Synthesis and Antiviral Evaluation of (−)-3′-Methylcarbovir, (−)-3′-Methylabacavir, and Modified Purine Analogues

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Abstract: Starting from ethyl (1S,4R)-4-hydroxy-2-methylcyclopent-2-ene-1-carboxylate, as a common enantiopure building block readily obtained by enzymatic kinetic resolution of the corresponding racemic derivative, 3′-methylcarbovir, 3′-methylabacavir, and three modified analogues were synthesized via the linear construction of the purine heterocycles. These derivatives were evaluated as potential agents against HIV-1, HIV-2, and other important viral pathogens. None of the new compounds had significant antiviral activity at a concentration of 100 μg·mL⁻¹, which was the highest concentration tested.

Key words: stereoselective synthesis, azides, carbocyclic nucleosides, nucleobases, antiviral agents

Nucleoside analogues have received great attention as synthetic targets because of the successful use of this class of compounds in the treatment of viral infections and cancer. Among those examples reported, the replacement of the furanose ring oxygen by a methylene has resulted in carbocyclic nucleoside analogues. This modification is of particular interest because it engenders a greater metabolic stability to phosphorylases that cleave the glycosidic bond of nucleosides, and the substances present potent activities in the area of antiviral and antitumor chemotherapy. In this class of compounds, 2′,3′-dideoxy-2′,3′-didehydrocarbocyclic nucleosides, such as carbovir and abacavir, are prominent representatives. Carbovir shows significant anti-HIV activity via its 5′-O-triphosphate by inhibiting HIV reverse transcriptase. It was, nevertheless, eliminated as a candidate for anti-HIV therapy because of its pharmacokinetic and toxicological deficiencies. In contrast, its congener abacavir, with higher oral bioavailability and the ability to penetrate the central nervous system, has been approved by the Food and Drug Administration for treatment of AIDS (acquired immunodeficiency syndrome).

The success of abacavir attracted a number of research groups to conduct related synthetic and pharmacological studies. Several carbasugar-branched and/or base-modified analogues of these molecules were synthesized and evaluated. Among them, 6′-hydroxymethyl- and 1′,2′-fluoroadenosine analogues of carbovir, and 3′-fluorocarbovir, norcarbovir and norabacavir (5′-demethylene derivatives), 6-modified purine analogues of abacavir, and Schiff bases of abacavir were reported to exhibit potent anti-HIV activity.

Encouraged by these findings, and as part of our ongoing program directed towards the discovery of less toxic and more effective antiviral agents, we herein describe the synthesis and antiviral evaluation of the hitherto unknown carbovir, abacavir, and modified purine analogues substituted with a methyl group at the C-3′ position of the carbasugar moiety (Figure 2).

In synthesis of carbocyclic nucleosides, particularly for dideoxydidehydro analogues, such as carbovir and abacavir, one highly useful strategy for the convergent attachment of the heterocyclic base to the appropriately functionalized carbocyclic ring is the palladium(0)-catalyzed substitution of allylic esters or carbonates.

Figure 1 Structures of carbovir and abacavir

Figure 2 Structures of target derivatives 1–5
substitution reaction occurs with retention of the configuration. In this respect, cis-diacetate (+)-7a and cis-dicarbonate (+)-7b were synthesized starting from ethyl cis-4-hydroxy-2-methylcyclopent-2-ene-1-carboxylate [(–)-6] (Scheme 1), obtained through the enzymatic resolution of the racemic mixture as previously reported by us.13 Unfortunately, the palladium-catalyzed coupling of compound (+)-7a or (+)-7b with 2-amino-6-chloropurine failed, probably because of steric hindrance.14,15 Although several parameters were explored, embracing the variation of the palladium catalyst [Pd(PPh₃)₄, Pd₂(dba)₃], the ligand [PPh₃, P(O-i-Pr)₃], and the salt of the purine base (from NaH, AlEt₃), no reaction was observed and the unreacted starting material was recovered. Because this approach was unsuccessful, we elected to use a Mitsunobu coupling, another frequently used method for the direct connection of a cyclic carbohydrates and a heterocyclic base.1b In this procedure, the activation of a secondary alcohol by a complex formed from an azodicarboxylate and triphenylphosphine allows the direct substitution of the hydroxy group with inversion of the configuration.

Consequently, our desired target became derivative (–)-9 (Scheme 1). For this purpose, the absolute configuration of the hydroxy group in (–)-6 was inverted to afford diester (–)-8. The sequential lithium aluminum hydride (LAH) reduction of the ester groups in (–)-8 and selective neutral monoacylation of the primary hydroxy group in the crude sensitive diol, using porcine pancreatic lipase (PPL) and vinyl acetate, afforded the desired trans-derivative (–)-9. With the appropriate derivative (–)-9 in hand, the Mitsunobu procedure was attempted. The reaction of (–)-9 under Mitsunobu conditions (Ph₃P, DIAD, 2-amino-6-chloropurine, THF, 0 °C then r.t.) led to a complex mixture of compounds with no evidence of the presence of the expected carbocyclic nucleoside derivative or the unreacted starting material. It must be noted at this point that attempts to synthesize the corresponding mesylate from (–)-9 also afforded a mixture of products that did not allow the isolation of the expected mesylate.

As the most promising direct couplings of the base with the cyclic carbohydrates failed, the linear construction of the heterocyclic moiety from an amine substituent on the car-
bicycle was mandatory. Using this protocol, the amino group becomes N-9 of the purine subunit. For this approach, the starting material was compound (–)-9 (Scheme 2). The reaction of (–)-9 with diphenylphosphoryl azide, according to Thompson et al. ’s procedure, proceeded in a stereo- and regiospecific fashion to give (+)-10 in 92% yield. The stereochemistry of (+)-10 was assigned unambiguously by the observed difference between the chemical shifts of H₃₋₅ (2.46 ppm) and H₅₋₅ (1.67 ppm) which is equal to 0.79 ppm, the corresponding coupling constants (Karplus rule), and nuclear Overhauser effect (NOE) experiments (Figure 3).

The acetate and azido groups in (+)-10 were reduced using LAH to give the target amino alcohol 11. Crude derivative 11 underwent a condensation reaction with 4,6-dichloropyrimidin-2-amine to afford the pyrimidinylamine derivative (+)-12. Diazotization of (+)-12 with 4-chlorobenzenediazonium chloride gave azopyrimidine 13, which was then reduced with triethyl orthoformate in the presence of acetic acid to provide the dianinopyrimidine derivative (+)-14. Finally, the reaction with triethyl orthoformate in the presence of acid converted (+)-14 into 2-amino-6-chloropurine (–)-15, which is a common precursor to compounds (–)-1 and (–)-2. Refluxing derivative (–)-15 in aqueous sodium hydroxide gave (–)-3’-methylcarbovir [(–)-1], and treatment of the same substrate with cyclopropylamine in methanol provided (–)-3’-methylabacavir [(–)-2].

Besides the modification of the sugar moity of nucleosides, alterations to the heterocyclic base have been proven to profoundly change the biological effects of such compounds. In view of this fact and with compound 11 in hand, we decided to synthesize modified purine analogues in anticipation of their interesting antiviral activity. For this purpose, and following a well-established procedure, crude 11 was coupled to 4,6-dichloropyrimidin-5-amine to afford (+)-16 in 76% yield [two steps from (+)-10]; (b) i. HC(OEt)₃, concd HCl (cat.), DMF; ii. 0.5 M aq HCl, 78% yield; (c) 0.33 M aq NaOH, reflux, 2 h, 68% yield; (d) cyclopropylamine, MeOH, 50 °C, 12 h, 82% yield; (e) NH₃, MeOH, 100 °C, 24 h, 86% yield.

The synthesized compounds were tested against HIV and several other viruses to determine their spectrum of antiviral activity. None of these compounds had any significant activity or cytotoxicity at concentrations up to 100 μg·mL⁻¹.

In summary, an efficient enantioselective synthesis of 3’-methylcarbovir, 3’-methylabacavir, and modified purine analogues has been accomplished from a common enantiopure building block. It should be noted that all the final nucleosides are novel compounds. Although, none of the new compounds had significant antiviral activity or cytotoxicity at concentrations up to 100 μg·mL⁻¹, we hope that the information obtained in the presented study will be useful for the synthesis of novel nucleoside antiviral agents.

All air- and/or moisture-sensitive reactions were carried out under an argon atmosphere with dry, freshly distilled solvents using standard syringe-cannula/septa techniques. All glassware was oven-dried (80 °C) and/or carefully dried in line with a flameless heat gun. All solvents were distilled under an argon atmosphere: THF from a blue solution of sodium benzophenone ketyl radical prior to use; CH₂Cl₂, DMF, and toluene from CaH₂. Routine monitoring of the reactions was performed using Merck silica gel 60 F254, aluminum-supported TLC plates; spots were visualized using UV light and an ethanolic acidic p-anisaldehyde or ethanolic phosphomolybdic solution, followed by heating. Column chromatography was performed with silica gel 60 (230–400 mesh) and gradients of EtO₉–petroleum ether or CH₂Cl₂–MeOH as eluent, unless otherwise stated.
ed. Melting points are uncorrected. The $^1$H and $^{13}$C NMR spectra were recorded in CDCl$_3$, CD$_3$OD, or DMSO-$d_6$ solutions on a Bruker AM-300 spectrometer at 300 and 75 MHz, respectively, using residual nondeuterated solvents as the internal reference. Infrared spectra were obtained using a Perkin-Elmer 1600 FTIR spectrophotometer (film or KBr pellets). Optical rotations were measured on a Perkin-Elmer 341 polarimeter with a path length of 1 dm and are in units of $10^{-1}$ deg cm$^{-1}$ g$^{-1}$; concentrations are in g per 100 mL. Microanalyses were performed at our university.

**Disubstituted Methyleclopent-2-ene Derivatives 7; General Procedure**

A solution of (–)–4 (200 mg, 1.18 mmol) in dry Et$_2$O (10 mL) was slowly added at –20 °C to a stirred slurry of LAH (89 mg, 2.35 mmol, 1.5 equiv) followed by a catalytic amount of DMAP (15 mg, 0.12 mmol) for 3 h at 0 °C under argon. The acylating agent (3.53 mmol, 3.0 equiv) was then added dropwise followed by a catalytic amount of DBU (1.2 mL, 7.65 mmol, 2.0 equiv) in dry Et$_2$O (10 mL). After 1 h at 0 °C, Celite (3 g) and Na$_2$SO$_4$·10H$_2$O (3 g) were added, and the solution was allowed to warm to r.t. and was stirred for a further 1 h. The mixture was filtered through a pad of MgSO$_4$ and then concentrated. The oily residue was used for the next reaction without further purification. The crude diol was dissolved in CH$_2$Cl$_2$–py (1:1, 10 mL) and stirring at 0 °C under argon. The acylating agent (3.53 mmol, 3.0 equiv) was then added dropwise followed by a catalytic amount of DMAP (15 mg, 0.12 mmol) for 7a. The resulting mixture was stirred for 12 h at r.t. and then was poured into dil HCl (100 mL), extracted with Et$_2$O (2 × 50 mL), washed, and dried (MgSO$_4$). Concentration in vacuo gave a residue that was purified by column chromatography to give azide (–)–9 as a colorless oil. Yield: 397 mg (83%).

Using Ac$_2$O. Yield: 220 mg (88%).

1H NMR (300 MHz, CDCl$_3$): δ = 5.52 (m, 1 H), 5.50–5.46 (m, 1 H), 4.16 and 3.98 (ABX, $J = 10.9, 7.0, 5.0$ Hz, 2 H), 2.73–2.63 (m, 1 H), 2.45 (ddd, $J = 14.5, 8.2, 7.5$ Hz, 2 H), 2.02 (s, 3 H), 1.97 (s, 3 H), 1.77 (br s, 3 H), 1.63 (br dt, $J = 14.5, 3.5$ Hz, 1 H). 13C NMR (75 MHz, CDCl$_3$): δ = 171.1 (C), 171.0 (C), 147.3 (C), 126.2 (CH), 78.8 (CH), 66.5 (CH$_2$), 46.4 (CH), 34.6 (CH$_3$), 21.4 (CH$_3$), 21.0 (CH$_3$), 15.4 (CH$_3$).


**Ethyl (1S,4S)-4-Acetoxy-2-methyleclopent-2-ene Carboxylate (–)–8**

A stirred solution of (–)–6 (1.00 g, 5.88 mmol), AcOH (0.44 mL, 7.68 mmol), and Ph$_3$P (2.00 g, 7.62 mmol) in THF (40 mL) was cooled in an ice bath, and DIAD (1.52 mL, 7.72 mmol) was slowly added. After removing the ice bath, the mixture was allowed to warm to r.t. and was further stirred for 1 h. The solvent was removed in vacuo, and the residue was directly purified by column chromatography to afford (–)–8 as a colorless oil. Yield: 1.11 g (89%).

IR (neat): 3031, 1763, 1751 cm$^{-1}$.

1H NMR (300 MHz, CDCl$_3$): δ = 5.66 (m, 1 H), 5.56 (m, 1 H), 4.12 and 4.08 (ABX, $J = 10.8, 7.2$ Hz, 2 H), 3.50 (m, 1 H), 2.55 (ddd, $J = 14.4, 7.6, 4.9$ Hz, 1 H), 2.02 (d, $J = 14.4, 8.5, 3.2$ Hz, 1 H), 1.95 (s, 3 H), 1.75 (d, $J = 1.1$ Hz, 3 H), 1.20 (ABX, $J = 7.2$ Hz, 3 H).

13C NMR (75 MHz, CDCl$_3$): δ = 173.4 (C), 170.7 (C), 145.1 (C), 127.2 (CH), 79.6 (CH), 60.7 (CH$_2$), 52.8 (CH$_3$), 35.2 (CH$_2$), 21.1 (CH$_3$), 15.3 (CH$_3$), 14.1 (CH$_3$).


Anal. Calcd for C_{12}H_{15}N_{5}O: C, 55.16; H, 5.79; N, 26.80. Found: C, 55.09; H, 6.68; N, 21.79.


[(1S,4R)-4-[[2-Amino-6-chloropyrimidin-4-yl]amino]-2-methylcyclopent-2-yl]methanol (–)-12

Under an argon atmosphere, 1.0 M LAH in THF (9.20 mL, 9.20 mmol) was added to the crude amino alcohol (11 (590 mg) in BuOH (30 mL) and DIMEA (15 mL) was used in the next step. To a stirred solution of the crude amino alcohol (11 (590 mg) in BuOH (30 mL) and DIMEA (15 mL) was added 4,6-dichlorohydroxyquinoline-2-amine (1.51 g, 9.22 mmol, 2.0 equiv) under argon. The resulting mixture was stirred at reflux for 1 h, then filtered and concentrated to afford crude (1S,4R)-4-[[2-Amino-6-chloropyrimidin-4-yl]amino]-2-methylcyclopent-2-yl]methanol (11), which was directly used in the next step. To a stirred solution of crude amino alcohol (11 (590 mg) in BuOH (30 mL) and DIMEA (15 mL) was added 4,6-dichlorohydroxyquinoline-2-amine (1.51 g, 9.22 mmol, 2.0 equiv) under argon. The resulting mixture was stirred at reflux for 12 h and then was diluted with CH\textsubscript{2}Cl\textsubscript{2} (100 mL) and poured into H\textsubscript{2}O (100 mL). The layers were separated, and the aqueous layer was repeatedly extracted with CH\textsubscript{2}Cl\textsubscript{2} (2 × 100 mL). The combined organic layers were dried (MgSO\textsubscript{4}) and concentrated in vacuo to give a residue, which was purified by column chromatography (silica gel) to afford pyrimidine (–)-12 as a foam. Yield: 942 mg (80%).

[\alpha\textsubscript{D}\textsubscript{25} +13.6 (c 1.0, MeOH).

IR (KBr): 3402, 3053, 1068 cm\textsuperscript{-1}.

[\textsuperscript{1}H] NMR (300 MHz, CD\textsubscript{3}OD): \(\delta = 5.80\) (br s, 1 H), 5.42 (q, J = 1.5 Hz, 1 H), 4.90 (m, (partially overlapped, 1 H), 3.65 and 3.57 (ABX, J = 10.8, 5.2, 4.0 Hz, 2 H), 2.59 (ABX, m, 1 H), 2.52 (dt, J = 12.8, 8.5 Hz, 1 H), 1.76 (br s, 3 H), 1.52 (dt, J = 12.8, 4.6 Hz, 1 H).

[\textsuperscript{13}C] NMR (75 MHz, CD\textsubscript{3}OD): \(\delta = 164.9\) (C), 164.2 (C), 158.7 (C), 145.4 (C), 128.1 (CH), 95.0 (CH), 64.4 (CH\textsubscript{2}), 56.1 (CH), 50.8 (CH\textsubscript{2}), 36.5 (CH\textsubscript{2}), 15.2 (CH).

Anal. Calcd for C\textsubscript{11}H\textsubscript{16}ClN\textsubscript{5}O: C, 48.98; H, 5.98; N, 25.96. Found: C, 48.81; H, 6.02; N, 25.70.

[(1S,4R)-4-[[2-Amino-6-chloropyrimidin-9H-purin-9-yl]amino]-2-methylcyclopent-2-yl]methanol (–)-15

To an ice-cold solution of pyrimidine (–)-14 (500 mg, 1.85 mmol) in DMF (10 mL) were added, under argon, HCl(OEt\textsubscript{2}) (20 mL, 120 mmol) and a catalytic amount of concd HCl soln. The resulting mixture was stirred at r.t. for 12 h and then was concentrated in vacuo. Then, 0.5 M aq HCl (30 mL) was added, the solution was stirred at r.t. for 1 h, and the acid was neutralized with 1.0 M aq NaOH. The mixture was filtered and concentrated to give a residue, which was purified by column chromatography to afford pyrimidine (–)-15 as a white powder. Yield: 388 mg (76%); mp >106 °C (dec.).

[\alpha\textsubscript{D}\textsubscript{25} –20.8 (c 0.5, MeOH–CH\textsubscript{2}Cl\textsubscript{2}, 1:1).

IR (KBr): 3418, 3036, 1609 cm\textsuperscript{-1}.

[\textsuperscript{1}H] NMR (300 MHz, DMSO-d\textsubscript{6}): \(\delta = 10.60\) (br s, 1 H, D\textsubscript{2}O exchangeable), 7.62 (s, 1 H), 6.48 (br s, 2 H, D\textsubscript{2}O exchangeable), 5.48 (br s, 1 H), 5.25 (m, 1 H), 4.67 (t, J = 5.1 Hz, 1 H, D\textsubscript{2}O exchangeable), 3.55–3.48 (m, 2 H), 2.65–2.55 (m, 2 H), 1.80 (br s, J = 1.0 Hz, 3 H), 1.74–1.67 (m, 1 H).

[\textsuperscript{13}C] NMR (75 MHz, DMSO-d\textsubscript{6}): \(\delta = 156.9\) (C), 153.5 (C), 150.7 (C), 147.4 (C), 135.3 (CH), 124.3 (CH\textsubscript{2}), 116.6 (C), 61.9 (CH\textsubscript{3}), 57.3 (CH\textsubscript{2}), 49.7 (CH), 35.0 (CH\textsubscript{3}), 15.0 (CH\textsubscript{2}).

Anal. Calcd for C\textsubscript{11}H\textsubscript{16}ClN\textsubscript{5}O: C, 48.98; H, 5.98; N, 25.96. Found: C, 48.81; H, 6.02; N, 25.70.
IR (KBr): 3403, 3047, 1600 cm⁻¹.

IR (KBr): 3420, 3043, 1609 cm⁻¹.

Anal. Calcd for C₁₂H₁₄N₄O₂: C, 58.53; H, 5.73; N, 22.75. Found: C, 146.4 (CH), 140.6 (CH), 125.2 (CH), 63.4 (CH₂), 59.6 (CH), 51.1 (CH), 36.1 (CH₂), 24.3 (CH), 15.1 (CH₃), 7.6 (2 CH₂).

[(1S,4R)-4-[5-Amino-6-chloropyrimidin-4-yl]amino]-2-methylcylopent-2-enyl]methanol [(+)16]

Compound (+)16 was obtained from azide (+)10 (432 mg, 2.21 mmol) via amino alcohol 11 in the same manner as described for the synthesis of compound (+)12. 4,6-Dichloropyrimidin-5-amine (725 mg, 4.42 mmol) was used as the substrate in 11 in the second step to give the product as a foam. Yield: 428 mg (76%).

[(1S,4R)-4-[6-Chloro-9H-purin-9-yl]-2-methylcyclopent-2-enyl]methanol [(+)17]

A stirred solution of purine (+)17 (100 mg, 0.38 mmol) in MeOH (5 mL) saturated with NH₃ was heated at 100 °C for 24 h in a Parr stainless steel sealed reaction vessel. After concentration in vacuo, column chromatography (silica gel) of the residue gave purine (+)17 as a foam. Yield: 79 mg (86%).

IR (KBr): 3420, 3042, 1603 cm⁻¹.

1H NMR (300 MHz, CD₃OD): δ = 0.86 (s, 1 H), 5.34 (s, 1 H), 5.77–5.55 (m, 1 H), 6.07 (dt, J = 10.6, 3.3 Hz, 1 H), 7.66–7.82 (m, 2 H), 8.07 (s, 1 H).

[(1S,4R)-4-[6-(Cyclopropylamino)-9H-purin-9-yl]-2-methylcyclopent-2-enyl]methanol [(+)18]

Compound (+)18 was obtained as a foam from purine (+)17 (100 mg, 0.38 mmol) in the same manner as described for the synthesis of compound (+)12. Yield: 88 mg (82%).

13C NMR (75 MHz, CD₃OD): δ = 137.8 (CH), 125.2 (CH), 63.2 (CH₂), 59.6 (CH), 51.3 (CH), 36.1 (CH₂), 24.3 (CH), 15.1 (CH₃), 7.6 (2 CH₂).

Anal. Calcd for C₁₂H₁₄N₄O₂: C, 58.53; H, 5.73; N, 22.75. Found: C, 58.49; H, 5.77; N, 22.50.
read in an eight-channel computer-controlled photometer (Multi-
scan Ascent Reader, Labsystems, Helsinki, Finland) at two wave-
lengths (540 and 690 nm). All data were calculated using the
median OD (optical density) value of three wells. The 50% cytoxi-
ic concentration (CC$_{50}$) was defined as the concentration of the test
compound that reduced the absorbance (OD540) of the mock-
infected control sample by 50%. The concentration achieving 50%
protection from the cytopathic effect of the virus in infected cells
was defined as the 50% effective concentration (EC$_{50}$).

Compounds 1–5 were also tested against herpes simplex virus type
1 and type 2 (HSV-1 strain KOS and HSV-2 strain G), vaccinia vi-
rus (VV), vesicular stomatitis virus (VSV), and thymidine kinase-
deficient herpes simplex virus type 1 (TK− HSV-1, strain KOS,
AcV) in HEL cell cultures; VSV, Coxsackie virus B4, and respir-
atory syncytial virus (RSV) in HeLa cell cultures; Coxsackie virus
B4, Sindbis virus, and Punta Toro virus in Vero cell cultures, and
feline corona virus (FIPV) and feline herpes virus in CRFK cell cul-
tures, as well as for their cytotoxicity. These antiviral assays were
performed as previously described.25–27

Supporting Information for this article is available online at

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References


(18) It is a general observation that the cis-1,4-disubstituted cyclopentenes present differences in the range of 1 ppm in the chemical shifts of their H-5 protons with the upfield proton assigned as syn to both substituents. Alternatively, the difference does not usually exceed 0.3 ppm for the trans-isomer, see: Marino, J. P.; Fernandez de la Pradilla, R.; Laborde, J. E. J. Org. Chem. 1987, 52, 4893; and references cited therein.


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