Synthesis of 1-(Phosphonomethoxyalkoxy)pyrimidines, a Novel Series of Acyclonucleotide Analogues

Michael R. Harnden, L. John Jennings, Ann Parkin*
SmithKline Beecham Pharmaceuticals, Yew Tree Bottom Road, Epsom, Surrey KT18 5XQ, England

A convenient synthesis of 1-(2-phosphonomethoxyalkoxy)uracil and thymine derivatives, 4–7 by Mitsunobu coupling of 1-hydroxypyrimidines with suitably functionalized alcohols is described. The uracil analogues were converted to the corresponding 1-(phosphonomethoxyalkoxy)cytosines 8, 9 via intermediate 4-(1,2,4-triazol-1-yl) derivatives. The antiviral activity of this series of compounds is reported.

Acyclic analogues of naturally occurring nucleotides have received considerable attention in recent years, following reports of the antiviral activity of a series of phosphonate derivatives of purines and pyrimidines.1–3 (S)-9-(3-Hydroxy-2-phosphonomethoxypropyl)adenine, [(S) HPMPA (1)], exhibits antiviral activity against a range of DNA viruses. The cytosine analogue 3 is reported to have selective activity against human cytomegalovirus (CMV) [a major opportunistic infection observed in acquired immunodeficiency syndrome (AIDS) patients]4 and 9-(2-phosphonomethoxyethyl)-adenine (PMEA, 2) shows activity against human immunodeficiency virus (HIV), the aetiological agent of AIDS.5

We recently described the synthesis of a series of 1-hydroxalkoxyxpyrimidines in which the alkoxy substituent is structurally related to a portion of a nucleoside ribose moiety.6 These derivatives were obtained by cyclisation of appropriately functionalised ureas with either ethyl 3,3-diethoxy-2-methylpropionate or methyl 3,3-dimethoxypropionate. We wished to extend our studies to the biological evaluation of phosphonomethoxy derivatives of representative members of this series, 4–9, and chose to develop a short synthetic route to these compounds, involving a direct alkylation reaction of 1-hydroxyuracil (10) or 1-hydroxythymine (11).

Attempted O-alkylation of hydroxypyrimidines with alkyl halides under basic conditions led to complex mixtures of O- and N-alkylated products. We were able to obtain 14–17 in good yields, however, by coupling 10 or 11 with suitably functionalised alcohols under Mitsunobu conditions. Thus, reaction of 10 or 11 with the alcoholic 12,7,8 or 13,7,8 in dimethylformamide using triphenylphosphine/diethyl azodicarboxylate (DEAD) afforded the uracil and thymine analogues 14–17 in 59%–86% yields. Under analogous conditions, 1-hydroxyuracil and 1-hydroxythymine have been converted to their 1-(β-D-ribofuranosyl)uracil and thymine derivatives in moderate 24%–34% yields.5 Deprotection of the alcohol group in 16 and 17 with sodium ethoxide in ethanol gave reasonable yields of 20 and 21, with the chromatographically separable cyclic phosphate esters 18 and 19 also being formed in small quantities. De-esterification of 14, 15, 20, and 21 using trimethylsilyl bromide gave the phosphonic acids 4–7 in 50%–93% yields.

Conversion of the uracil derivatives 14 and 16 to the cytosine analogues 22 and 23 was achieved by reaction with 1,2,4-triazole and 4-chlorophenyl phosphorodichloridate in pyridine.6,10,11 The 4-(1,2,4-triazol-1-yl) derivatives thus formed were not isolated, but converted to the cytosine derivatives 22, 23 in situ by reaction with ammonia in methanol or dioxane. De-esterification of 22 with trimethylsilyl bromide gave the phosphonic acid 8 in 60% yield.

Attempted deprotection of the alcohol group in 23 by treatment with sodium ethoxide in ethanol led to the formation of a complex mixture of products. However, treatment of 23 with aqueous ethanolic hydrogen chloride at 80°C followed by reaction with trimethylsilyl bromide afforded the phosphonic acid 9 in 74% overall yield.

Compounds 4–9 were screened against viruses of the herpes family and visna virus, a lentivirus related to HIV. In these screens the thymine and cytosine derivatives 7 and 9 showed activity against the herpes virus varicella zoster virus (VZV) at 19 and 66 μg/mL, respectively. Both of these compounds were also active against visna virus at 3 μg/mL.

NMR spectra were recorded on a JEOL GX270 spectrometer, and mass spectrometry was performed using a VG 70-70 instrument. Column chromatography was carried out using Merck 7736 60H silica gel, and elemental analyses were obtained on a Carlo Erba model 1106 analyser. Compounds 14–23 were homogenous by TLC on silica gel 60F254 coated aluminium sheets.
1-[2-(Diethoxymethylthio)ethoxy]uracil (14), 1-[2-(Diethoxymethylthio)ethoxy]thymine (15), 1-[(RS)-3-Acetoxy-2-(diethoxymethylthio)propoxy]uracil (16), and 1-[(R,R)-3-Acetoxy-2-(diethoxymethylthio)propoxy]thymine (17).

**General Procedure:**
To a mixture of 1-hydroxyuracil 10 or 11 (10 mmol), Ph$_3$P (11 mmol) and alcohol 12 or 13 (10 mmol) in dry DMF (20 mL) at 0°C is added diethyl azodicarboxylate (DEAD, 11 mmol), and the resulting red solution is stirred at rt. for 20 h. The solvent is evaporated at reduced pressure and the resulting yellow oil is column chromatographed on silica gel.

**Compound 14:** yield after chromatography (MeOH/CHCl$_3$, 2:98): 84%; pale yellow oil.

C$_{14}$H$_9$N$_2$O$_4$P calc. C 41.00 H 5.94 N 8.70 (322.1) found 40.83 6.03 8.68

MS (70 eV): $m/z$ = 322 (M$^+$).

$^1$H-NMR (CDCl$_3$/TMS): $\delta$ = 1.36 (t, 6H, $J$ = 7.15 Hz, 2CH$_3$), 1.93 (d, 3H, $J$ = 1.1 Hz, CH$_3$-$O$), 3.85 (d, 2H, $J$ = 8.5 Hz, CH$_2$P), 3.85 (m, 2H, OCH$_2$), 4.20 (m, 4H, 2CH$_2$), 4.35 (m, 2H, CH$_2$ON), 7.50 (q, 1H, $J$ = 1.1 Hz, H-$E$), 8.40 (br s, 1H, NH).

**Compound 15:** yield after chromatography (MeOH/CHCl$_3$, 4:96): 86%; colourless gum.

C$_{15}$H$_{13}$N$_2$O$_4$P calc. C 42.86 H 6.29 N 8.33 (398.8) found 42.85 6.24 8.31

MS (70 eV): $m/z$ = 394.

$^1$H-NMR (CDCl$_3$/TMS): $\delta$ = 1.35 (t, 6H, $J$ = 7.0 Hz, 2CH$_3$), 2.09 (s, 3H, CH$_3$OCH$_2$), 3.85-4.50 (m, 11H, 5CH$_2$CH$_2$), 5.60 (dd, 1H, $J$ = 8.3, 2.2 Hz, H-$E$), 7.85 (d, 1H, $J$ = 8.3 Hz, H-$E$), 8.36 (s, 1H, NH).

**Compound 17:** yield after chromatography (MeOH/EtOAc, 1:20): 76%; colourless oil.

C$_{14}$H$_{14}$N$_2$O$_4$P·0.3 H$_2$O calc. C 43.54 H 6.24 N 7.77 (413.7) found 43.63 6.29 6.43

MS (70 eV): $m/z$ = 408.

$^1$H-NMR (CDCl$_3$/TMS): $\delta$ = 1.35 (t, 6H, $J$ = 7.0 Hz, 2CH$_3$), 1.94 (d, 3H, $J$ = 1.2 Hz, CH$_3$-$O$), 2.10 (s, 3H, CH$_3$O), 3.85-4.48 (m, 11H, 5CH$_2$CH$_2$), 7.62 (1H, $J$ = 1.2 Hz, H-$E$), 8.79 (s, 1H, NH).
Compound 20: yield after chromatography (MeOH/CHCl₃, 1:50): 73%; colourless glass.

C₂₀H₂₀N₂O₇P · 0.5H₂O calc. C 40.60 H 6.44 N 7.46 (375.3) found 40.63 6.52 7.10

MS (70 eV): m/z = 367 (M⁺).

1H-NMR (CDCl₃/TMS): δ = 1.356 (3 H, J = 6.2 Hz, CH₃), 1.360 (t, 3 H, J = 6.9 Hz, CH₃), 1.93 (d, 3 H, J = 1.2 Hz, CH₃), 3.64-4.45 (m, 12 H, 5CH₂, CH, OH), 7.44 (q, 1 H, J = 1.2 Hz, H-6), 8.79 (s, 1 H, NH).
(MeOH/CH₂Cl₂, 1:9). The resulting colourless gum (0.2 g, 0.56 mmol) is dissolved in dry DMF, and treated with Me₂SiBr (0.75 mL, 5.4 mmol) under dry N₂. After stirring at r.t. for 3 h, the solvent is evaporated at reduced pressure and the residue treated with MeOH (10 mL) and evaporated at reduced pressure. This procedure is repeated four times. The residue is column chromatographed on C18 reverse phase silica gel (H₂O), to give 9 as a solid; yield 0.14 g (73%); mp 173–176°C (EtOH).

C₁₇H₂₆N₂O₇P·0.5H₂O calc. C 31.58 H 4.97 N 13.81 (304.2) found 31.22 4.66 13.90

UV (MeOH): λ<sub>max</sub> = 276 nm (ε 8142).

¹H-NMR (DMSO-d₆/TMS): δ = 2.80–4.40 (br s, 3H, 2HOP, OH), 3.61 (d, 2H, J = 5.2 Hz, CH₃), 3.70–3.90 (m, 3H, CH₂P, CH), 4.15 (dd, 1H, J = 6.8, 11 Hz, CH of CH₂ON), 4.4 (dd, 1H, J = 3.3, 11 Hz, CH of CH₂ON), 5.8 (d, 1H, J = 5.3 Hz, H-5), 7.3 (br s, 2H, NH₂), 8.01 (d, 1H, J = 5.3 Hz, H-6).

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