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First synthesis of enantio-uracil dinucleotide, comparison of physicochemical properties of their enantiomers, and separation by chiral column chromatography

Takanori Miyashita, Shinji Sakata and Hiroyuki Hayakawa*

Chemistry & Pharmacology Laboratory, Biochemicals Division, Yamasa Corporation, 10-1, Araoicho 2-Chome, Choshi, Chiba-Ken 288-0056, Japan

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Abstract—Enantio-uracil dinucleotide **5**, which consists of two L-uridylic acids and one pyrophosphate, was synthesized for the first time in our laboratory. Benzolyated L-uridine was prepared by a stereoselective glycosylation of silylated uracil with L-1-*O*-acetyl-2,3,5-tri-*O*-benzoylribose (L-ABR **7**). After deprotection, L-uridine **9** was converted to P^1,P^4-di(L-uridine 5′-) tetraphosphate tetrasodium salt (L-UP_4U **5**) by treatment of L-UMP morpholidate **10c** with triethylammonium pyrophosphate (TEA-PPi **11b**). Spectral data of synthesized L-UP_4U **5** are given in the references. All spectral data were identical with those of UP_4U **3** except the specific rotation, which showed a positive value compared to UP_4U **3** having a negative value. Furthermore, the separation by chiral column chromatography was investigated.

Purine and pyrimidine (P2) receptors are attractive drug targets.[1] We are interested in dinucleotide derivatives because they have both P2 receptor agonist and antagonist activities. Among them, we also have focused on the preparation of uracil dinucleotides **1**–**4** (Fig. 1) and elucidated their structures including physicochemical properties.[2]

P^1,P^4-Di(uridine 5′-) tetraphosphate (**3**, uracil dinucleotide, UP_4U) has been developed as a drug for the treatment of Dry Eyes disease and chronic obstructive pulmonary disease.[3]

However, it is known that these dinucleotides are unstable to enzymatic degradation. Additionally, new methods for the analysis of stereoisomers in relation to impurities were required.

On the contrary, it is considered that enantio dinucleotides will be probably stable to enzymatic degradation. Furthermore, both enantio dinucleotides and *meso* dinucleotides are also considered as attractive targets for the exploration of new probes of P2Y ligands. However, there are no reports on the synthesis and analysis of these dinucleotides.

As a part of our program to study both chirality and physicochemical properties of these compounds, we planned to make L-UP_4U **5** (Fig. 2). The present report

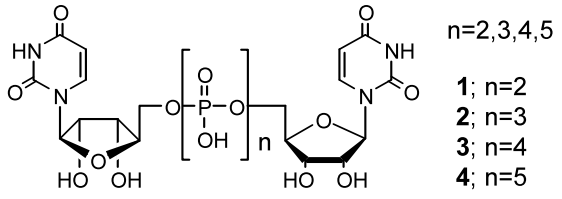

Figure 1.

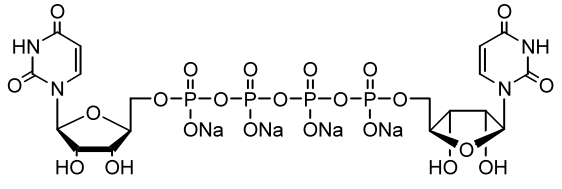

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Figure 2.

Keywords: enantio-uracil dinucleotide; uracil dinucleotide; L-uridylic acids; TEA-PPi; specific rotation; separation; HPLC; chiral column chromatography; stereoisomers.

* Corresponding author. Tel.: 81-479-22-0095; fax: 81-479-22-9821; e-mail: hayakawa@yamasa.com

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describes the first synthesis of L-UP_4U **5** bearing the L-ribose moiety, comparison of physicochemical properties in enantiomers, and then separation of enantiomers by chiral column chromatography.

Synthesis of L-UP_4U 5

Preparation of L-ABR 7 and L-uridylic acid (L-UMP 10b): L-1-*O*-Acetyl-2,3,5-tri-*O*-benzoylribose (L-ABR **7**) was made by the modified method used for the preparation of peracylated sugars from L-ribose **6**, and then it was derived to protected L-uridine **8** by stereoselective glycosylation of silylated uracil with L-ABR **7**.[4]

After deprotection of L-2,3,5-tri-*O*-benzoyluridine **8** with sodium methoxide (NaOMe) to give L-uridine **9**, this compound was converted to the corresponding L-uridylic acid **10** by the improved method[5] used for the preparation of uridine 5′-monophosphophoric acid (UMP) (Scheme 1).

Preparation of L-UMP morpholidate 10c: L-Uridine 5′-monophosphoric acid, triethylammonium salt (L-UMP, 2TEA **10a**) in deionized water was acidified to uridine-5′-monophosphoric acid (L-UMP, free, **10b**) by passing through a cation-exchange resin (H^+ form). L-UMP, free was converted to its morpholidate **10c** with morpholine and dicyclohexyl carbodiimide (DCC).

Preparation of TEA-PPi 11b: In a similar manner, pyrophosphoric acid, tetrasodium salt (PPi, 4Na, **11a**) was acidified by passing through a cation-exchange resin, then neutralized with triethylamine to give a triethylammonium salt (TEA-PPi **11b**).

Dehydrated L-UMP morpholidate **10c** was treated with TEA-PPi **11b** in pyridine to afford a crude mixture containing L-UP_4U **5**.

L-UP_4U **5** was purified twice by chromatography, first on an anion-exchange resin, and next on charcoal. The appropriate fractions containing L-UP_4U **5**, after adjusting pH with NaOH, were concentrated to crystallize L-UP_4U **5** (Scheme 2).

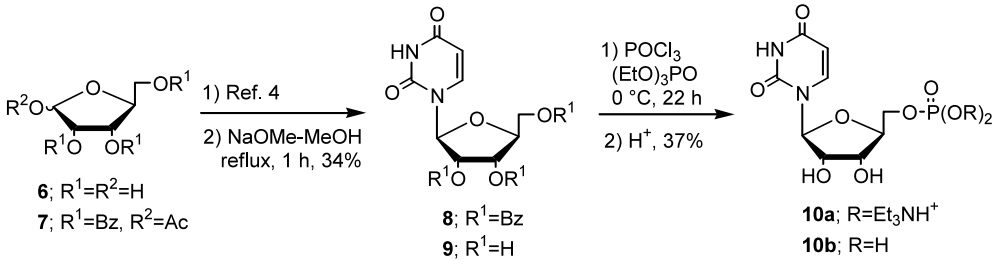
After recrystallization from aqueous ethanol, L-UP_4U **5** was obtained in 0.4% overall yield, HPLC (99.4%).[6,7]

Elucidation of the structure of L-UP_4U 5: The structure of L-UP_4U **5** was confirmed by ^1H, ^{13}C, ^{31}P NMR MS, UV, IR, elemental analysis (EA), and specific rotation. The spectral data of L-UP_4U **5** are summarized in Ref. 6, and other data such as mp and EA are noted in Ref. 7.

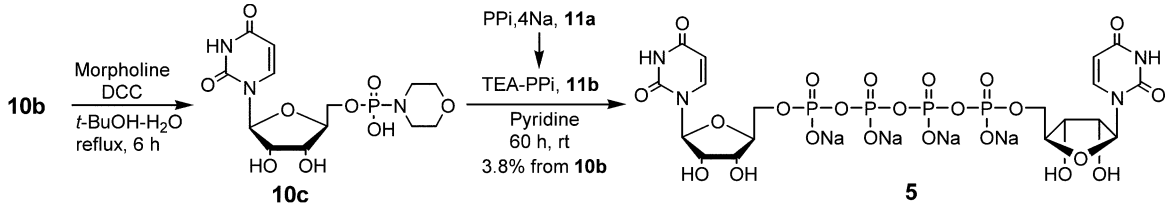
All spectral data were identical with those of UP_4U **3** except the specific rotation, which showed +8.5° (*c* 1, water, 20°C) compared to that of UP_4U (–9.5°). The melting point of L-UP_4U **5** showed a slightly higher temperature (228°C).

Separation of enantiomers (L-UP_4U 5 and UP_4U 3) by HPLC

Initially, we tried to distinguish several enantiomers (nucleosides or 5′-mononucleotides, which were components of the desired dinucleotides) by HPLC through these studies. However, we could not separate enantiomers such as UMP and L-UMP (uridine and L-uridine) by using even chiral column chromatography. Interestingly, enantiomer (L-UP_4U **5**) was separated from D-derivative **3** by the same technique. Mixtures of UP_4U **3** and L-UP_4U **5** were analyzed by HPLC using YMC chiral β-CD BR column (see operating condition). Figure 3 shows the HPLC profile of a mixture of L-UP_4U **5** and UP_4U **3** with the ratio of 1:1. L-UP_4U **5** was eluted at 18.49 min of retention time about 2 min faster than UP_4U **3**.



Scheme 1.



Scheme 2.

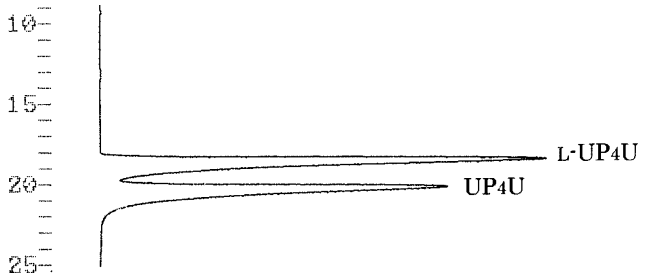


Figure 3. Operating condition. Detector: spectrophotometer (wavelength: 262 nm). Column: A stainless steel column (4.6 mm I.D.×25 cm L) (5 μm, CHIRAL β-CD BR, YMC). Column temperature: room temperature. Mobile phase: mixture of K-P (potassium phosphate 80 mM) and TBAS (tetrabutylammonium hydrogen sulfate 20 mM) in water (pH 6.7). Flow rate: 0.5 mL/min.

These results suggest the possibility that chiral HPLC will become a useful technique to elucidate the stereoisomers of these dinucleotides. Therefore, we can identify a large number of impurities and the desired products related to these dinucleotides.

Stability of L-UP_4U 5 to enzymatic degradation

Enantiomers were treated with two enzymes. UP_4U **3** was completely degraded when exposed to 5′-phosphodiesterase[8] and subsequent acid phosphatase in aqueous ammonium acetate (37°C, pH 5.0). On the contrary, L-UP_4U **5** was stable to these enzymatic reactions.

In summary, we have prepared enantio-uracil dinucleotide **5** for the first time. All spectral data were identical with those of UP_4U **3** except the specific rotation. The melting point of the enantiomer showed slightly higher temperature. The chiral column chromatography was effective to separate these enantiomers. Enantio-uracil dinucleotide **5** is resistant to enzymatic degradation. According to these results, L-dinucleotides might be new probes of P2Y ligands as agonists and/or antagonists.

Further investigations are in progress in our laboratory.

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6. Spectral data: IR (KBr) 3380 cm^{-1}, 1690 cm^{-1}, 1250 cm^{-1}, 1140 cm^{-1}, 1113 cm^{-1}, 890 cm^{-1}; FAB$^+$MS 879 $(M+H)^+$; ^{1}H NMR (D_2O) δ_{ppm} 7.97 (2H, d, H-6), 6.00 (2H, d, H-1′), 5.98 (2H, d, H-5), 4.43 (2H, m, H-3′), 4.39 (2H, m, H-2′), 4.29 (2H, m, H-4′), 4.25 (2H, m, H_2-5′); ^{13}C NMR (D_2O) δ_{ppm} 169.3 (C-4), 154.9 (C-2), 144.5 (C-6), 105.6 (C-5), 91.0 (C-1′), 86.4 (C-4′), 76.6 (C-2′), 72.6 (C-3′), 67.9 (C-5′); ^{31}P NMR (D_2O) δ_{ppm} −10.6 (P-1), −22.2 (P-2); UV (0.01N HCl) λmax 262 nm. $[\alpha]_D^{20}=+8.5°$ (*c* 1, water, 20°C).
7. Other data. Anal. calcd for ($C_{18}H_{22}N_4Na_4O_{23}P_4\cdot 3.5H_2O$): C, 22.97; H, 3.11; N, 5.95. Found: C, 22.96; H, 3.13; N, 5.92; mp 228°C (dec.).
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