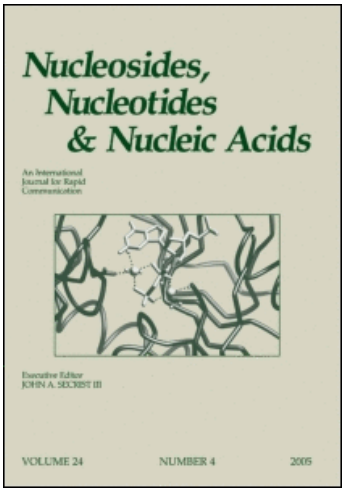

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Efficient Chemo-Enzymatic Syntheses of Pharmaceutically Useful Unnatural 2'-Deoxynucleosides

Hironori Komatsu[a]; Tadashi Araki[a]
[a] Catalysis Science Laboratory, Mitsui Chemicals, Inc., Chiba, Japan

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EFFICIENT CHEMO-ENZYMATIC SYNTHESES OF PHARMACEUTICALLY USEFUL UNNATURAL 2′-DEOXYNUCLEOSIDES

Hironori Komatsu and Tadashi Araki ▫ *Catalysis Science Laboratory, Mitsui Chemicals, Inc., Chiba, Japan*

▫ *Our chemo-enzymatic method was successfully applied to the synthesis of 2-chloro-2′-deoxyadenosine (CdA, cladribine) in two ways: 1) direct conversion of chemically synthesized 2-deoxy-α-D-ribose 1-phosphate (dRP) to CdA; 2) a two-step route via 9-(2-deoxy-β-D-ribos-1-yl)-2,6-dichloropurine (Cl_2Pu-dR, **5**).*

Keywords 2-Deoxy-α-D-Ribose 1-Phosphate, dRP, 2-Chloro-2′-Deoxyadenosine, Cladribine, Purine Nucleoside Phosphorylase, Glycosylation

INTRODUCTION

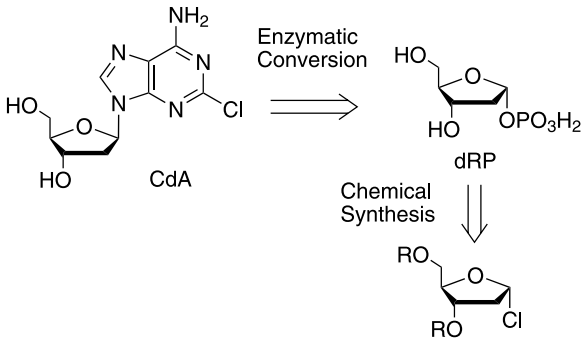
Efficient synthetic methods for 2′-deoxynucleosides (dNus) has been under development over the past few decades. We previously reported a chemo-enzymatic method and its application to the syntheses of natural dNus.[1,2] The method consists of three distinctive technologies: 1) stereoselective synthesis of 2-deoxyribose 1-α-phosphate (dRP) by crystallization-induced asymmetric transformation;[3] 2) an efficient method to expedite an enzymatic conversion by adding $Mg(OH)_2$; 3) development of a new enzyme for 2′-deoxycytidine. To expand the application of this method,[4] syntheses of various pharmaceutically useful unnatural dNus such as CdA* have been examined (Scheme 1).

RESULTS AND DISCUSSIONS

Phosphate (**1**) was stereoselectively synthesized as described.[3] Preparation of dRP, however, was slightly modified, since its cyclohexylammonium salt was highly soluble in MeOH and tedious isolation steps were required. Deprotection of **1** by

*For previous syntheses of CdA, see: Ref. [5].

Address correspondence to Hironori Komatsu, Catalysis Science Laboratory, Mitsui Chemicals, Inc., Sodegaura-shi, Chiba, Japan.



SCHEME 1

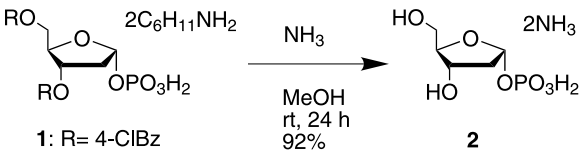
NH_3/MeOH gave an ammonium salt of dRP (**2**) as crystals directly from the reaction solution (Scheme 2).

A direct enzymatic glycosylation pathway was first examined using **2** (Scheme 3). Glycosylation of 2-chloroadenine (**4**) with **2** would be the simplest synthetic route to CdA. Difficulties in the preparation of dRP prevented its application. Our chemo-enzymatic method accomplished this synthetic strategy. As previously reported,[6] **4** was prepared by amination of **3** in NH_3/MeOH. The reaction required high temperature (160°C) in a sealed tube and long reaction time (24 h). Unlike the reported method, simple filtration was sufficient to obtain **4** in pure form. Enzymatic glycosylation of **4** with **2** was performed in H_2O at 45°C in the presence of purine nucleoside phosphorylase (PNPase, self-cloned in *E. coli*)† and $Mg(OH)_2$ in 98% HPLC yield. Recrystallization from MeOH afforded pure CdA in 88% isolated yield.

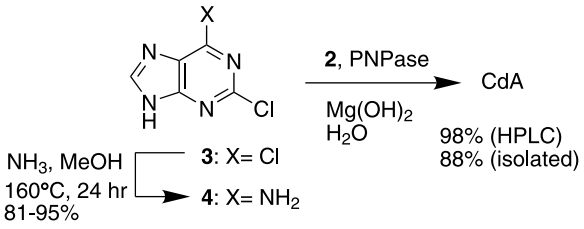
A two-step synthetic route via Cl_2Pu-dR (**5**) was next investigated (Scheme 4). The same glycosylation condition as described above was carried out first in the presence of $Mg(OH)_2$. The slightly alkaline condition partially hydrolyzed or ammonolyzed the 6-Cl group of **3**. Without $Mg(OH)_2$, sparingly soluble **5** crystallized directly from the reaction solution, which facilitated the enzymatic conversion. Thus, enzymatic glycosylation of **3** with **2** in H_2O at 45°C in the presence of PNPase gave **5** in high isolated yield (91%). Ammonolysis of **5** was performed in NH_4OH/CH_3CN. In contract to the ammonolysis of **3** or acetylated **5**,* the reaction proceeded at moderate temperature to give 98% HPLC yields. The relative hydrophilic property of **5**, compared to **3** or acetylated **5**, increased its reactivity in a polar reaction medium (NH_4OH/CH_3CN). This made two-step route advantageous. Finally, treatment with anion exchange resin [IER ($^-$OH)] followed by recrystallization from EtOH gave CdA in 79% isolated yield.

In summary, synthesis of CdA was successfully performed using our novel chemo-enzymatic strategy. Two synthetic routes were demonstrated. One is a direct

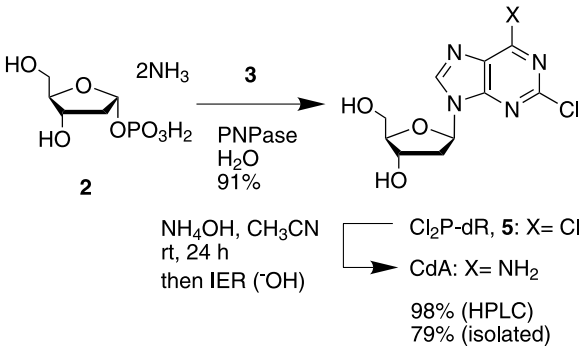
†For a preparation of PNPase, see: Refs. [7,8].



SCHEME 2



SCHEME 3



SCHEME 4

enzymatic glycosylation pathway, and the other, a glycosylation-amination pathway. This strategy will be useful as an efficient alternative method for the syntheses of various unnatural 2′-deoxynucleosides.

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