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# First Stereoselective Synthesis of 2-Deoxy-α-D-ribosyl-1-phosphate: Novel Application of Crystallization-Induced Asymmetric Transformation

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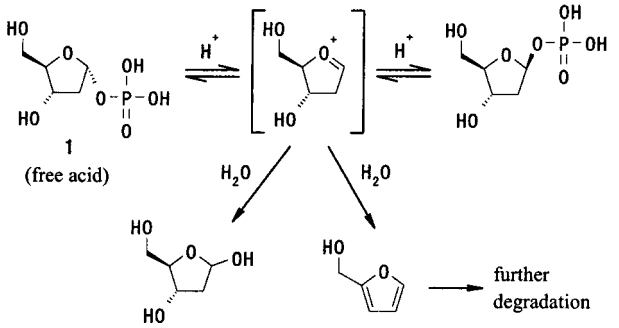
**Abstract:** A first stereoselective synthesis of bis(cyclohexylamine) 2-deoxy-α-D-ribosyl-1-phosphate has been achieved. The synthesis features a key crystallization-induced asymmetric transformation (AT) to generate a desired α-anomer in 99% yield at a 98.8:1.2 ratio of α/*β*.

Chemical synthesis of 2-deoxy-α-D-ribosyl-1-phosphate (**1**)[1] has been an attractive target as a key substrate in enzymatic preparations of 2′-deoxynucleosides[1c,2] that are currently produced from natural resources such as DNA in salmon milt. Large-scale production of 2′-deoxynucleosides is the focus of growing attention for the development of DNA-relating drugs due to the increased demand of the starting raw materials. Since the known synthetic methods of 2′-deoxynucleosides[3] are not satisfactory in yield and show low stereoselectivity at the anomeric position, particularly the synthetic methods for purine 2′-deoxynucleosides, the enzymatic conversion of **1** into 2′-deoxynucleoside should be the most expedient strategy for its practical manufacture.[4] Even though several methodologies in pyranosyl-1-phosphate or furanosyl-1-phosphate chemistry[5] have been reported during the past decades, to our knowledge, no application to the synthesis of **1** has been demonstrated. Its extreme acid lability[6] and lack of a neighboring group at the C2-position, important for increasing the anomeric selectivity,[5a] have prevented success. Only one application based on the known methodology reported by MacDonald[6a] resulted in modest selectivity and low yield.[7] These results suggest the urgent need for a new concept for sugar 1-phosphate synthesis that is applicable to the synthesis of **1**.

**SCHEME 1**




Crystallization-induced asymmetric transformation (AT)[8] is a promising methodology for syntheses of unnatural amino acids,[9] stereogenic heteroelements,[10] and other chiral molecules.[11] Crystallization of one isomer from an equilibrating diastereomeric mixture would likely provide a realistic solution for syntheses of the sugar 1-phosphates. However, this reaction requires highly restrictive conditions. One requirement is the faster interconversion of diastereomers than crystallization of one diastereomer.[10] The instability of **1** is based on the rapid dephosphorylation to afford an oxonium cation form under low pH[1a,12] that would accelerate the interconversion between the α- and *β*-anomers. As illustrated in Scheme 1, in the presence of water, the oxonium cation form is rapidly hydroxylated to stop the interconversion.[13] It is also immediately transformed to a furan derivative[14] by dehydration and aromatization, even at room temperature, that undergoes further degradation dependent on the reaction conditions.[15] Thus, strict control of the anhydrous condition, low temperature, and proper pH will enable a rapid interconversion suitable for AT for the synthesis of **1**. Despite many examples of acidic isomerization of sugar 1-phosphates,[16] no further development according to this strategy has yet been examined. Another requirement for AT is the

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(2) Friedkin, M. *J. Biol. Chem.* **1950**, *184*, 461.

(3) For a review, see: Zorbach, W. W. *Synthesis* **1970**, *7*, 329.

(4) Komatsu, H.; Awano, H.; Tanikawa, H.; Itou, K.; Ikeda, I. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1291.

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(7) The yield and the specific rotation of the product in ref 6a were 26% and $[\alpha]^{20}_D$ +23.6 (*c* 2, $H_2O$), respectively, after a preliminary purification. An enzymatically prepared sample in ref 1c showed $[\alpha]^{20}_D$ +38.8 (*c* 2, $H_2O$).

(8) For a review, see: Caddick, S.; Jenkins, K. *Chem. Soc. Rev.* **1996**, 447.

(9) For most recent reports: (a) Kolarovic, A.; Berkes, D.; Baran, P.; Povazanec, F. *Tetrahedron Lett.* **2001**, *42*, 2579. (b) Boesten, W. H. J.; Seerden, J.-P. G.; de Lange, B.; Dielemans, H. J. A.; Elsenberg, H. L. M.; Kaptein, B.; Moody, H. M.; Kellogg, R. M.; Broxterman, Q. B. *Org. Lett.* **2001**, *3*, 1121.

(10) For most recent reports: Vedejs, E.; Chapman, R. W.; Lin, S.; Muller, M.; Powell, D. R. *J. Am. Chem. Soc.* **2000**, *122*, 3047.

(11) For most recent reports, see: (a) Kanomata, N.; Ochiai, Y. *Tetrahedron Lett.* **2001**, *42*, 1045. (b) Olszewska, T.; Milewska, M. J.; Gdaniec, M.; Maluszynska, H.; Polonski, T. *J. Org. Chem.* **2001**, *66*, 501.

(12) In ref 6b, it is reported that 2-deoxyribosyl-1-α-phosphate is dephosphorylated within a few minutes at pH 4 at rt while ribosyl-1-α-phosphate is stable under the same conditions. In our observation, the pH for the stable state was more than 8.

(13) For a mechanism of acidic hydrolysis, see: Bunton, C. A.; Humeres, E. *J. Org. Chem.* **1969**, *34*, 572 and references therein.

(14) For an example of pyrolytic elimination to a furan derivative, see: Ness, R. K.; MacDonald, D. L.; Fletcher, Jr., H. G. *J. Org. Chem.* **1961**, *26*, 2895.

(15) When McDonald's direct condensation with phosphoric acid (see: MacDonald, D. L. *J. Org. Chem.* **1962**, *27*, 1107) was applied to the synthesis of **1** starting from triacetyl 2-deoxyribose, the reaction immediately turned to black tar right above the room temperature.

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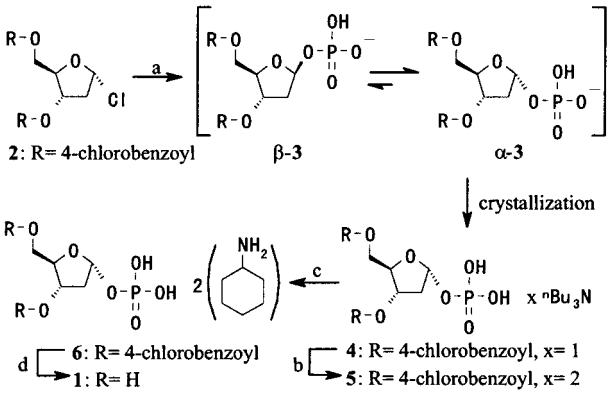
**SCHEME 2**[a]




[a] Reagents and conditions: (a) 3.5 equiv of *o*-$H_3PO_4$, 1.2 equiv of $^nBu_3N$, 4 Å MS, acetonitrile, −5 °C, α-**3**/*β*-**3** = 98.5:1.5; (b) 3.5 equiv of $^nBu_3N$; (c) cyclohexylamine, 99%; (d) cyclohexylamine, MeOH, 92% (85% for recrystallization).

specific crystallization of the target diastereomer.[10] A proper salt formation of the phosphate and selection of a crystallizable protective group for the sugar hydroxyl groups can be key factors to apply AT to sugar 1-phosphate syntheses. Here, we report a novel application of AT to the first stereoselective synthesis of **1**.

As depicted in Scheme 2, reaction of chlorosugar **2**, prepared from 2-deoxy-D-ribose according to the reported method,[17] with orthophosphoric acid (3.5 equiv) and tri(*n*-butyl)amine (1.2 equiv) in various solvents in the presence of 4 Å molecular sieves (pellets)[18] at −5 °C initially produced α-phosphate (α-**3**) and *β*-phosphate (*β*-**3**) in a ratio of 49:51 with no anomeric selectivity according to HPLC analysis. The result reflects the low selectivity observed in a previous report.[6a] The reaction proceeds by $S_N2$ displacement of Cl by phosphoric acid with inversion at the anomeric carbon of **2**, but rapid anomerization of **2** in polar solvents results in low selectivity.[19] However, the possibility of a complete inversion followed by rapid anomerization of the resulting *β*-**3** cannot be excluded. Under acidic conditions due to excess phosphoric acid, equilibration between α-**3** and *β*-**3** occurred, gradually inclining to the thermodynamically more stable α-**3**. In DMF solvent, the reaction equilibrated at a ca. 2:1 ratio of α-**3**/*β*-**3**. In 4-methyl-2-pentanone, the ratio was further upgraded by crystallization of mono(tributylamine) phosphate (**4**) out of the reaction solution, giving α-**3** and *β*-**3** at a ratio of 91:9. Clear evidence of AT is shown in Figure 1. In various solvents, the α-anomer ratio increased dependent on the reaction time. However, beyond the ratio of ca. 2:1 (α-**3**/*β*-**3**), the selection of amine and solvent was crucial for the induction of the α/*β* selectivity at the anomeric position. AT was not observed using either triethylamine

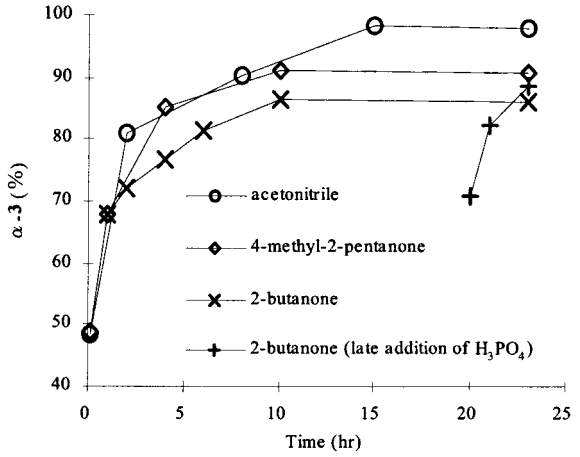



**FIGURE 1.** Time-course of crystallization-induced asymmetric transformation in various solvents in the presence of orthophosphoric acid (3 equiv) and tri(*n*-butyl)amine (1 equiv): acetonitrile (○), 4-methyl-2-pentanone (◇), 2-butanone (×), 2-butanone, after 20 h of reaction time with 1 equiv of orthophosphoric acid, 2 equiv of orthophosphoric acid was added (+).

or pyridine in lieu of tri(*n*-butyl)amine. In acetonitrile, the reaction resulted in the highest selectivity (a 98.5:1.5 ratio of α-**3**/*β*-**3**) as a consequence of solidification of the reaction mixture. Increased acidity accelerated the reaction as shown in Figure 1 [compare the result of (×) with (+)]. When 1.2 equiv of orthophosphoric acid was initially used in 2-butanone, the α-**3**/*β*-**3** ratio was 71:29 even after 20 h of reaction time, and no crystallization was observed. However, immediately after the addition of 2.3 equiv of orthophosphoric acid into the reaction mixture, the crystallization of **4** out of the reaction solution occurred, and subsequently increased the selectivity to a 89:11 ratio of α-**3**/*β*-**3** within 3 h.

Since mono(tributylamine) phosphate (**4**) was too labile to isolate directly from the reaction mixture, 3.5 equiv of tri(*n*-butyl)amine was added to neutralize the reaction mixture before the workup.[12] The resulting bis(tributylamine) phosphate (**5**) was stable enough for an aqueous workup. Extraction with 4-methyl-2-pentanone followed by rinsing of the organic phase with water to remove phosphate impurities was performed. From the organic solution, phosphate **6** was isolated as a bis(cyclohexylamine) salt in 99% yield, and the α- and *β*-anomer ratio was 98.8:1.2 in HPLC assay. The resulting **6** contained no phosphate impurities according to $^{31}P$ NMR analysis. Without further purification, **6** was debenzoylated by using cyclohexylamine in methanol at room temperature to afford bis(cyclohexylamine) salt of the desired 2-deoxy-α-D-ribosyl-1-phosphate (**1**) in 92% yield. The resulting phosphate (**1**) contained no phosphate impurities according to $^{31}P$ NMR analysis, and its specific rotation indicated $[\alpha]^{25}_D$ +37.3 (*c* 3, $H_2O$).[7] Recrystallization from aqueous methanol−diethyl ether afforded analytically pure **1** as bis(cyclohexylamine) salt in 85% yield, and its specific rotation was improved to $[\alpha]^{25}_D$ +41.4 (*c* 3, $H_2O$) that was comparable with the reported value of the sample synthesized by an enzymatical degradation of 2′-deoxyguanosine.[1,7] The structure was confirmed by spectroscopic methods ($^1H$, $^{13}C$, and $^{31}P$ NMR and IR), mass spectral analysis, and elemental analysis in comparison

(16) (a) MacDonald, D. L. *Carbohydr. Res.* **1966**, *3*, 117. (b) Perchemlides, P.; Osawa, T.; Davidson, E. A.; Jeanloz, R. W. *Carbohydr. Res.* **1967**, *3*, 463.

(17) (a) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777. (b) Wang, Z.-X.; Duan, W.; Wiebe, L. I.; Balzarini, J.; De Clercq, E.; Knaus, E. E. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 11.

(18) Fine powder molecular sieves was not suitable to induce the desired crystallization of the target diastereomer (**4**).

(19) For a mechanism of $S_N2$ attack of nucleoside bases to **2**, see: Hubbard, A. J.; Jones, A. S.; Walker, R. T. *Nucleic Acids Res.* **1984**, *12*, 6827.

with commercially available **1** [bis(cyclohexylamine) salt] that was enzymatically prepared.[20]

Crystallization-induced asymmetric transformation has been applied to the stereoselective synthesis of bis(cyclohexylamine) 2-deoxy-α-D-ribosyl-1-phosphate (**1**) that is a key compound for the enzymatic preparation of 2′-deoxynucleosides.[1c,2] Further modification to establish a more practical and scalable method is under investigation and will be reported elsewhere.

## Experimental Section

**General Methods.** Melting points are uncorrected. The $^1$H NMR chemical shifts are described as $\delta$ values in ppm relative to TMS as an internal standard. The $^{13}$C NMR chemical shifts are reported as $\delta$ values in ppm relative to methanol. The $^{31}$P NMR chemical shifts are described as $\delta$ values in ppm downfield from 85% $H_3PO_4$ as an external standard.

**Synthesis of Bis(cyclohexylamine) 3′,5′-*O*-Bis(4-chlorobenzoyl)-2-deoxy-α-D-ribosyl-1-phosphate (6).** A mixture of 98% *o*-$H_3PO_4$ (6.92 g, 6.92 mmol), $Bu_3N$ (5.51 mL, 23.1 mmol), and 4 Å MS (10 g, pellets) in $CH_3CN$ (80 mL) was stirred at rt overnight. 3′,5′-*O*-Bis(4-chlorobenzoyl)-2-deoxy-α-D-ribosyl 1-chloride (**2**)[17] (8.5 g, 19.8 mmol) was added at −5 °C, and the reaction mixture was stirred for 23 h. $Bu_3N$ (16.5 mL, 69.3 mmol) was added at −5 °C. The molecular sieves were filtered off, and the filtrate was concentrated at rt. The residue was dissolved in 4-methyl-2-pentanone and rinsed with purified water three times. After filtration of the organic phase to remove very fine impurities, cyclohexylamine (5.66 mL, 49.5 mmol) was added at 0 °C, and the mixture was stirred for 2 h. The resulting precipitates were collected, rinsed with 4-methyl-2-pentanone and acetone successively, and dried in vacuo at 40 °C to give 13.5 g (99%) of **6** as a colorless solid: mp 179−180 °C dec; $[\alpha]^{25}_D$ 45.6 (*c* 1.23, methanol); IR (KBr) 2938, 2859, 1721 cm$^{-1}$; NMR $\delta_H$ (400 MHz, $CD_3OD$) 8.05 (2 H, ddd, $J$ = 2.2, 2.2, 8.8 Hz), 7.99 (2 H, ddd, $J$ = 2.2, 2.2, 8.8 Hz), 7.456 (2 H, ddd, $J$ = 2.2, 2.2, 8.8 Hz), 7.455 (2H, ddd, $J$ = 2.2, 2.2, 8.8 Hz), 5.98 (1H, ddd, $J$ = 0.8, 5.1, 6.3 Hz), 5.46 (1H, ddd, $J$ = 1.8, 3.3, 7.7 Hz), 4.65 (1H, ddd, $J$ = 4.2, 4.2, 4.2 Hz), 4.62 (1H, dd, $J$ = 4.2, 11.5 Hz), 4.51 (1H, dd, $J$ = 4.2, 11.5 Hz), 2.96 (2H, m), 2.56 (1H, dddd, $J$ = 0.8, 5.1, 7.7, 14.4 Hz), 2.37 (1H, dddd, $J$ = 0.8, 0.8, 1.8, 14.4 Hz), 1.98 (4H, m), 1.76 (4H, m), 1.61 (2H, m), 1.28−1.1 (8H, m), 1.17 (2H, m); NMR $\delta_C$ (100 MHz, $CD_3OD$) 167.1, 166.8, 140.7, 140.6, 132.5, 132.2, 129.9, 129.8, 101.1 (d, $J$ = 4.1 Hz), 83.0, 76.9, 65.9, 51.2, 41.3 (d, $J$ = 6.6 Hz), 32.8, 26.1, 25.6; NMR $\delta_P$ (162 MHz, $CD_3OD$) 1.35; MS [APCI (−)] *m*/*z* 489 (M − 2)$^-$; HPLC $t_R$ 14.8 min (α-isomer); $t_R$ 18.0 min (β-isomer) [column: YMC Pack AM312 (ODS-AM) (150 cm × 6.0 mm) (from YMC, Co. Ltd.), mobile phase: 10 mM $NaH_2PO_4$−MeOH (35:65), flow rate: 1.0 mL/min, UV wavelength: 254 nm, column temperature: 40 °C], α-**3**/β-**3** = 98.8:1.2.

**Synthesis of Bis(cyclohexylamine) 2-Deoxy-α-D-ribosyl-1-phosphate (1).** A solution of **6** (7.05 g, 10.2 mmol) and cyclohexylamine (2.92 mL, 25.6 mmol) in MeOH (106 mL) was stirred at rt for 72 h. The reaction mixture was concentrated at 10 °C, and EtOH was added. The resulting precipitates were collected, rinsed with EtOH, and dried in vacuo at rt to give 3.87 g (92%) of crude **1** as a colorless solid: $[\alpha]^{25}_D$ 37.3 (*c* 3.0, $H_2O$). The crude **1** (495 mg, 1.20 mmol) was recrystallized from MeOH−$Et_2O$ at −15 °C and dried in vacuo at rt for 2 days to give 423 mg (85%) of **1** as a colorless solid: mp 168−169 °C dec; $[\alpha]^{25}_D$ 41.4 (*c* 3.0, $H_2O$); IR (KBr) 2940, 2725, 2239, 1631 cm$^{-1}$; NMR $\delta_H$ (400 MHz, $D_2O$) 5.60 (1H, dd, $J$ = 5.2, 5.2 Hz), 4.07 (1H, ddd, $J$ = 2.9, 3.6, 7.2 Hz), 4.04 (1H, ddd, $J$ = 3.6, 3.6, 3.6 Hz), 3.56 (1H, dd, $J$ = 3.6, 12.2 Hz), 3.45 (1H, dd, $J$ = 3.6, 12.2 Hz), 2.99 (2H, m), 2.20 (1H, ddd, $J$ = 5.2, 7.2, 14.4 Hz), 1.93 (1H, ddd, $J$ = 1.3, 2.9, 14.4 Hz), 1.83 (4H, m), 1.64 (4H, m), 1.50 (2H, m), 1.26−1.1 (8H, m), 1.12 (2H, m); NMR $\delta_C$ (100 MHz, $D_2O$) 100.2 (d, $J$ = 4.1 Hz), 86.7, 72.0, 62.4, 51.1, 42.5 (d, $J$ = 5.0 Hz), 31.1, 25.1, 24.6; NMR $\delta_P$ (162 MHz, $D_2O$) 1.48; MS [APCI (−)] *m*/*z* 213 (M − 1)$^-$. Anal. Calcd: C, 49.50; H, 9.04; N, 6.79; P, 7.51. Found: C, 49.26; H, 8.81; N, 6.64; P, 7.29.

**Supporting Information Available:** Spectral data of compounds **1** [bis(cyclohexylamine) salt] and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Available from Sigma-Aldrich Co.