-H. Wong, *Bioorg. Med. Chem.* **1997**, *5*, 661–672.
[8] L. Ballell, R. J. Young, R. A. Field, *Org. Biomol. Chem.* **2005**, *3*, 1109–1115.
[9] H. Yuasa, T. Izumi, N. Mitsuhashi, Y. Kajihara, H. Hashimoto, *Chem. Eur. J.* **2005**, *11*, 6478–6490.
[10] H. Yuasa, N. Miyagawa, M. Nakatani, M. Izumi, H. Hashimoto, *Org. Biomol. Chem.* **2004**, *2*, 3548–3556.
[11] X. Liu, C. B. Reese, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2227–2236.
[12] The $^4C_1/^1C_4$ ratios were calculated by a multiple regression analysis with a least-squares fitting of the *J* values calculated for model structures to observed values. For details, see ref.[9]
[13] J. T. Powell, K. Brew, *Biochemistry* **1976**, *15*, 3499–3505.
[14] D. D. Perrin, W. L. Armarego, D. R. Perrin, *Purification of Laboratory Compounds*, 2nd ed., Pergamon Press, London, **1980**.

Received: December 26, 2008
Published Online: February 18, 2009

