Thermophilic Esterases/Lipases as an Effective Tool for the Resolution of Nucleoside Diastereoisomers: Convenient One-Pot Synthesis of α-L-Taluronamide and β-D-Alluronamide Nucleosides

Jian-qiang Wang,† Christopher Archer,† Jun Li,† Simon G. Bott,‡ and Peng George Wang*,†

Department of Chemistry, Wayne State University, Detroit, Michigan 48202, and Department of Chemistry, University of North Texas, P.O. Box 5068, Denton, Texas 76203

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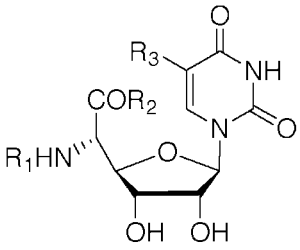
Introduction

Enzymatic reactions as alternatives to chemical methods in organic synthesis are currently well-recognized. Enzymes from a diverse range of microorganisms in extreme environments regarding temperature, pH, and salt concentration represent a new class of catalytic entities which very often possess new types of activity and substrate specificity for synthetic application.[1,2] In this report, esterase/lipase CloneZyme (ESL-001) library, a thermophilic enzyme library developed by Diversa Corp. (San Diego, CA) based on recombinant technology and robotic screening approaches, was employed in the resolution of diastereoisomeric nucleoside derivatives.

Polyoxins and nikkomycins which exhibit selective activity against certain pathogenic fungi are two typical families of nucleoside antibiotics (Chart 1).[3,4] 1-(5′-Amino-5′-deoxy-β-D-allofuranosyluronic acid)uracil (uracil polyoxin C) (**1**) constitutes the basic terminal amino acid nucleoside common to most members of polyoxin and nikkomycin dipeptides. To prepare these nucleoside cores, many synthetic approaches have been reported during the synthesis of corresponding antibiotics.[3,5] However, the efficient synthesis of such compounds is far from being a trivial problem despite their simple structures.[6] In the direct synthesis of uracil polyoxin C from uridine,

Chart 1. Polyoxin and Nikkomycin Nucleoside Antibiotics



Polyoxins and Nikkomycins
R_1=C-terminal amino acid
R_2=N-terminal amino acid or OH
R_3=H, CH_3, CH_2OH, CO_2H

Uracil Polyoxin C
R_1, R_3=H; R_2=OH **1**

the problem has been that the intermediate, 2′,3′-*O*-cyclohexylideneuridine-5′-aldehyde or 2′,3′-*O*-isopropylideneuridine-5′-aldehyde, underwent hydration readily and was unstable to purification by conventional chromatography on silica gel.[7,8] Another problem encountered by Moffatt and co-workers in the direct synthesis of polyoxin C from uridine via cyanohydrin formation at the 5′-aldehyde was that the resulting mixture of β-D-allo- and α-L-talofuranuronic acid derivatives was difficult to separate.[9]

Here we report a one-pot preparation of a mixture of α-L-taluronamide and β-D-alluronamide nucleoside derivatives. Such derivatives can be considered as intermediates[9] to uracil polyoxin C (**1**), capuramycin,[5] and other nucleoside antibiotics. This method provides a convenient approach to prepare α-L-taluronamide and β-D-alluronamide nucleosides starting directly from the corresponding 5′-OH nucleosides.[10] Since the resulting two diastereoisomeric nucleosides were difficult to separate, the esterase/lipase CloneZyme (ESL-001) library was used to selectively deacylate one diastereoisomer of its 5′-O-acylated form. One thermophilic esterase/lipase mutant from the CloneZyme library has proved to be the best enzyme for such resolution.

Results and Discussion

One-Pot Preparation of 1-(2′,3′-*O*-Isopropylidene-(α-L-talo and β-D-allo)furanosyluronamide)uracil and Other Related Nucleoside Derivatives. Starting from 2′,3′-*O*-isopropylidene nucleosides, our strategy for the preparation of α-L-taluronamide and β-D-alluronamide nucleosides **3** depicted in Scheme 1 involved a one-pot procedure which included oxidation (DMSO, DCC,

* To whom correspondence should be addressed.
† Wayne State University.
‡ To whom correspondence should be made regarding X-ray analysis, at University of North Texas.

(1) (a) Pennisi, E. *Science* **1997**, May 2, *V276*, 705. (b) Brennan, M. B. *Chem. Eng. News* **1996**, Oct 14, 31. (c) Robertson, D. E.; Mathur, E. J.; Swanson, R. V.; Marrs, B. L.; Short, J. M. *Sim News* **1996**, *46*, 3. (d) Adams, M. W. W.; Kelly, R. M. *Chem. Eng. News* **1995**, Dec 18, 32.

(2) Our recent work with the thermophilic enzymes: (a) Xie, W. H.; Li, J.; Chen, D.; Wang, P. G. *Polym. Prepr.* **1997**, *38* (2), 279. (b) Li, J.; Wang, P. G. *Tetrahedron Lett.* **1997**, *38*, 7967. (c) Xie, W. H.; Li, J.; Chen, D.; Wang, P. G. *Macromolecules* **1997**, *30*, 6997. (d) Fang, J. W.; Li, J.; Xie, W. H.; Wang, P. G. *Tetrahedron Lett.* **1998**, *39*, 919.

(3) For reviews on nucleoside antibiotics, see: (a) Knapp, S. *Chem. Rev.* **1995**, *95*, 1859. (b) Lerner, L. M. Synthesis and Properties of Various Disaccharide Nucleosides. In *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum Press: New York, 1991; Vol. 2, pp 27−79. (c) Isono, K. *Pharmacol. Ther.* **1991**, *52*, 269. (d) Isono, K. *J. Antibiot.* **1988**, *41*, 1711. (e) Garner, P. Synthetic Approaches to Complex Nucleoside Antibiotics. In *Studies in Natural Product Chemistry*; Atta-uhr-Rahman, Ed.; Elsevier: Amsterdam, 1988; Part A, Vol. 1, pp 397−434.

(4) (a) Chapman T.; Kinsman, O.; Houston, J. *Antimicrob. Agents Chemother.* **1992**, *36*, 1909. (b) Milewski, S.; Mignini, F.; Borowski, E. *J. Gen. Microbiol.* **1991**, *137*, 2155. (c) Cabib, E. *Antimicrob. Agents Chemother.* **1991**, *35*, 170. (d) Isono, K. *J. Antibiot.* **1988**, *41*, 1711 and references therein.

(5) Knapp, S.; Nandan, S. R. *J. Org. Chem.* **1994**, *59*, 281.

(6) (a) Dondoni, A.; Franco, S.; Junquera, F.; Merchán, F. L.; Merino, P.; Tejero, T. *J. Org. Chem.* **1997**, *62*, 5497. (b) Evina, C. M.; Guillerm, G. *Tetrahedron Lett.* **1996**, *37*, 163. (c) Dondoni, A.; Junquera, F.; Merchán, F. L.; Merino, P.; Tejero, T. *Tetrahedron Lett.* **1994**, *35*, 9439. (d) Chida, N.; Koizumi, K.; Kitada, Y.; Yokoyama, C.; Ogawa, S. *J. Chem. Soc., Chem. Commun.* **1994**, 111. (e) Chen, A.; Savage, I.; Thomas, E. J.; Wilson, P. D. *Tetrahedron Lett.* **1993**, *34*, 6769. (f) Mukaiyama, T.; Suzuki, K.; Yamada, T.; Tabusa, F. *Tetrahedron* **1990**, *46*, 265. (g) Barrett, A. G. M.; Lebold, S. A. *J. Org. Chem.* **1990**, *55*, 3853. (h) Garner, P.; Park, J. M. *J. Org. Chem.* **1990**, *55*, 3772. (i) Auberson, Y.; Vogel, P. *Tetrahedron* **1990**, *46*, 7019 and references therein.

(7) Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 4697.

(8) Corey, E. J.; Samuelsson, B. *J. Org. Chem.* **1984**, *49*, 4735.

(9) Damodaran, N. P.; Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1971**, *93*, 3812.

(10) Tronchet, J. M. J.; Valero, M. J. *Helv. Chim. Acta* **1979**, *62*, 2788.

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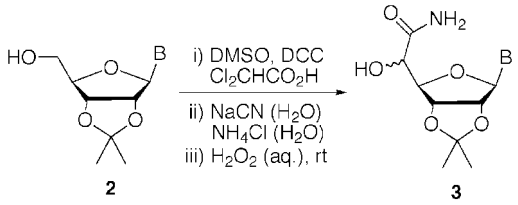
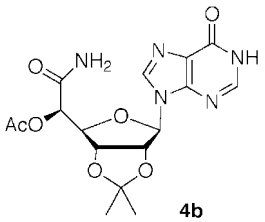
Table 1. One-Pot Preparation of 5′-Hydroxy Amide Nucleoside Derivatives with Different Bases

entry	substrate	product	yield[a] (%)	de selectivity[b] (%)
1	2′,3′-*O*-isopropylideneuridine (**2a**)	**3a**	64	9 (*R*)[c]
2	2′,3′-*O*-isopropylideneinosine (**2b**)	**3b**	66	one diastereomer (*R*)
3	2′,3′-*O*-isopropylideneadenosine (**2c**)	**3c**	50	32 (*R*)[c]

[a] Isolated yield. [b] Determined by ^{1}H NMR. [c] Determined by ^{1}H NMR and X-ray crystal structural analysis.

Scheme 1





$Cl_2CHCOOH$),[10,11] nucleophilic addition (NaCN),[9,12] and hydrolysis (30% hydrogen peroxide solution).[13] The three reactions occurred sequentially to give **3** in a reasonable yield (Table 1). Ammonium chloride in the second step of the reaction was essential because the yield (37% with no ammonium chloride; 64% with ammonium chloride) decreased without it when uridine **2a**[14] was used as the substrate. The formation of product **3** indicated that the intermediate formed before hydrolysis is 2′,3′-*O*-isopropylideneuridine-5′-hydroxycyanide instead of 2′,3′-*O*-isopropylideneuridine-5′-aminocyanide.

2′,3′-*O*-Isopropylideneinosine (**2b**) and 2′,3′-*O*-isopropylideneadenosine (**2c**) underwent the same reaction sequence to give products **3b**,**c** in 66% and 50% yield, respectively (Table 1). Products **3a**,**c** were diastereoisomeric mixtures of *β*-D-alluronamide and α-L-taluronamide nucleosides in which the α-L-taluronamide with *R* configuration at the new chiral carbon (C5′) was the major isomer. This suggests that the attack of nucleophile CN^- took place mainly at the Re face. This may be contributed to the shielding of the Si face by the nucleoside base or other factors during the reaction.

For determination of the configuration at the newly formed C5′, we first resorted to the X-ray analysis of compound **4b**. **4b** was derived from **3b** with an acylation reaction in 84% yield. Crystallization of **4b** from methanol gave crystals of the *R* configuration at C5′. For determination of the configuration at C5′ in **3a,c**, see below.

Resolution of 5′-Acyl(α-L-taluronamide and *β*-D-alluronamide) Nucleosides with CloneZyme. Two approaches, esterification and hydrolysis, are commonly used in enzymatic resolution of enantiomers. For the diastereoisomers **3a**, selective enzymatic esterification of the 5′-hydroxyl group with vinyl acetate was tried first but no reaction occurred in the presence of porcine pancreas lipase (PPL; 25 °C, DMF, 48 h) or CloneZyme (ESL-001-02; 70 °C, DMF, 48 h). Therefore, we converted both diastereoisomers of **3a** into their corresponding esters **4a** (Scheme 2). The enzymatic hydrolysis of ester **4a** with PPL in sodium phosphate buffer (50 mM, pH = 7.0) at 28 °C did not show any preference toward the two diastereoisomers. In sharp contrast, the enzymatic hydrolysis reaction of **4a** catalyzed by ESL-001-02 at 60 °C in sodium phosphate buffer (50 mM, pH = 7.0, [enzyme] = 126 *μ*g/mL, [substrate] = 27 mM) yielded 1-(2′,3′-*O*-isopropylidene-α-L-talofuranosyluronamide)uracil (**3a-*R***) and 1-(2′,3′-*O*-isopropylidene-5′-*O*-acetyl-*β*-D-allofuranosyluronamide)uracil (**4a-*S***) in diastereoisomerically pure form (Table 2). **3a-*R*** and **4a-*S*** were then easily separated by conventional chromatography on silica gel. Other enzymes in the CloneZyme library were screened for the resolution of **4a** under the same conditions. The results of the screening are summarized in Table 2.

Although ESL-001-02 exhibited good activity and selectivity for the hydrolysis of C′5(*R*)-**4a**, all of the other enzymes in the library preferred to catalyze the hydrolysis of the C′5(*S*)-**4a** with low de values. At room temperature, the thermophilic enzymes did not catalyze the reaction. This provided us a convenient way to stop the reaction at any time. In a detailed study, the relationship of de values of products **3a-*R*** and **4a-*S*** versus the reaction time was studied for the hydrolysis catalyzed by ESL-001-02 under the same conditions shown in row 2, Table 2. The results demonstrated that the hydrolysis of **4a-*R*** with ESL-001-02 was fast and complete within 1 h. **4a-*S*** started to hydrolyze slowly 2 h after reaction. Hence, the diastereoisomerically pure compound was obtained by stopping the reaction within 1−2 h.

Similarly, product **3c** could be resolved by ESL-001−02 as depicted in Scheme 3. Here **3c** was converted into its dibutyryl form **5** first. Hydrolysis of **5** with ESL-001-02 gave **6** in 100% de value and unhydrolyzed ester **5-*S*** in 78% de value.

The configuration of C5′ in **3a-*R***, **4a-*S***, **5-*S***, and **6** was assigned based on ^{1}H NMR spectroscopy along with X-ray crystal structure analysis of **3a-*R***. For this class of compound, the resonance of C4′H was greatly affected by the configuration of C5′. We can conclude the absolute configuration of C5′ according to chemical shifts of the C4′H. Diastereoisomerically pure compound **3a-*R*** shows a chemical shift for C4′H at 4.27 ppm, and its X-ray crystal structure analysis indicated that the absolute configuration of C5′ was *R*. Hence, the absolute configuration of C5′ in the other enzymatically resolved product **4a-*S***, with a chemical shift of C4′H at 4.43 ppm, was *S*.

(11) (a) Montgomery, J. A.; Thomas, H. J. *J. Org. Chem.* **1981**, *46*, 594. (b) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661.

(12) (a) Williams, R. B. *Synthesis of Optically Active* α*-Amino Acids*; Pergamon: Elmsford, NY, 1989; pp 208−229. (b) Fiandor, J.; Garcia-López, M.-T.; De las Heras, F. G.; Méndez-Castrillón, P. P. *Synthesis* **1987**, 978. (c) Boehm, J. C.; Kingsbury, W. D. *J. Org. Chem.* **1986**, *51*, 2307. (d) Robins, M. J.; Perker, J. M. R. *Can. J. Chem.* **1983**, *61*, 312. (e) Tsuchida, K.; Mizuno, Y.; Ikeda, K. *Nucleic Acids Res. Symp. Series* **1980**, *8*, 49.

(13) (a) Katritzky, A. R.; Pilarski, B.; Urogdi, L. *Synthesis* **1989**, 949. (b) Kornblum, N.; Singaram, S. *J. Org. Chem.* **1979**, *44*, 4727. (c) Denney, D. B.; Rosen, J. D. *Tetrahedron* **1964**, *20*, 271. (d) Kharasch, M. S.; Fono, A.; Nudenberg, W.; Bischof, B. *J. Org. Chem.* **1952**, *17*, 207.

(14) Hampton, A. *J. Am. Chem. Soc.* **1961**, *83*, 3640.

Scheme 2

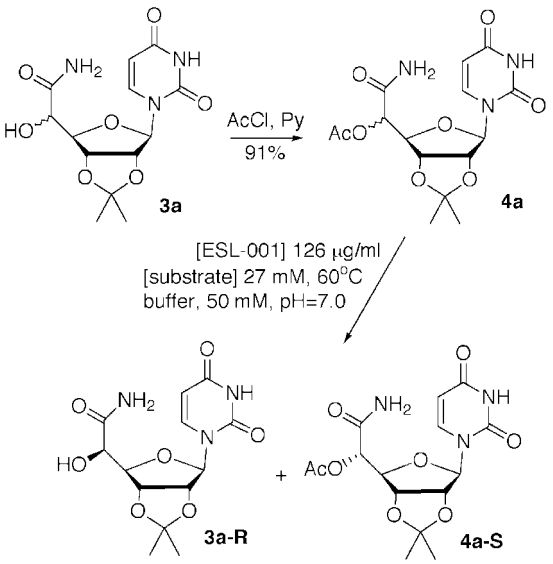
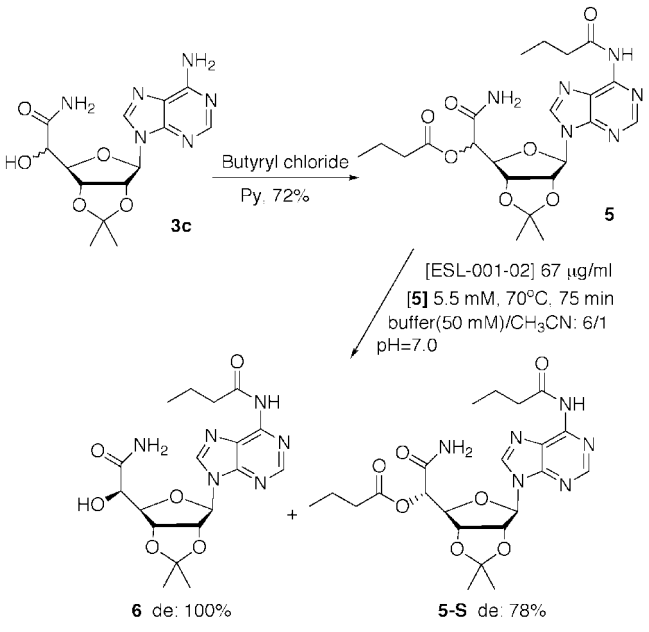


Table 2. Screening of Enzymatic Deacetylation of 4a

			de[b] (%) (config at C′[5])[c]	
enzyme[a]	time (h)	temp (°C)	**3a**	**4a**
ESL-001-01	24	60	23 (*S*)	26 (*R*)
ESL-001-02	1	60	100 (*R*)	100(*S*)
ESL-001-02	1	rt	no reaction	no reaction
ESL-001-03	24	60	21 (*S*)	24 (*R*)
ESL-001-04	24	60	25 (*S*)	27(*R*)
ESL-001-05	24	60	23 (*S*)	25 (*R*)
ESL-001-06	24	60	22 (*S*)	26 (*R*)

[a] [Enzyme] = 2.1 mg/mL. [b] Determined by ^{1}H NMR. [c] Determined by X-ray crystal structural analysis and ^{1}H NMR.

Scheme 3



Therefore, in the ^{1}H NMR spectroscopy of diastereoisomeric mixture **4a** with its C4′H signals at 4.27 and 4.43 ppm, the lower chemical shift at 4.27 ppm was caused by C4′-H attached to (*R*)-C5′, and the higher chemical shift at 4.43 ppm was caused by C4′-H attached to (*S*)-C5′. According to the trend of chemical shift shown in diastereoisomeric mixture **4a**, the proton signals of diastereoisomeric mixture **5** at 4.48 and 4.71 ppm were assigned as C4′H attached to (*R*)-C5′ and (*S*)-C5′, respectively. Enzymatic hydrolysis resolution product **5-*S*** from dibutyryl nucleoside **5** had its C4′H resonance at 4.71 ppm. So, the absolute configuration of C5′ in **5-*S*** was assigned as *S* and then the configuration of C5′ in **6** as *R*. These results were consistent with the *R* stereoselectivity of ClonZyme ESL-001-02 toward the hydrolysis of analogue **4a**.

In summary, α-L-taluronamide and β-D-alluronamide nucleoside derivatives were readily prepared via a one-pot reaction sequence of oxidation, nucleophilic addition, and hydrolysis. The resulting diastereoisomers, which are difficult to separate by conventional chromatography, were resolved by stereoselective deacylation of their 5′-O-acylated forms with thermophilic enzymes. Thermophilic enzyme ESL-001-02 in the esterase/lipase library exhibited good selectivity in the hydrolysis of (*R*)-C5′ substrates. This work demonstrates that enzymes from organisms of extreme environments exhibit unique activity and selectivity useful for enzymatic transformation. Work is in progress on using such an enzymatic approach for the synthesis of novel optically pure nucleoside derivatives.

Experimental Section

General. Baker silica gel (40 μm) for column chromatography and E. Merck precoated TLC plates for thin-layer chromatography were used. ^{1}H and ^{13}C NMR spectra were recorded on a 400-MHz NMR spectrometer. Mass spectra were run at the mass spectrometry facility at the University of California, Riverside. X-ray structural analysis was completed at the University of North Texas.

Synthesis of 3a−c. General Procedure. To a solution of **2a−c** (2 mmol) and DCC (6 mmol) in DMSO (2.5 mL) was gently added dichloroacetic acid (1 mmol). The reaction mixture was stirred vigorously for 1 h at 25 °C. Then a solution of oxalic acid dihydrate (2 mmol) in 10 mL of methanol was added slowly to quench the extra DCC. After gas was completely excluded, an aqueous solution of NH_4Cl (2.2 mmol) in 2 mL of water and NaCN (2.2 mmol) in 2 mL of water was added. Three hours later, an excess of 35% hydrogen peroxide (usually 6 mmol) was added. Strong stirring was continued for an additional 4−6 h at room temperature. The reaction mixture was diluted with MeOH (25 mL), filtered, and then concentrated. The residue was chromatographed with silica gel, eluting with chloroform/methanol in a ratio of 25/1, 10/1, 5/1 sequentially, to give the products.

1-(2′,3′-*O*-Isopropylidene(α-L-talo and β-D-allo)furanosyluronamide)uracil (3a). Reaction of **2a** (820 mg, 2.88 mmol) according to the general procedure gave diastereoisomers **3a** (600 mg, 64%, de: 9%, C5′(*R*)) as a white solid (for the spectral data of diastereoisomerically pure C5′(*R*)-**3a**, see the data of **3a-*R*** in this section): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.26 (s)/1.28 (s), 1.46 (s)/1.48 (s), 4.27 (dd)/4.37 (dd), 4.78 (m)/4.81 (m), 5.62 (d, J = 8.0 Hz), 5.87 (d, J = 2.8 Hz)/5.90 (d, J = 2.8 Hz), 6.00 (d, J = 5.6 Hz)/6.07 (d, J = 6.0 Hz), 7.30 (s), 7.35 (s), 7.38 (s), 7.45 (s), 7.73 (d, J = 8.0 Hz)/7.89 (d, J = 8.0 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.1/25.2, 27.0/27.1, 70.8/71.2, 78.9/80.1, 83.4/83.6, 85.9/86.6, 89.8, 101.9, 113.0, 141.0/141.1, 150.3/150.4, 162.0, 172.6/172.9; LRMS (CI, NH_3) *m*/*z* (%) 328 (MH^+, 100), 283 (18), 270 (13), 253 (12), 216 (72), 195 (13), 158 (13), 113 (39); HRMS (CI, NH_3) calcd for $C_{13}H_{18}N_3O_7$ (MH^+) 328.1143, found 328.1145.

1-(2′,3′-*O*-Isopropylidene-α-L-talofuranosyluronamide)-hypoxanthine (3b). Reaction of **2b** (420 mg, 1.36 mmol), according to the general procedure, gave **3b** (360 mg, 66%) as a white solid: mp 196 °C (turn to dark); $[\alpha]^{25}_D$ −0.5 (*c* 0.51, DMF); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.27 (s, 3H), 1.51 (s, 3H), 4.54 (dd, J = 4.2, 4.2 Hz, 1H), 4.95 (m, 1H), 5.04 (m, 1H), 6.03 (d, J = 3.2 Hz, 1H), 6.10 (d, J = 5.6 Hz, 1H), 7.24 (s, 1H), 7.32 (s, 1H), 8.05 (s, 1H), 8.35 (s, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.2, 27.1, 71.1, 81.0, 84.1, 86.4, 89.7, 113.1, 124.1, 138.4, 145.9,

147.6, 156.4, 172.7; LRMS (CI, NH_3) *m*/*z* (%) 352 (MH^+, 5), 252 (14), 233 (18), 233 (18), 216 (100), 198 (42), 166 (50), 137 (54), 100 (19), 56 (20); HRMS (CI, NH_3) calcd for $C_{14}H_{18}N_5O_6$ (MH^+) 352.1260, found 352.1257.

9-(2′,3′-*O*-Isopropylidene(α-L-talo and β-D-allo)furanosyluronamide)adenine (3c). Reaction of **2c** (800 mg, 2.61 mmol), according to the general procedure, gave diastereoisomers **3c** (462 mg, 50%, de: 32%, C5′(*R*)) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.26 (s)/1.28 (s), 1.50 (s)/1.52 (s), 4.45 (dd)/4.54 (dd), 4.89 (m)/5.00 (m), 5.08 (m)/5.13 (m), 6.05 (m), 6.39 (d, J = 8.0 Hz), 6.59 (d, J = 4.4 Hz), 7.30 (m), 8.10 (s)/8.13 (s), 8.26 (s)/8.34 (s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.2/25.3, 27.1/27.2, 71.5/71.9, 79.7/81.1, 83.3/83.4, 85.8/86.7, 89.9, 113.2, 139.4, 148.6, 152.5, 152.6, 156.1/156.2, 172.3/173.0; LRMS (CI, NH_3) *m*/*z* (%) 351 (MH^+, 100), 306 (13), 252 (32), 225 (32), 136 (89), 79 (4), 56 (8); HRMS (CI, NH_3) calcd for $C_{14}H_{18}N_5O_6$ (MH^+) 351.1422, found 351.1417.

1-(2′,3′-*O*-Isopropylidene-5′-*O*-acetyl(α-L-talo and β-D-allo)furanosyluronamide)uracil (4a). Uridine derivative **3a** (0.20 g, 0.61 mmol) was dissolved in 5 mL of anhydrous pyridine, and the mixture was cooled to 0 °C. Acetyl chloride (44 μL, 0.73 mmol) was added, and the reaction solution was warmed to room temperature. The reaction mixture was kept stirring until the starting material had disappeared from the TLC plate. After removal of pyridine in vacuo, the residue was purified with silica gel column chromatography (eluent: $CHCl_3$/MeOH, 40/1 and 30/1) to give a white solid product **4a** (0.21 g, yield 91%, de: 9%) (for the spectral data of diastereoisomerically pure C5′(*S*)-**4a**, see the data of **4a-*S*** in this section): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.27 (s), 1.47 (s)/1.48 (s), 2.08 (s), 4.27 (dd, J = 3.6 Hz)/4.43 (dd, J = 3.2, 3.2 Hz), 4.90 (s)/4.95 (m), 5.04 (m), 5.63 (d, J = 8.4 Hz)/5.65 (d, J = 8.4 Hz), 5.82 (d, J = 2.4 Hz)/5.85 (d, J = 2.0 Hz), 7.41 (s)/7.43 (s), 7.53 (d, J = 8 Hz), 7.63 (s)/7.67 (s), 7.75 (d, J = 8.0 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 20.5, 25.1, 26.9, 72.3/72.5, 79.3/79.8, 82.7/83.7, 84.4/85.0, 90.4/91.1, 101.5/102.1, 113.1/113.4, 141.2/141.5, 150.2, 162.9/163.0, 167.9/168.0, 169.3/169.4; LRMS (CI, NH_3) *m*/*z* (%) 370 (MH^+, 69), 328 (7), 312 (16), 283 (4), 258 (65), 240 (26), 216 (10), 113 (100), 60 (36); HRMS (CI, NH_3) calcd for $C_{15}H_{20}N_3O_8$ (MH^+) 370.1251, found 370.1250.

9-(2′,3′-*O*-Isopropylidene-5′-*O*-acetyl-α-L-talofuranosyluronamide)hypoxanthine (4b). Using the procedure described for **4a**, **3b** (156 mg, 0.44 mmol) was converted to acetylated inosine derivative **4b** (146 mg, 84%) as a white solid. Recrystallization of **4b** from methanol gave single crystals for X-ray structural analysis: mp 245 °C (turn to dark); $[\alpha]^{25}_D$ −17.6 (*c* 0.71, DMF); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.31 (s, 3H), 1.54 (s, 3H), 1.99 (s, 3H), 4.65 (dd, J = 2.8, 3.2 Hz, 1H), 5.03 (m, 2H), 5.20 (dd, J = 2.8, 6.4 Hz, 1H), 6.12 (d, J = 2.0 Hz, 1H), 7.41 (s, 1H), 7.62 (s, 1H), 8.09 (s, 1H), 8.33 (s, 1H); LRMS (CI, NH_3) *m*/*z* (%) 394 (MH^+, 12), 334 (2), 275 (24), 258 (26), 240 (29), 200 (26), 154 (15), 137 (100), 91 (14); HRMS (CI, NH_3) calcd for $C_{16}H_{20}N_5O_7$ (MH^+) 394.1358, found 394, 1362.

Resolution of Diastereoisomer 4a. Preparation of 1-(2′,3′-*O*-Isopropylidene-α-L-talofuranosyluronamide)uracil (3a-*R*) and 1-(2′,3′-*O*-Isopropylidene-5′-*O*-acetyl-β-D-allofuranosyluronamide)uracil (4a-*S*). Diastereoisomer **4a** (25 mg) and 0.15 mL of CloneZyme (ESL-001-02; 2.1 mg/mL in water) were dissolved in sodium phosphate buffer (2.5 mL, 50 mM, pH 7.0). The mixture was incubated at 60 °C for 1−2 h and then cooled to room temperature. A portion of **3a-*R*** was obtained by filtration. The aqueous solution was concentrated in vacuo. The residue was purified with silica gel column chromatography (eluent: $CHCl_3$/MeOH, 40/1 and 25/1) to give white solid **3a-*R*** (12 mg, quantitative, de: 100%) and **4a-*S*** (11 mg, quantitative, de: 100%). Single crystals of **3a-*R*** which is suitable for X-ray structural analysis were obtained by slow evaporation of a solution of **3a-*R*** in methanol at room temperature. **3a-*R***: mp 278 °C (turn to dark); $[\alpha]^{25}_D$ −66.3 (*c* 0.30, DMF); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.26 (s, 3H), 1.46 (s, 3H), 4.27 (dd, J = 3.2, 3.2 Hz, 1H), 4.81 (m, 2H), 5.63 (d, J = 8.0 Hz, 1H), 5.90 (d, J = 3.2 Hz, 1H), 6.10 (d, J = 5.2 Hz, 1H), 7.38 (s, 1H), 7.45 (s, 1H), 7.73 (d, J = 8.0 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.1, 27.0, 70.8, 79.0, 83.5, 86.6, 89.8, 102.0, 113.2, 141.1, 150.5, 163.1, 172.7. **4a-*S***: mp 196 °C (turn to dark); $[\alpha]^{25}_D$ 7.5 (*c* 0.82, DMSO- d_6); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.26 (s, 3H), 1.48 (s, 3H), 2.08 (s, 3H), 4.43 (dd, J = 3.2, 3.2 Hz, 1H), 4.90 (m, 2H), 5.03 (m, 1H), 5.63 (d, J = 8.0 Hz, 1H), 5.82 (d, J = 1.6 Hz, 1H), 7.41 (s, 1H), 7.62 (s, 1H), 7.75 (d, J = 8.0 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 20.6, 25.2, 27.0, 72.6, 79.9, 83.8, 85.1, 91.2, 101.5, 113.1, 141.7, 150.3, 163.2, 168.2, 169.6.

6-*N*-Butyryl-9-(2′,3′-*O*-isopropylidene-5′-*O*-butyryl(α-L-talo and β-D-allo)furanosyluronamide)adenine (5). To a solution of **3c** (100 mg, 0.29 mmol) in anhydrous pyridine (2 mL) was added butyryl chloride (66 μL, 0.64 mmol) at 0 °C. Then the mixture was warmed to room temperature slowly and stirred for 2 h. After removal of pyridine in vacuo, the residue was chromatographed, eluting with chloroform and methanol (50/1 and 30/1) sequentially, to give dibutyladenine **5** (102 mg, 72%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.76 (t, J = 7.2 Hz), 0.92 (t, J = 7.2 Hz), 1.00−1.60 (m), 2.52 (m), 4.48 (dd, J = 3.2, 3.2 Hz, 2H)/4.70 (dd, J = 3.2, 3.2 Hz), 5.04 (m)/5.06 (m), 5.16 (m)/5.34 (m), 5.56 (d, J = 2.8 Hz)/6.25 (d, J = 2.0 Hz), 7.41 (s), 7.63 (s)/7.66 (s), 8.52 (s), 8.65 (s)/8.66 (s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 13.5/13.7, 18.1/18.4, 25.3/25.4, 21.2/27.3, 35.6/35.8, 39.7, 72.2/72.7, 80.9/81.6, 83.8/84.2, 85.6/85.7, 90.4/90.8, 114.9/115.1, 121.8/122.2, 141.8/142.2, 149.5, 151.0/151.3, 152.5/152.6, 169.4/169.8, 171.8/171.9, 173.6/173.9; LRMS (CI, NH_3) *m*/*z* (%) 491 (MH^+, 37), 421 (10), 405 (5), 303 (13), 268 (10), 206 (100), 136 (35), 105 (15), 88 (40), 71 (6); HRMS (CI, NH_3) calcd for $C_{22}H_{32}N_6O_7$ (MH^+) 491.2246, found 491.2253.

Enzymatic Resolution of Diastereoisomer 5. Preparation of 6-*N*-Butyryl-9-(2′,3′-O-isopropylidene-5-*O*-butyryl-β-D-allofuranosyluronamide)adenine (5-*S*) and 6-*N*-Butyryl-9-(2′,3′-O-isopropylidene-α-L-talofuranosyluronamide)-adenine (6). Diastereoisomer **5** (15 mg) and 0.18 mL of CloneZyme (ESL-001-02; 2.1 mg/mL in water) was dissolved in the mixture of sodium phosphate buffer (5.6 mL, 50 mM, pH 7.0)/CH_3CN (6/1). The reaction mixture was incubated at 70 °C for 75 min and then cooled to room temperature. The water was removed in vacuo. The residue was purified with silica gel column chromatography (eluent: $CHCl_3$/MeOH, 50/1 and 30/1) to give white solid **5-*S*** (4 mg, de: 78%) and **6** (6 mg, conversion 67%, de: 100%). **5-*S*** (de: 78%, *S* at C5′): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.76 (t, J = 7.2 Hz, 3H), 0.92 (t, J = 7.2 Hz, 3H), 1.33 (s, 3H), 1.55 (s, 3H), 1.33−1.64 (m, 4H), 2.15 (m, 2H), 2.53 (t, J = 7.2 Hz, 2H), 4.71 (dd, J = 3.2, 3.2 Hz, 1H), 5.06 (m, 2H), 5.34 (dd, J = 3.2, 6.0 Hz, 1H), 6.25 (d, J = 2.4 Hz, 1H), 7.41 (s, 1H), 7.63 (s, 1H), 8.65 (d, J = 4.0 Hz, 1H). **6**: mp 200−202 °C; $[\alpha]^{25}_D$ −58.6 (*c* 0.31, DMF); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.93 (t, J = 7.6 Hz, 3H), 1.30 (s, 3H), 1.54 (s, 3H), 1.61 (m, 2H), 2.52 (t, J = 7.6 Hz, 2H), 4.48 (dd, J = 2.4, 2.4 Hz, 1H), 4.97 dd, J = 2.4, 6.0 Hz, 1H), 5.30 (m, 1H), 6.14 (d, J = 5.2 Hz, 1H), 6.22 (d, J = 4.0 Hz, 1H), 7.40 (s, 1H), 7.46 (s, 1H), 8.60 (s, 1H); LRMS (CI, NH_3) *m*/*z* (%) 421 (MH^+, 4), 376 (3), 351 (17), 306 (3), 288 (4), 206 (100), 158 (12), 136 (28), 88 (8), 71 (4); HRMS (CI, NH_3) calcd for $C_{18}H_{25}N_6O_6$ (MH^+) 421.1840, found 421.1835.

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Supporting Information Available: X-ray structural analysis of **3a-*R*** and **4b** (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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