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The Synthesis of Amino Acid Methyl Ester 5'-Phosphoamidates of Protected Uridine

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THE SYNTHESIS OF AMINO ACID METHYL ESTER 5'-PHOSPHOAMIDATES OF PROTECTED URIDINE

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In this article, we used the Atherton–Todd reaction to synthesize amino acid methyl ester 5'-phosphoamidates of uridine as prodrugs. Their structures were confirmed by ¹H NMR, ³¹P NMR, ¹³C NMR, IR, and mass spectrometry.

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements to view the free supplemental file.

Keywords Amino acid methyl ester 5'-phosphoamidates of protected uridine; Atherton–Todd; spectra characterization; synthesis

INTRODUCTION

Nucleosides and their analogues as potential anticancer and antivirus agents have been studied extensively in recent years.^{1–3} For example, several purine and pyridine bases and nucleoside analogs were used as a chemotherapeutic arsenal. However, the biological activity of most analogs requires intracellular metabolism to 5'-mononucleotides by kinase.⁴ In order to overcome the problem of drug resistance and improve membrane penetration, a series of amino acid phosphoramidate di- and tri-esters of nucleotides have been developed to deliver phosphorylated nucleoside analogues as neutral derivatives into the cell.⁵ These types of compounds exhibit enhancing antiviral activity and reducing cytotoxicity as compared to the corresponding parent nucleosides.^{6,7} For the purpose of developing new types of prodrugs, a new type of amino acid methyl ester 5'-phosphoamidates of nucleoside was synthesized, and is reported in this article.

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RESULTS AND DISCUSSION

Synthesis

The reported synthesis of O-nucleoside N-phosphoryl amino acid methyl esters involved the coupling of amino acid methyl esters with nucleoside monophosphates in the presence of condensing reagents such as dicyclohexylcarbodiimide (DCC) and 2,4,6triisopropylbenzenesulfonyl chloride,⁸ or with nucleoside phosphite triesters in the presence of iodine.⁹ Phosphorochloridate approach was also reported in the preparation of aryl phosphate derivative of nucleoside analogs.¹⁰ The amino acids chosen, however, were usually the nonpolar ones without active side chains. In the present article, we report the application of the Atherton–Todd¹¹ reaction in the synthesis of O-alkyl O-2',3'-isopropylideneuridine N-phosphoryl amino acid methyl ester (Scheme 1). The present methodology was applicable to amino acid methyl esters with different side chains and with either of the nucleosid-3' (or 5')-yl H-phosphonates. Owing to the low nucleophilic reactivity of amino groups on the nucleobases and the hydroxyl on both the amino acid and the nucleoside compared with the α -amino group, it was unnecessary to protect them in the coupling reaction.



Scheme 1 Synthetic route of amino acid methyl ester 5'-phosphoamidates of protected uridine. Reagents and conditions: (i) NEt₃, dry CH₂Cl₂, 0° C for 0.5 h, then room temperature for 3 h; (ii) dry CH₂Cl₂, menthol, *tert*-butyl alcohol, 0° C, then room temperature for 0.5 h; (iii) NEt₃, dry CH₂Cl₂, 0° C, then room temperature for 10 min; (iv) CCl₄, NEt₃, H₂O, CH₃CN, amino acid methyl ester, room temperature.

³¹P NMR Spectra

The ³¹P nucleus is a very sensitive probe for structural studies of organophosphorus compounds. The ³¹P NMR spectra of 2',3'-isopropylidenenucleoside-5'-O-yl-Hphosphonate and O-alkyl O-2',3'-isopropylideneuridine N-phosphoryl amino acid methyl ester are in the range of 5 to about 10 ppm. Due to the chirality at the phosphorus center, they both exhibit two different chemical shift values in the ³¹P NMR spectra (Figures 1a and 1b), and the integral areas of ³¹P NMR are basically the same if protons are decoupled.

But if the protons are not decoupled, nucleoside-5'-O-yl-H-phosphonate (Figure 1a) has a distinct different chemical shift value from uridine N-phosphoryl amino acid methyl ester (Figure 2a). It exhibits four different chemical shift values in the ³¹P NMR spectra. This is because the phosphorus in nucleoside-5'-O-yl-H-phosphonate was coupled by the hydrogen on the phosphorus. The coupling constant ${}^{1}J_{PH}$ is about 700 Hz in nucleoside-5'-O-yl-H-phosphonate. For example, the coupling constant ${}^{1}J_{PH}$ is 703 Hz in menthyl 2',3'-isopropylideneuridin-5'-yl-H-phosphonate (**3**). But in O-alkyl O-2',3'-isopropylideneuridine N-phosphoryl amino acid methyl ester, although the hydrogen on the nitrogen has a coupling effect on the phosphorus, ${}^{2}J_{PH}$ is much smaller than ${}^{1}J_{PH}$, and the coupling splitting is not distinct. This is the difference between ${}^{31}P$ NMR spectra of nucleoside-5'-O-yl-H-phosphonate and O-alkyl O-2',3'-isopropylideneuridine N-phosphorate Albara Al

¹H NMR Spectra

2',3'-Isopropylideneuridine-5'-O-yl-H-phosphonate and O-alkyl O-2',3'-isopropyli deneuridine N-phosphoryl amino acids methyl esters are a pair of diastereoisomers due to the chirality at the phosphorus center. Their ¹H NMR spectra are all pairs of peaks,



Figure 1 The ³¹P NMR spectrum of menthyl 2',3'-isopropylideneuridin-5'-yl-H-phosphonate (3).



Figure 2 The ³¹P NMR spectrum of O-nucleoside N-phosphoryl amino acids methyl esters (4a).

especially the H-6 of the nucleoside and methyl ester of the amino acid. For example, OCH_3 on the compound **4h** exhibits two single peaks. Their chemical shift values are 3.71 and 3.70.

¹³C NMR Spectra

Due to the chirality at the phosphorus center, 2',3'-isopropylideneuridine-5'-O-yl-Hphosphonate and O-alkyl O-2',3'-isopropylideneuridine N-phosphoryl amino acids methyl esters are a pair of diastereoisomer, so some carbon atoms exhibit double peaks in the ¹³C NMR spectra. For example, C-6 on the compound **4h** exhibits two single peaks. Their chemical shift values are 141.69 and 142.00.

Mass Spectra

Positive-ion mode electrospray ionization (ESI) mass spectral fragmentation pathways of 2',3'-isopropylideneuridine phosphates were analyzed by multistage tandem mass spectrometry. Tables S1 and S2 (Supplemental Materials, available online) list the fragment ions observed in multistage tandem mass spectra of the protonated and sodium ion adducts of **4a–j**, respectively.

Because these compounds displayed very similar fragmentation patterns in positiveion mode ESI multistage tandem mass spectrometry, the mass spectral fragmentation pathways of compound **4h** were discussed as a typical example (Scheme S1, Supplemental Materials). The protonated molecule has richer fragmentation pathways than the sodium ion adduct. The abundant characteristic fragment ions were observed by loss of uracil (112), amino acid methyl ester (195), menthene (138), and acetone (58), corresponding to the cleavage of glycosyl C-N between uracil and sugar, P-N, and C-O bonds. The fragment ions almost always contained a phosphoryl group. In addition, it was interesting that the fragmentation pathways of the sodium ion adduct showed some differences from that of the protonated molecule. The methoxy group of amino acid methyl ester could migrate from the carbonyl group to the phosphoryl group only for the sodium ion adduct, but not for the protonated molecule $[M + H]^+$. The ion at m/z 252 in the sodium ion adduct of **4h** might yield in this way.¹² The reason is that metal ions such as the sodium ion could coordinate with both carbonyl oxygen and phosphoryl oxygen to form a seven-membered ring, which might help the attack of the methoxy group on phosphorus. In contrast, with the smaller size, a proton could only attach to either the phosphoryl oxygen or the carbonyl oxygen. These characteristic ions in ESI mass spectra and the rearrangement mechanism are useful for the structural determination of amino acid methyl ester 5'-phosphoramidate of nucleosides.

CONCLUSION

In summary, we use a convenient and efficient approach to synthesize a series of amino acid methyl ester 5'-phosphoamidates of protected uridine under mild conditions. The reaction process was traced by ³¹P NMR. In addition, the structures of these compounds were verified and fragmentation pathways were analyzed by positive-ion mode ESI-MSⁿ.

EXPERIMENTAL

Material and Methods

All glassware was dried in an oven for at least 3 h at 140 °C prior to use. Air-sensitive materials were transferred under a nitrogen atmosphere. CH₂Cl₂ and NEt₃ were dried over CaCl₂ and KOH, respectively. PCl₃ was distilled. ¹H NMR and ¹³C NMR spectra were recorded in a Bruker AM 500 spectrometer. TMS ($\delta = 0.0$) and CDCl₃ ($\delta = 7.24$ ppm) were references for ¹H and ¹³C NMR spectra, respectively. ¹³C NMR spectra were all taken under ¹H-decoupled and ³¹P-coupled conditions. ³¹P NMR spectra were taken on a Bruker AM 500 spectrometer under ¹H decoupled conditions. ³¹P NMR chemical shifts are reported in ppm downfield (+) or upfield (-) from external 85% H₃PO₄ as reference. ESI were determined with a Bruker ESQYIRE 3000 plus. Operating conditions for ESI in the positive ion mode were as follows: spray voltage, 4000 V; capillary temperature, 300°C; dry gas (N₂), 10 L/min; nebulizer (N₂), 30 psi. HRMS was recorded on a Micromass-LCT Premier Time of Flight (TOF) mass spectrometer (Waters, USA).

Synthesis of Amino Acid Methyl Ester Hydrochloride and Protected Nucleoside (1)

The preparations of amino acid methyl ester hydrochloride and protected nucleoside were carried out according to the literature.^{13,14} All physical constants and spectroscopic data of the products synthesized were in agreement with the literature.

General Procedure for Synthesis of Aryl Phosphoramidate Derivatives of Nucleosides¹⁵

To a solution of PCl₃ (10 mmol) in anhydrous dichloromethane (10 mL), 2',3'isopropylideneuridine (**1**) was added (1 mmol) at -30° C under a nitrogen atmosphere, and the solution was stirred for 30 min at this temperature and then for 3 h at room temperature. The solvent and excess of PCl₃ were removed under reduced pressure. The residue was dissolved in anhydrous dichloromethane (10 mL). Menthol and tert-butyl alcohol (2.5 mmol, 1:1) in anhydrous dichloromethane (5 mL) was then added dropwise to the solution at 0°C, and the reaction was completed within 30 min. A solution of triethylamine (2 mmol) in anhydrous dichloromethane (5 mL) was added to the resulting solution. After 10 min, the solvent was removed by rotary evaporation, and the product was purified by chromatography on silica gel using CH₂Cl₂–MeOH (50:1) as eluent. The final product 2',3'-isopropylideneuridine-5'-O-yl-H-phosphonate (**3**) was obtained in 90% yield.

To a solution of amino acid ester (1.2 mmol), water (0.5 mL), tetrachloromethane (0.5 mL), triethylamine (0.5 mL), and acetonitrile (5 mL), a solution of 2',3'-Isopropylideneuridine-5'-O-yl-H-phosphonate (3) (1 mmol) in acetonitrile (5 mL) was added dropwise with an ice-bath. The solution was then stirred at room temperature for 10 min. The solvent was removed by rotary evaporation to a gum below 40 °C. The residual syrup was subjected to silica gel column chromatography by elution with 5% methanol in chloroform. Pooling and evaporation of appropriate fractions gave amino acid methyl ester 5'-phosphoamidates of protected uridine **4a–j** in 81–94% yields.

Menthyl 2',3'-osopropylideneuridin-5'-yl H-phosphonate (3). Yield 4.37 g (90%). IR (film) *v*: 3193, 3058, 2431, 1695, 1624, 1249, 1063, 954 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 8.89, 8.04. ¹H NMR (CDCl₃, 500 MHz): δ 9.40 (br, 1H, H-3), 7.52, 6.12 (d, d, 1H, J_{PH} = 703.0 Hz, P-H), 7.27, 7.26 (d, d, 1H, J = 8.0 Hz, H-6), 5.65–5.67 (m, 2H, H-1', H-5), 4.86–4.87 (m, 1H, H-2'), 4.75–4.82 (m, 1H, H-3'), 4.19–4.26 (m, 4H, H-4', H-5', OCHR), 2.06–2.13 (1H, m, H_{menthyl}), 1.92–2.01 (1H, m, H_{menthyl}), 1.81–1.90 (1H, m, H_{menthyl}), 1.58–1.60 (2H, m, H_{menthyl}), 1.49 (s, 3H, >CCH₃), 1.36–1.40 (1H, m, H_{menthyl}), 1.27 (s, 3H, >CCH₃), 1.12–1.19 (2H, m, H_{menthyl}), 0.87–1.00 (1H, m, H_{menthyl}), 0.84 (d, 6H, J = 7.0 Hz, 2CH₃), 0.72 (d, 3H, J = 7.0 Hz, CH₃). ¹³C NMR (CDCl₃, 100 MHz): δ 163.63 (C-4), 150.18 (C-2), 142.12 (C-6), 114.70 (>CMe₂), 102.79, 102.69 (C-5), 94.27, 94.17 (C-1'), 85.63, 85.53, 85.47 (C-4'), 84.35 (C-2'), 80.73, 80.65 (C-3'), 78.95, 78.89 (OCHR), 64.82 (C-5'), 48.24 (C_{menthyl}), 43.28, 42.79 (C_{menthyl}), 33.82 (C_{menthyl}), 31.52(C_{menthyl}), 27.08 (C_{menthyl}), 25.87, 25.69, 25.22 (CH₃), 22.86 (C_{menthyl}), 21.87, 21.85 (CH₃), 20.84, 20.81 (CH₃), 15.65 (CH₃); HRMS (ESI) calcd for C₂₂H₃₆N₂O₈P: 567.2621, found 567.2616.

Glycine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine **(4a).** Yield 496 mg (86.6%). IR (film) *v*: 3231, 1751, 1696, 1618, 1217, 1068, 990 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 8.12, 7.32. ¹H NMR (CDCl₃, 500 MHz): δ 9.46, 9.40 (br, br, 1H, H-3), 7.38, 7.34 (d, d, 1H, J = 8.0 Hz, H-6), 5.72–5.76 (m, 1H, H-1'), 5.66 (d, J = 8.0 Hz, 1H, H-5), 4.79–4.86 (m, 2H, H-2', H-3'), 4.24–4.32 (m, 1H, OC<u>H</u>R), 4.06–4.23 (m, 3H, H-4', H-5'), 3.68, 3.67 (s, s, 3H, OCH₃), 3.39–3.65 (m, 3H, P-NH, H-α,), 2.13–2.20 (m, 1H, H_{menthyl}), 1.92–1.98 (m, 2H, H_{menthyl}), 1.58–1.60 (m, 2H, H_{menthyl}), 1.51 (s, 3H, >CCH₃), 1.33–1.44 (m, 1H, H_{menthyl}), 1.28 (s, 3H, >CCH₃), 1.16–1.25 (m, 2H, H_{menthyl}), 0.89–1.01 (m, 1H, H_{menthyl}), 0.84 (d, 6H, J = 6.5 Hz, 2CH₃), 0.74 (t, 3H, J = 7.0 Hz, CH₃). ¹³C NMR (CDCl₃, 125 MHz): δ 171.16 (COOMe), 163.59 (C-4), 150.11 (C-2), 141.69, 141.33 (C-6), 114.09 (>CMe₂), 102.27 (C-5), 92.64, 93.15 (C-1'), 85.45, 84.66 (C-4'), 84.03 (C-2'), 80.52 (C-3'), 78.38 (OCHR), 65.45, 65.22 (C-5'), 51.97 (OCH₃), 48.16, 48.09

 $\begin{array}{l} (C_{menthyl}), 42.44, 42.14 (C-\alpha, C_{menthyl}), 33.66 (C_{menthyl}), 31.13 (C_{menthyl}), 26.79 (>CCH_3), \\ 25.31 (>CCH_3), 24.92 (C_{menthyl}), 22.45 (C_{menthyl}), 21.67 (CH_3), 20.68 (CH_3), 15.33, 15.27 (CH_3). HRMS (ESI) calcd for C_{25}H_{40}N_3O_{10}PNa [M+Na]: 596.2349, found 596.2350. \end{array}$

Alanine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4b). Yield 450 mg (76.7%). IR (film) v: 3224, 1742, 1698, 1630, 1213, 1066, 1004 cm^{-1.31}P NMR (CDCl₃, 202 MHz): δ 7.05, 6.39. ¹H NMR (CDCl₃, 500 MHz): δ 8.98, 8.92 (br, br, 1H, H-3), 7.44, 7.39 (d, d, 1H, J = 8.0 Hz, H-6), 5.82, 5.76 (br, br, 1H, H-1'), $5.74 (d, 1H, J = 7.0 Hz, H-5), 4.86 \sim 4.91 (m, 2H, H-2', H-3'), 4.22 \sim 4.35 (m, 1H, OCHR),$ 4.16~4.25 (m, 3H, H-5', H-4'), 3.90~3.98 (m, 1H, H-α), 3.74, 3.73 (s, 3H, OCH₃)7~3.51 (m, 1H, P-NH), 2.19~2.24 (m, 1H, H_{menthyl}), 2.01~2.12 (m, 1H, H_{menthyl}), 1.73~1.78 (m, 2H, H_{menthyl}), 1.64~1.67 (m, 2H, H_{menthyl}), 1.58 (s, 3H, CH₃), 1.40~1.48 (m, 1H, $H_{menthyl}$, 1.39, 1.38 (d, d, 3H, J = 4.0 Hz, H- β), 1.35 (s, 3H, >CCH₃), 1.23-1.33 (m, 1H, $H_{menthyl}$, 0.96~1.15 (m, 1H, $H_{menthyl}$), 0.91 (t, 6H, J = 6.0 Hz, 2CH₃), 0.81 (d, 3H, J = 7.0 Hz, CH₃).¹³C NMR (CDCl₃, 125 MHz): δ 174.19 (COOMe), 163.57 (C-4), 150.13 (C-2), 141.71 (C-6), 114.35 (>CMe₂), 102.49-5), 93.35 (C-1'), 85.45 (C-4'), 84.18 (C-2'), 80.64 (C-3'), 78.75 (OCHR), 65.41 (C-5'), 52.19 (OCH₃), 49.28, 48.88 (C_{menthyl}), 42.34 (C-α), 33.78 (C_{menthyl}), 31.28 (C_{menthyl}), 26.91 (>CCH₃), 25.46 (>CCH₃), 25.04 (C_{menthyl}), 22.60 (C_{menthyl}), 21.79 (CH₃), 20.80 (CH₃), 15.49 (CH₃). HRMS (ESI) calcd for C₂₆H₄₃N₃O₁₀P: 588.2686, found 588.2688.

Valine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4c). Yield 364 mg (59.2%). IR (film) v: 3237, 1734, 1694, 1622, 1214, 1063, 998 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 8.02, 7.78. ¹H NMR (CDCl₃, 500 MHz): δ 9.02, 8.42 (br, br, 1H, H-3), 7.45, 7.38 (d, d, 1H, J = 8.0 Hz, H-6), 5.84, 5.77 (d, d, 1H, J = 3.0 Hz, H-1'), 5.76, 5.73 (d, d, 1H, J = 8.0 Hz, H-5), 4.81–4.90 (m, 2H, H-2', H-3'), 4.31–4.34 (m, 1H, OCHR), 4.12-4.25 (m, 3H, H-4', H-5'), 3.72 (s, 4H, H- α , OCH₃), 3.31, 3.37 (t, t, 1H, J = 10.0 Hz, P-NH), 2.19–2.24 (m, 1H, H-β), 1.99–2.11 (m, 2H, H_{menthyl}), 1.71–1.81 (m, 1H, H_{menthyl}), 1.64–1.66 (m, 2H, H_{menthyl}), 1.58 (s, 3H, >CCH₃), 1.38–1.45 (m, 1H, H_{menthyl}), 1.35 (s, 3H, >CCH₃), 1.24–1.33 (m, 1H, H_{menthyl}), 0.97–1.15 (m, 2H, H_{menthyl}), 0.95 (t, 3H, $J = 7.0 Hz, CH_3$, 0.91, 0.88 (d, d, 9H, $J = 7.0 Hz, 3CH_3$), 0.81 (d, 3H, $J = 7.0 Hz, CH_3$). ¹³C NMR (CDCl₃, 125 MHz): δ 173.34 (COOMe), 163.50 (C-4), 150.03 (C-2), 141.59, 141.18 (C-6), 114.38 (>CMe₂), 102.49 (C-5), 93.28, 92.62 (C-1'), 85.08 (C-4'), 84.62, 84.13 (C-2'), 80.69, 80.44 (C-3'), 78.65 (OCHR), 65.45 (C-5'), 59.71, 59.44 (OCH₃), 51.85 (C-α), 48.31 (C_{menthyl}), 42.46 (C_{menthyl}), 33.80 (C_{menthyl}), 32.10 (C-β), 31.25 (C_{menthyl}), 26.91 (>CCH₃), 25.38 (>CCH₃), 25.02 (C_{menthyl}), 22.57 (C_{menthyl}), 21.79 (CH₃), 20.82 (CH_3) , 18.79 $(C-\gamma)$, 17.38 $(C-\gamma')$, 15.44 (CH_3) . HRMS (ESI) calcd for $C_{28}H_{47}N_3O_{10}P$: 616.2999, found 616.2997.

Leucine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4d). Yield 562.4 mg (89.41%). IR (film) *v*: 3226, 1730, 1697, 1626, 1213, 1060, 999 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 7.41, 6.80. ¹H NMR (CDCl₃, 500 MHz): δ 9.00, 8.98 (br, br, 1H, H-3), 7.46, 7.38 (d, d, 1H, J = 8.0 Hz, H-6), 5.85, 576 (d, d, 1H, J = 2.5 Hz, H-1'), 5.75, 5.73 (d, d, 1H, J = 8.0 Hz, H-5), 4.82–4.91 (m, 2H, H-3', H-4'), 4.30–4.35 (m, 1H, OCHR), 4.16–4.23 (m, 3H, H-4', H-5'), 3.82–3.90 (m, 1H, H- α), 3.72 (s, 3H, OCH₃), 3.44, 3.33 (t, t, 1H, J = 10.0 Hz, P-NH), 2.05–2.22 (m, 2H, H- β), 1.693–1.771 (m, 1H, H- γ , H_{menthyl}), 1.66, 1.64 (br, 2H, H_{menthyl}), 1.58, 1.57 (s, s, 3H, >CCH₃), 1.40–1.51 (m, 1H, H_{menthyl}), 1.35 (s, 3H, >CCH₃), 1.26–1.32 (m, 2H, H_{menthyl}), 1.04–1.14 (m, 1H, H_{menthyl}), 0.98–1.16 (m, 4H, H_{menthyl}), 0.89–0.98 (m, 12H, 4CH₃), 0.81 (t, 3H, J = 7.0 Hz, CH₃). ¹³C NMR (CDCl₃, 125 MHz): δ 174.41 (COOMe), 163.08 (C-4), 150.03 (C-2), 141.62 (C-6), 114.62 (>CMe₂), 102.71 (C-5), 93.52 (C-1'), 85.17 (C-4'), 84.32 (C-2'), 80.78 (C-3'), 78.96

 $\begin{array}{l} (\text{OCHR}), 65.48 \ (\text{C-5}'), 52.87, 52.16 \ (\text{OCH}_3), 48.50 \ (\text{C}_{\text{menthyl}}), 43.94 \ (\text{C-}\alpha), 42.70 \ (\text{C}_{\text{menthyl}}), \\ \text{C-}\beta), 33.95 \ (\text{C}_{\text{menthyl}}), 31.47 \ (\text{C}_{\text{menthyl}}), 27.11 \ (>\text{CCH}_3), 25.63 \ (>\text{CCH}_3), 25.19 \ (\text{C}_{\text{menthyl}}), \\ 24.46 \ (\text{C-}\gamma), 22.74 \ (\text{C}_{\text{menthyl}}, \text{CH}_3), 21.96 \ (\text{CH}_3), 20.99 \ (\text{CH}_3), 15.61 \ (\text{CH}_3). \ \text{HRMS} \ (\text{ESI}) \\ \text{calcd for } C_{29}\text{H}_{49}\text{N}_3\text{O}_{10}\text{P}: 630.3156, \ \text{found} \ 630.3160. \end{array}$

Serine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4e). Yield 384 mg (63.7%). IR (film) v: 3256, 1750, 1693, 1630, 1216, 1070, 1012 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 7.75, 6.72. ¹H NMR (CDCl₃, 500 MHz): δ 9.34 (br, 1H, H-3), 7.31, 7.27 (d, d, 1H, J = 8.0 Hz, H-6), 5.70, 5.69 (d, d, 1H, J = 8.0 Hz, H-5), 5.60, 5.56 (d, d, 1H, J = 2.0 Hz, H-1'), 5.04, 5.00 (dd, dd, 1H, J = 6.5, 2.0 Hz, H-2'), 4.90 (dd, 1H, J = 6.5, 3.5 Hz, H-3'), 4.06–4.33 (m, 5H, H-4', H-5', H- β), 4.10 (q, 1H, $J = 7.0 \text{ Hz}, H-\alpha$, 3.89–3.98 (br, 2H, P-NH, OCHR), 3.73 (s, 3H, OCH₃), 3.46 (br, 1H, -OH), 2.18–2.20 (m, 1H, H_{menthyl}), 1.99–2.04 (m, 1H, H_{menthyl}), 1.70–1.80 (m, 2H, H_{menthyl}), 1.61-1.64 (m, 2H, H_{menthyl}), 1.54 (s, 3H, >CCH₃), 1.37-1.44 (m, 1H, H_{menthyl}), 1.33 (s, 3H, >CCH₃), 1.21–1.26 (m, 1H, H_{menthyl}), 1.02–1.12 (m, 1H, H_{menthyl}), 0.88, 0.87 (d, d, 6H, J = 6.0 Hz, 2CH₃), 0.78 (d, 3H, J = 7.0 Hz, CH₃). ¹³C NMR (CDCl₃, 125 MHz): δ 171.89 (COOMe), 163.71 (C-4), 150.35 (C-2), 142.34 (C-6), 114.23 (>CMe₂), 102.54 (C-5), 94.46 (C-1'), 85.42 (C-4'), 84.20 (C-2'), 80.95 (C-3'), 78.99 (OCHR), 65.48 (C-5'), 64.07 (C-β), 56.50 (OCH₃), 52.38 (C-α), 48.35 (C_{menthyl}), 42.34 (C_{menthyl}), 33.85 (C_{menthyl}), 31.33 (C_{menthyl}), 26.96 (>CCH₃), 25.46 (>CCH₃), 25.09 (C_{menthyl}), 22.62 (C_{menthyl}), 21.87 (CH₃), 20.87 (CH₃), 15.49 (CH₃). ESI-MS: 604 (M+H)⁺, 626 (M+Na)⁺. HRMS (ESI) calcd for C₂₆H₄₃N₃O₁₁P: 604.2635, found 604.2632.

Aspartic acid ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4f). Yield 440 mg (68.2%). IR (film) *v*: 3430, 1693, 1216, 1069, 1011 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 7.60, 6.89; ¹H NMR (CDCl₃, 500 MHz): δ 9.31, 9.24 (br, br, 1H, H-3), 7.41, 7.37 (d, d, 1H, J = 7.5 Hz, H-6), 5.59–5.76 (m, 2H, H-1', H-5), 4.85–5.06 (m, 2H, H-2', H-3'), 4.28–4.34 (m, 1H, H-4'), 4.13–4.26 (m, 2H, H-5'), 3.78–3.94 (m, 3H, H-α, P-NH, OC<u>H</u>R), 3.75 (s, 3H, OCH₃), 3.68 (s, 3H, OCH₃), 2.72–3.12 (m, 2H, H-β), 2.21–2.27 (m, 1H, H_{menthyl}), 2.01–2.13 (m, 1H, H_{menthyl}), 1.651–1.676 (m, 2H, H_{menthyl}), 1.58, 1.57 (s, 3H, CH₃), 1.40–1.48 (m, 1H, H_{menthyl}), 1.37, 1.35 (s, 3H, CH₃), 1.22–1.29 (m, 2H, H_{menthyl}), 0.96–1.15 (m, 2H, H_{menthyl}), 0.92 (d, 6H, J = 6.5 Hz, 2CH₃), 0.82 (d, 3H, J = 7.0 Hz, CH₃); ¹³C NMR (CDCl₃, 125 MHz): δ 171.06 (2COOMe), 163.08 (C-4), 150.01 (C-2), 141.61 (C-6), 114.34 (>CMe₂), 102.54 (C-5), 93.54 (C-1'), 85.34 (C-4'), 84.56 (C-2'), 80.39 (C-3'), 72.13 (OCHMe₂), 65.53 (C-5'), 52.79, 52.04 (OCH₃), 50.97 (C-α), 48.23 (C_{menthyl}), 42.60 (C_{menthyl}), 38.46 (C-β), 34.00 (C_{menthyl}), 31.48 (C_{menthyl}), 25.66, 25.31 (C_{menthyl}), 22.47 (C_{menthyl}), 21.75 (CH₃), 20.75 (CH₃), 15.37 (CH₃). HRMS (ESI) calcd for C₂₈H₄₄N₃O₁₂PNa [M+Na]: 668.2560, found 668.2556.

Phenylalanine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4g). Yield 503.8 mg (75.9%). IR (film) *v*: 3222, 1742, 1693, 1630, 1455, 1214, 1063, 998 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 7.50, 6.81. ¹H NMR (CDCl₃, 500 MHz): δ 8.81, 8.68 (br, br, 1H, H-3), 7.40, 7.33 (d, d, 1H, J = 7.5 Hz, H-6), 7.22–7.31 (m, 5H, H_{Ph}), 5.80, 5.75 (br, br, 1H, H-1'), 5.686 (d, 1H, J = 7.0 Hz, H-5), 4.767–4.861 (m, 2H, H-2', H-3'), 4.284–3.947 (m, 5H, H-4', H-5', H-α, OCHR), 3.70, 3.67 (s, s, 3H, OCH₃), 3.03 (d, 2H, J = 6.0 Hz, H-β), 3.31 (t, 1H, J = 10.0 Hz, P-NH), 1.98–2.24 (m, 2H, H_{menthyl}), 1.70–1.79 (m, 2H, H_{menthyl}), 1.64–1.66 (m, 2H, H_{menthyl}), 1.57 (s, 3H, >CCH₃), 1.36–1.47 (m, 1H, H_{menthyl}), 1.35 (s, 3H, >CCH₃), 1.22–1.30 (m, 1H, H_{menthyl}), 1.07–1.15 (m, 1H, H_{menthyl}), 0.905 (d, 6H, J = 6.0 Hz, 2CH₃), 0.784 (d, 3H, J = 7.0 Hz, CH₃). ¹³C NMR (CDCl₃, 125 MHz): δ 172.93 (COOMe), 163.37 (C-4), 150.03 (C-2), 141.52 (C-6), 135.77 (C_{Ph}), 129.32 (C_{Ph}), 128.40 (C_{Ph}), 126.94 (C_{Ph}), 114.48 (>CMe₂), 102.56 (C-5), 93.23 (C-1'), 84.96 (C-4'), 84.20 (C-2'), 80.64 (C-3'), 78.84 (OCHR), 65.33 (C-5'), 55.36 (OCH₃), 52.09 (C- α), 48.35 (C_{menthyl}), 42.58 (C_{menthyl}), 40.45 (C- β), 33.87 (C_{menthyl}), 31.33 (C_{menthyl}), 27.01 (>CCH₃), 25.53 (>CCH₃), 25.17 (C_{menthyl}), 22.67 (C_{menthyl}), 21.87 (CH₃), 20.92 (CH₃), 15.56 (CH₃); HRMS (ESI) calcd for C₃₂H₄₇N₃O₁₀P: 664.2999, found 664.3004.

Tyrosine ester menthyl phosphoramidates of 2', 3'-isopropylideneuridine (4h). Yield 442.1 mg (65.1%). IR (film) v: 3253, 1710, 1692, 1615, 1517, 1455, 1216, 1067, 1017 cm⁻¹.³¹P NMR (CDCl₃, 202 MHz): δ 7.56, 6.60; ¹H NMR (CDCl₃, 500 MHz): δ 9.79, 9.64 (br, br, 1H, H-3), 7.89–7.73 (br, 1H, -OH), 7.34, 7.30 (d, d, 1H, J = 8.0 Hz, H-6), 6.94-6.97 (m, 2H, H_{Ph}), 6.75-6.78 (m, 2H, H_{Ph}), 5.72, 5.67 (d, 1H, J = 2.0 Hz, H-1'), 5.66, 5.65 (d, d, 1H, J = 8.0 Hz, H-5), 4.917-4.96 (m, 2H, H-2', H-3'), 4.87-4.88 (m, 1H, H-4'),4.69–4.78 (m, 3H, H-5', OCHR), 3.90–3.99 (m, 1H, H-α), 3.71, 3.70 (s, s, 3H, OCH₃), 3.57 (t, 1H, J = 10.5 Hz, P-NH), 2.92–2.96 (m, 2H, H- β), 2.01–2.21 (m, 2H, H_{menthyl}), 1.69–1.78 (m, 2H, H_{menthyl}), 1.61–1.69 (m, 2H, H_{menthyl}), 1.55 (s, 3H, CH₃), 1.39–1.47 (m, 1H, H_{menthyl}), 1.34, 1.33 (s, s, 3H, CH₃), 1.22–1.30 (m, 1H, H_{menthyl}), 0.95–1.14 (m, 1H, $H_{menthyl}$), 0.90 (d, 3H, J = 7.0 Hz, CH₃), 0.87 (d, 3H, J = 7.0 Hz, CH₃), 0.79, 0.78 (d, d, 3H, J = 7.0 Hz, CH₃); 13 C NMR (CDCl₃, 125 MHz): δ 173.42 (COOMe), 163.88 (C-4), 155.88 (PhOH), 150.79 (C-2), 142.00, 141.69 (C-6), 130.41 (C_{Ph}), 126.82 (C_{Ph}), 126.43 (C_{Ph}), 115.66 (C_{Ph}), 114.45 (>CMe₂), 102.44 (C-5), 93.96, 93.44 (C-1'), 85.03 (C-4'), 84.23 (C-2'), 80.69 (C-3'), 79.28 (OCHR), 65.38 (C-5'), 55.97 (OCH₃), 52.21 (C-α), 48.38 (C_{menthyl}), 42.51 (C_{menthyl}), 39.52 (C-β), 33.87 (C_{menthyl}), 31.33 (C_{menthyl}), 27.01 (>CCH₃), 25.50 (>CCH₃), 25.12 (C_{menthyl}), 22.64 (C_{menthyl}), 21.87 (CH₃), 20.92 (CH₃), 15.56 (CH₃). HRMS (ESI) calcd for C₃₂H₄₇N₃O₁₁P: 680.2948, found 680.2942.

Histidine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4i). Yield 465.6 mg (71.3%). IR (film) v: 3246, 1705, 1693, 1610, 1217, 1054, 999 cm⁻¹. ³¹P NMR (CDCl₃, 202 MHz): δ 7.25, 6.40; ¹H NMR (CDCl₃, 500 MHz): δ 7.58 (s, 1H, H_{imidazole}), 7.41 (d, 1H, J = 8.0 Hz, H-6), 6.81, 6.77 (s, s, 1H, H_{imidazole}), 5.70 (br, 1H, H-1'), 5.65 (d, 1H, J = 8.0 Hz, H-5), 4.86–4.93 (m, 2H, H-2', H-3'), 4.25–4.31 (m, 2H, H-4', OCHR), 4.14 (br, 3H, H-5', P-NH), 4.05 (t, 1H, J = 5.5 Hz, H- α), 3.69 (s, 3H, OCH₃), 3.11, 3.06 (d, d, 2H, J = 3.5 Hz, H- β), 2.15-2.18 (m, 1H, H_{menthyl}), 2.03–2.12 (m, 1H, H_{menthyl}), 1.62–1.64 (m, 2H, H_{menthyl}), 1.56 (s, 3H, >CCH₃), 1.37–1.47 (m, 1H, H_{menthyl}), 1.34 (s, 3H, >CCH₃), 1.26–1.30 (m, 2H, H_{menthyl}), 0.93–1.09 (m, 2H, H_{menthyl}), 0.88, 0.87 (d, d, 6H, J = 6.5 Hz, 2CH₃), 0.78 (d, 3H, J = 7.0 Hz, CH₃); ¹³C NMR (CDCl₃, 125 MHz): δ 173.25 (COOMe), 164.08 (C-4), 150.54 (C-2), 141.93 (C-6), 135.41 (C_{imidazole}), 133.16 (C_{imidazole}), 119.58 (C_{imidazole}), 114.18 (>CMe₂), 102.49 (C-5), 93.88 (C-1'), 85.25 (C-4'), 84.35 (C-2'), 80.90 (C-3'), 78.96 (OCHR), 65.33 (C-5'), 54.83 (OCH₃), 52.19 (C-α), 48.43 (C_{menthyl}), 42.41 (C_{menthyl}), 35.82 (C_{menthyl}), 31.33 (C-β, C_{menthyl}), 27.01 (>CCH₃), 25.46 (>CCH₃), 25.12 (C_{menthyl}), 22.62 (C_{menthyl}), 21.87 (CH₃), 20.90 (CH₃), 15.51 (CH₃). HRMS (ESI) calcd for C₂₉H₄₅N₅O₁₀P: 654.2904, found 654.2899.

Methionine ester menthyl phosphoramidates of 2',3'-isopropylideneuridine (4j). Yield 472.3 mg (73.1%). IR (film) *v*: 3216, 1722, 1694, 1625, 1216, 1063, 1011 cm⁻¹.³¹P NMR (CDCl₃, 202 MHz): δ 7.49, 6.68; ¹H NMR (CDCl₃, 500 MHz): δ 8.91 (br, 1H, H-3), 7.36, 7.28 (d, d, 1H, J = 8.0 Hz, H-6), 5.63–5.74 (m, 2H, H-1', H-5), 4.77–4.89 (m, 2H, H-2', H-3'), 4.24–4.27 (m, 1H, H-4'), 4.06–4.21 (m, 3H, H-5', OC<u>H</u>R), 3.91–4.00 (m, 1H, H-α), 3.67 (s, 3H, OCH₃), 3.46–3.55 (m, 1H, P-NH), 2.49, 2.48 (d, 2H, J = 3.0 Hz, H-γ), 2.22, 2.15 (d, 2H, J = 10.5 Hz, H-β), 2.02 (s, 3H, SCH₃), 1.94–2.00 (m, 1H, H_{menthyl}), 1.80–1.91 (m, 1H, H_{menthyl}), 1.67–1.77 (m, 2H, H_{menthyl}), 1.54–1.63 (m, 2H, H_{menthyl}), 1.51 (s, 3H, >CCH₃), 1.33–1.43 (m, 1H, H_{menthyl}), 1.28 (s, 3H, >CCH₃), 1.19–1.23 (m, 1H, H_{menthyl}), 1.01–1.06 (m, 1H, H_{menthyl}), 0.84 (s, 6H, 2CH₃), 0.74 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 125 MHz): δ 173.32 (COOMe), 163.50 (C-4), 150.11 (C-2), 141.70, 141.38 (C-6), 114.26 (>CMe₂), 102.44 (C-5), 93.44, 92.94 (C-1'), 85.01, 84.74 (C-4'), 84.11 (C-2'), 80.69, 80.47 (C-3'), 78.72 (OCHR), 65.41 (C-5'), 53.20 (C-α), 52.19 (OCH₃), 48.23 (C_{menthyl}), 42.36 (C_{menthyl}), 33.68, 33.44 (C- β), 31.20 (C_{menthyl}), 29.44 (C- γ), 26.86 (>CCH₃), 25.31 (>CCH₃), 25.00 (C_{menthyl}), 22.47 (C_{menthyl}), 21.75 (CH₃), 20.75 (CH₃), 15.37 (CH₃), 15.08 (SCH₃). HRMS (ESI) calcd for C₂₈H₄₇N₃O₁₀PS: 648.2720, found 648.2717.

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