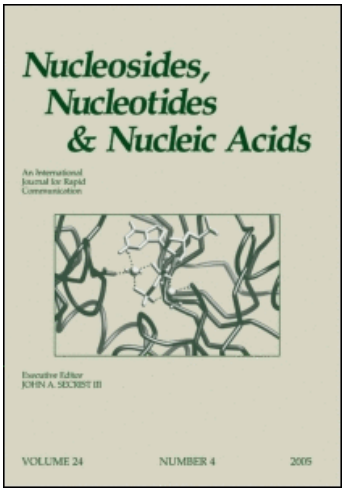

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Novel 6-Azapyrimidine Acyclic Nucleoside Analogues and Antiviral Evaluation

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Online publication date: 28 June 2010

To cite this Article Jasamai, Malina , Simons, Claire and Balzarini, Jan(2010) 'Synthesis of Novel 6-Azapyrimidine Acyclic Nucleoside Analogues and Antiviral Evaluation', Nucleosides, Nucleotides and Nucleic Acids, 29: 7, 535 — 541
To link to this Article: DOI: 10.1080/15257771003781634
URL: http://dx.doi.org/10.1080/15257771003781634

Nucleosides, Nucleotides and Nucleic Acids, 29:535–541, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003781634

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SYNTHESIS OF NOVEL 6-AZAPYRIMIDINE ACYCLIC NUCLEOSIDE ANALOGUES AND ANTIVIRAL EVALUATION

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□ *Acyclic nucleosides have been of considerable interest since the approval of aciclovir by the FDA to be used as an antiviral agent in the 1990s. The acyclic moieties and the bases used in the experiment were either available commercially or synthesized using literature methods. Vorbrüggen coupling method was utilized involving reaction of persilylated heterocyclic bases with the appropriate acyclic moiety in the presence of a Lewis acid catalyst. A series of novel 6-azapyrimidine acyclic oxosugar nucleosides was successfully synthesized with a promising yield (more than 50%). An efficient method of protection and deprotection was also investigated.*

Keywords Antiviral; 6-azapyrimidine acyclic nucleosides; coupling; deprotection

INTRODUCTION

Since the approval of aciclovir[1] as an antiviral agent by the U.S. Food and Drug Administration (FDA), there have been numerous publications concerned with the synthesis of acyclic nucleoside analogues with improved biological activity and selectivity compared with aciclovir. Aciclovir is used in the treatment of herpes simplex virus (HSV), varicella zoster virus (VZV), and cytomegalovirus (CMV) infections.[1] Several acyclic nucleoside analogues have been approved for clinical use as antiviral agents; ganciclovir,[2] penciclovir,[3] and valaciclovir.[4]

Synthesis of the first 6-azapyrimidine base, 6-azauracil was described by Seibert[5] in 1947. Despite having bacteriostatic[6] and antitumour activities,[7] 6-azauracil was found to be toxic to the central nervous system.[8] Alternatively, 6-azauridine which was synthesized later showed an absence of

Received 29 December 2009; accepted 15 March 2010.
M.J. acknowledges the Malaysian Government for research funding.
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toxicity[8] and beneficial responses in some hyperplastic diseases.[9] An alkyl substituent[10] at C-5 of nucleoside analogues, propyl group[11] in particular was shown to exhibit significant antiviral activity against HSV.

There are two main methods employed to synthesize the acyclic moiety. Robins et al.[12] reported a synthesis, which involved the preparation of (2-acyloxyethoxy) methyl halides as the acyclic moiety. This was achieved by acylation of the cyclic acetal, 1,3-dioxolane[13] by reacting it with either benzoyl chloride or acetyl bromide.[13] Keyser et al.[14] on the other hand, described the preparation of an acyclic moiety which involved the rapid reaction of 1,3-dioxolane with trimethylsilyl iodide. The cumbersome method to synthesize the conventional acyclic moiety is no longer required as the compound is commercially available as the protected benzoyloxyethylcloromethylether.

The aim of this study is to synthesize novel acyclic nucleosides using commercially available acyclic moiety, which will be coupled to the 6-azapyrimidine bases. The effective method of protection and deprotection will also be examined.

MATERIALS AND METHODS

Chemistry

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX500 spectrometer operating at 500 and 125MHz, with Me_4Si as internal standard. Mass spectra were determined by the EPSRC Mass Spectrometry Centre (Swansea, UK). Medac Ltd (Surrey, UK) performed the microanalyses. Flash column chromatography was performed with silica gel 60 (230–400 mesh; Merck, UK) and TLC was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Melting points were determined on an electrothermal instrument and are uncorrected. Compounds were visualized by illumination under ultraviolet (UV) light (254 nm) or by the use of vanillin stain followed by charring on a hotplate. All solvents were dried prior to use as described by the handbook, *Purification of Laboratory Chemicals*[15] and stored over 4 Å molecular sieves, under nitrogen.

Synthesis of Acyclic Nucleosides

Benzoic acid 1′-(5-ethyl-6-azauracil)methoxy-ethyl ether (4)

BSA (3.50 mL, 14.07 mmol) was added to a suspension of 5-ethyl-6-azauracil (**1**) (1 g, 7.03 mmol) in dry acetonitrile (20 mL) and the reaction mixture was stirred at room temperature under nitrogen for 30 minutes. A solution of benzoyloxyethylchloromethylether (**3**) (1.40 mL, 8.08 mmol) in dry acetonitrile (20 mL) was then added. The reaction mixture was cooled in an ice bath before TMSOTf (2.40 mL, 13.36 mmol) was added dropwise. The reaction mixture was stirred at room temperature under nitrogen for 3

hours, diluted with chloroform (150 mL) and washed with saturated aqueous sodium bicarbonate (2 × 100 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give yellowish syrup. Purification by column chromatography (ethyl acetate-petroleum ether 1:1 v/v) yielded compound **4** as a thick white syrup, which gave a white solid on standing (1.25 g, 56%). R_F 0.62 (ethyl acetate-petroleum ether 1:2 v/v); m.p.: 80–84°C; ^{1}H NMR ($CDCl_3$) δ 10.59 (bs, 1, NH), 9.57 (m, 2, *o*-Ph), 7.44 (m, 1, *p*-Ph), 7.33 (m, 2, *m*-Ph), 5.32 (s, 2, H-1′), 4.41 (m, 2, H-4′), 3.96 (t, 2, J = 4.3 Hz, H-3′), 2.50 (q, 2, J = 7.4, CH_2), 1.07 (t, 3, J = 7.3, CH_3). ^{13}C NMR ($CDCl_3$) δ 166.68 (C=O, PhCO), 156.94 (C=O, C-2), 149.77 (C, C-5), 148.47 (C=O, C-4), 133.40 (CH, *p*-Ph), 130.16 (C, COCPh), 129.91 (CH, 2 x *o*-Ph), 128.66 (CH, 2 x *m*-Ph), 79.91(CH_2, C-1′), 68.44 (CH_2, C-3′), 64.37 (CH_2, C-4′), 22.96 (CH_2), 10.60 (CH_3); $IR_{Vmax/cm^{-1}}$ (NaCl, film) 1719.7 (C=O), 1451.8 (C=C aromatic stretch), 1275.8–1094.1 (C—O) stretch; MS (ES+) *m/z*: 342.1 $[M+Na]^+$; Microanalysis calculated for $C_{15}H_{17}N_3O_5$ (319.3164). C, 56.42%, H, 5.37%, N, 13.16%. Found C, 56.46%, H, 5.46%, N, 12.89%.

Benzoic acid 1′-(5-propyl-6-azauracil)methoxy-ethyl ether (5)

BSA (2.20 mL, 8.96 mmol) was added to a suspension of 5-propyl-6-azauracil (**2**) (0.70 g, 2.18 mmol) in dry acetonitrile (20 mL) and the reaction mixture was stirred at room temperature under nitrogen for 30 minutes. A solution of benzoyloxyethylchloromethylether (**3**) (0.90 mL, 5.15 mmol) in dry acetonitrile (20 mL) was then added. The reaction mixture was cooled in an ice bath before TMSOTf (1.50 mL, 8.51 mmol) was added dropwise. The reaction mixture was stirred at room temperature under nitrogen for 3 hours, diluted with chloroform (150 mL) and washed with saturated aqueous sodium bicarbonate (2 × 100 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give yellowish syrup. Purification by column chromatography (ethyl acetate-petroleum ether 3:2 v/v) yielded compound **5** as a thick clear syrup, (0.36 g, 49%). R_F 0.56 (ethyl acetate-petroleum ether 1:2 v/v); ^{1}H NMR ($CDCl_3$) δ 10.23 (bs, 1, NH), 8.08 (m, 2, *o*-Ph), 7.59 (m, 1, *p*-Ph), 7.46 (m, 2, *m*-Ph), 5.43 (s, 2, H-1′), 4.52 (t, 2, J = 4.6 Hz, H-4′), 4.05 (t, 2, J = 5.0 Hz, H-3′), 2.58 (t, 2, J = 7.6 Hz, CH_2), 1.66 (q, 2, J = 7.5 Hz, CH_2), 0.99 (t, 3, J = 7.3 Hz, CH_3).^{13}C NMR ($CDCl_3$) δ 166.93 (C=O, PhCO), 156.94 (C=O, C-2), 149.48 (C, C-5), 147.87 (C=O, C-4), 133.57 (CH, *p*-Ph), 130.32 (C, COCPh), 130.10 (CH, 2 x *o*-Ph), 128.82 (CH, 2 x *m*-Ph), 80.05 (CH_2, C-1′), 68.62 (CH_2, C-3′), 64.21 (CH_2, C-4′), 31.97, (CH_2), 20.02 (CH_2) 14.11 (CH_3); $IR_{Vmax/cm^{-1}}$ (NaCl, film) 1719.9 (C=O), 1451.8 (C=C aromatic stretch), 1275.1–1095.6 (C—O stretch); LRMS (ES+) *m/z*: 168.1 $[M-OCH_2CH_2OCOPh]^+$, 181.2 [M-heterocyclic base]$^+$, 290.2 [M-propyl]$^+$, 304.1 [M-ethyl]$^+$, 333.3 $[M]^+$, 356.1 $[M+Na]^+$. LRMS (CI+) *m/z*: 149.0 $[M-CH_2CH_2OCOPh]^+$, 167.1 $[M-CH_2$ heterocyclic base$]^+$, 184.1 $[M-OCH_2$ heterocyclic base$]^+$, 212.1 $[M-OCOPh]^+$, 334.2 $[M+H]^+$.

HRMS (ES+) *m/z*: Calculated mass: 351.1663 $[M+NH_4]^+$. Accurate mass: 351.1662

2-(5-Ethyl-6-azauracil)methoxy-ethanol (6)

Aqueous methylamine (25 mL) was added to compound **4** (1.09 g, 3.40 mmol) and the resulting lilac solution was heated on a steam bath for 15 minutes. The lilac colour faded on heating. The crude product was concentrated in vacuo. Purification by column chromatography (methanol-dichloromethane 3:97 v/v) yielded white solids. Further purification by recrystallization using ethyl acetate gave compound **6** as a white solid (0.61 g, 83%). R_F 0.32 (methanol-dichloromethane 1:9 v/v); m.p.: 91–93°C; ^{1}H NMR (DMSO-d_6) δ 12.15 (bs, 1, NH), 5.17 (s, 2, H-1′), 4.64 (bs, 1, OH), 3.55 (t, 2, J = 4.8 Hz, H-4′), 3.46 (t, 2, J = 4.4 Hz, H-3′), 2.48 (q, 2, J = 7.4 Hz, CH_2), 1.08 (t, 3, J = 7.4 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 157.19 (C=O, C-2), 149.31 (C, C-5), 147.21 (C=O, C-4), 79.32 (CH_2, C-1′), 71.39 (CH_2, C-3′), 60.43 (CH_2, C-4′), 22.96 (CH_2), 10.61 (CH_3); $IR_{Vmax/cm}{}^{-1}$ (KBr, disc) 3464.1–3429.3 (O—H stretch), 2984.7–2778.7 (C-H stretch), 1713.8 (C=O), 1444.7 (C—O stretch). MS (ES+) *m/z*: 238.1 $[M+Na]^+$; Microanalysis calculated for $C_8H_{13}N_3O_4$ (215.2085). C, 44.65%, H, 6.09%, N, 19.52%. Found C, 44.83%, H, 6.17%, N, 19.28%.

2-(5-Propyl-6-azauracil)methoxy-ethanol (7)

Aqueous methylamine (17 mL) was added to compound **5** (0.73 g, 2.18 mmol) and the resulting clear solution was heated on a steam bath for 15 minutes. The crude product was concentrated in vacuo. Purification by column chromatography (methanol-dichloromethane 3:97 v/v) yielded white solids. Further purification by recrystallization using ethyl acetate gave compound **7** as a white crystalline solid (0.42 g, 84%). R_F 0.38 (methanol-dichloromethane 1:9 v/v); m.p.: 90–91°C; ^{1}H NMR (DMSO-d_6) δ 10.13 (bs, 1, NH), 5.39, (s, 2, H-1′), 3.79 (m, 4, H-4′ & H-3′), 2.82 (t, 1, J = 7.5 Hz, OH), 2.60 (t, 2, J = 7.5 Hz, CH_2), 1.68 (q, 2, J = 7.5 Hz, CH_2), 1.00 (t, 3, J = 7.3 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 156.95 (C=O, C-2), 149.68 (C, C-5), 147.80 (C=O, C-4), 80.26 (CH_2, C-1′), 71.74 (CH_2, C-3′), 61.99 (CH_2, C-4′), 32.02, (CH_2), 20.08 (CH_2) 14.11 (CH_3); $IR_{Vmax/cm}{}^{-1}$ (KBr, disc) 3433.7 (O—H), 2966.2–2833.1 (C-H stretch), 1704.2 (C=O), 1441.6 (C—O stretch); LRMS (ES+) *m/z*: 252.1 $[M+Na]^+$, 267.9 $[M+K]^+$. LRMS (EI+) *m/z*: 156.2 [M-acyclic moiety]$^+$, 169.2 $[MOCH_2CH_2OH]^+$, 199.2 $[M\text{-}CH_2OH]^+$, 229.1 $[M]^+$. LRMS (CI+) *m/z*: 187.2 [M-propyl]$^+$, 216.2 [M-ethyl]$^+$. HRMS (ES+) *m/z*: Calculated mass: 247.1401 $[M+NH_4]^+$. Accurate mass: 247.1398

Antiviral Assay

CEM cells in complete medium infected with 100 μL of human immunodeficiency virus (HIV) type 1 and 2 were seeded at 4×10^4 cells into

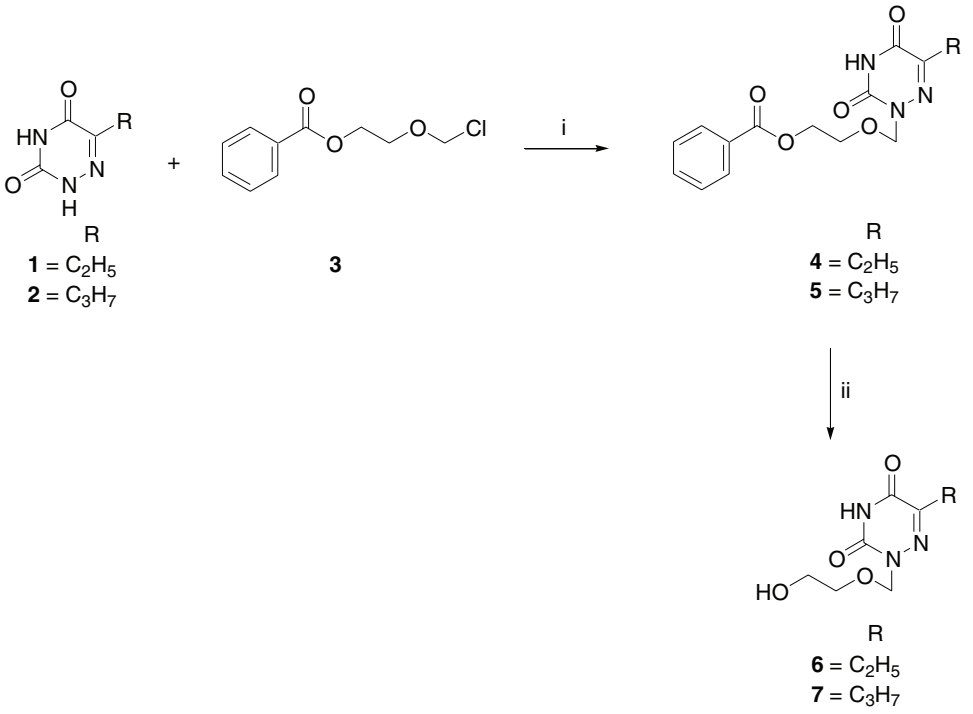
0.32 cm^2 wells of a 96-well microplate containing 100 μL various concentrations of test compounds (100 μM, 20 μM, 4 μM, and 0.8 μM). Virus stock used in the experiment had a titre of $10^{3.7}$ $CCID_{50}$/mL. In the control wells, 200 μL of medium containing CEM cells and the virus with no test compound was pipetted. To assess toxicity of the test compound, 100 μL of CEM cells alone without the viruses was seeded into the wells containing 100 μL of medium and test compound at different concentrations. The microplates were then incubated at 37°C for 4 days.

RESULTS AND DISCUSSION

Chemistry

The synthesis involved coupling of the heterocyclic 6-azapyrimidine bases (compound **1** and **2)** synthesized as reported by Chang[16] with commercially available acyclic moiety (**3**) using the Vorbrüggen coupling method.[17] Deprotection of the benzoyl protecting group gave the desired acyclic nucleoside analogues (**6** and **7**) (Scheme 1).

The 6-azapyrimidine bases exist as tautomers, where the lactim form can participate in the coupling reaction, making it necessary to temporarily



SCHEME 1 *Reagents and Conditions*: i) dry acetonitrile, BSA, under nitrogen, room temperature, TMSOTf, 3 hours; ii) aq. methylamine, steam bath, 15 minutes.

protect these groups using bis(trimethylsilyl)acetamide (BSA) as described in the literature.[18] Silylated **1** and **2** obtained are soluble in acetonitrile yeilding a clear solution on completion of the reaction. The completion of the reaction could not be monitored by thin layer chromatography (TLC) as the R_F value of the reactant (**3**) and the products (**4** and **5**) were very close. From preliminary study, the reaction was completed after 3 hours.

The presence of the aromatic group was confirmed by the aromatic signals at 7.33–8.11 ppm in the ^{1}H NMR spectrum and at 128.70–133.60 ppm in the ^{13}C NMR spectrum. Importantly, the occurrence of the H-1 signals at 5.32–5.83 ppm in the ^{1}H NMR spectrum and C-1 signals at 79.91–84.52 ppm in the ^{13}C NMR spectrum indicates that the acyclic moiety had coupled to the respective bases forming the *N*-glycosidic bond.[19]

Deprotection of the benzoyl group was achieved by reacting compound **4** and **5** with methylamine on a steam bath for 15 minutes.[20] The reaction was monitored by TLC The impurities, including *N*-methylbenzamide, were removed from the crude product by column chromatography (ethyl acetate-petroleum ether). Pure **6** and **7** were obtained by recrystallization with ethyl acetate. The disappearance of the benzoyl protecting group in the ^{1}H NMR and ^{13}C NMR spectra confirmed the presence of the desired compounds **6** and **7**.

A series of novel 6-azapyrimidine acyclic nucleosides was successfully synthesized by coupling the commercially available acyclic moiety with the respective 6-azapyrimidine bases using the Vorbrüggen coupling procedure.[17] An effective deprotection of the benzoyl group was achieved by employing method described by Kelly et al.[20]

Antiviral Activity

The acyclic nucleoside analogues synthesized are neither toxic to CEM cells at concentrations up to 100μM nor active against HIV-1 and HIV-2 virus. The acyclic analogues (**6** and **7**) obtained have potential to be developed as antiviral drug due to their non-toxic property in vitro. Further studies on the relevant functional groups are needed to improve antiviral activity of the nucleoside analogues synthesized.

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