Syntheses and anti-HIV activities of (±)-norcarbovir and (±)-norabacavir

Weiqiang Huang,[a] Marvin J. Miller,*[a,b] Erik De Clercq[c] and Jan Balzarini[c]

Received 9th January 2007, Accepted 14th February 2007
First published as an Advance Article on the web 1st March 2007
DOI: 10.1039/b700321h

Norcarbovir (1) and norabacavir (2), the desmethylene derivatives of anti-HIV agents carbovir and abacavir, were efficiently synthesized from a common intermediate 4. Their antitumor and antiviral activities were evaluated and the results indicate norabacavir showed comparable anti-HIV activity to that of abacavir.

Introduction

Nucleoside analogs have received great interest as synthetic targets due to their potent antitumor and antiviral activities.[1] Replacement of the furanose ring oxygen of nucleosides by a methylene group results in carbocyclic nucleosides. The absence of the normal acetal group leads to increased stability to both chemical and enzymatic hydrolyses. In some cases, syntheses of carbocyclic nucleosides provide analogs with decreased toxicity and higher biological activity than nucleosides themselves.[2] As a representative of biologically active synthetic carbocyclic nucleosides, carbovir (Fig. 1)[3] was reported to show significant anti-HIV activity *via* its triphosphate by inhibiting HIV-1 reverse transcriptase (RT). Its congener, abacavir (1592U89), which has higher oral bioavailability than carbovir, has been approved by the FDA for the treatment of HIV infection.[4] This significant biological activity attracted a number of research groups to conduct related synthetic studies. However, most of the reported syntheses of carbovir and abacavir required multiple steps to construct the substituted cyclopentene ring and sometimes the purine nucleobase.[1a,5]

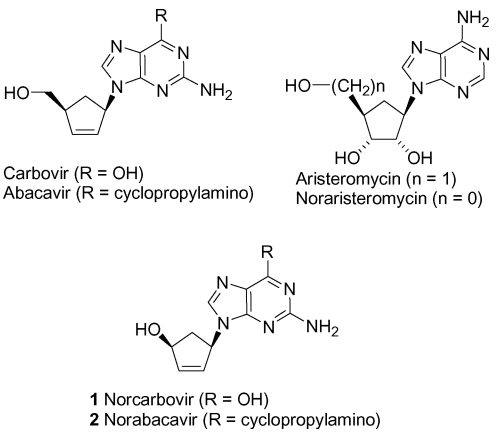
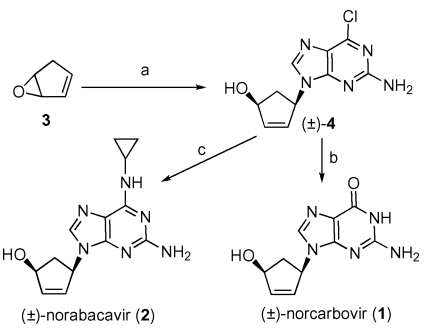


Fig. 1 Structures of carbocyclic nucleosides.

Inspired by the fact that both aristeromycin[6] and 5′-noraristeromycin[7] are inhibitors of *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase), which likely accounts for their wide spectrum of antiviral activity, we considered the syntheses of norcarbovir (**1**) and norabacavir (**2**) with the anticipation that these two desmethylene derivatives might have anti-HIV activity similar to carbovir and abacavir. Besides the possible changes in biological activity, including cytotoxicity, the removal of the 5′-methylene group would also greatly shorten synthetic routes. To date, only the syntheses of phosphate analogs of **1** have been reported,[8] but not the parent compound **1** itself. The synthesis and biological studies of norabacavir (**2**) surprisingly have not been previously described. We herein report highly efficient syntheses of **1** and **2**, along with their biological activities.

Results and discussion

The syntheses of norcarbovir and norabacavir started with allylic epoxide **3**, which could be easily obtained in large quantity by peracetic acid oxidation of cyclopentadiene.[9] Epoxide **3** underwent Pd(0)-catalyzed allylic nucleophilic substitution to afford compound **4** with 1,4-*cis* selectivity.[8] Utilizing 2-amino-6-chloro-9*H*-purine has two advantages over guanine as the nucleophile: it has good solubility and the resulting product **4** is a common precursor to **1** and **2**. Refluxing compound **4** in aqueous sodium hydroxide gave norcarbovir (**1**), and treatment of **4** with cyclopropylamine provided norabacavir (**2**) (Scheme 1).[10]



Scheme 1 Synthesis of norcarbovir and norabacavir. *Reagents and conditions*: a) $Pd(OAc)_2$, PPh_3, 2-amino-6-chloro-9*H*-purine, THF, DMSO, 51%; b) 0.33 N NaOH, reflux, 59%; c) EtOH, cyclopropylamine, 91%.

The biological activities of racemic norcarbovir and norabacavir were evaluated by testing their inhibitory effects on the proliferation of murine leukemia cells (L1210) and human T-lymphocyte cells (Molt4/C8, CEM), and also antiviral activity against HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells

[a]*Department of Chemistry & Biochemistry, University of Notre Dame, 251 Nieuwland Science Hall, Notre Dame, Indiana, 46556, USA. E-mail: mmiller1@nd.edu; Fax: +1 574 631 6652; Tel: +1 574 631 7571*
[b]*Leibniz Institute for Natural Products Research and Infection Biology-Hans Knoell Institute, Beutenbergstrasse 11a, 07745, Jena, Germany*
[c]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000, Leuven, Belgium*

Table 1 Anti-HIV activities in human T-lymphocyte (CEM) cells

Compound	EC50[a]/μg mL^{-1} HIV-1	HIV-2
(±)-Norcarbovir	>100	>100
(±)-Norabacavir	5.0 ± 1.4	≥20
Carbovir	2.0 ± 0.0	2.3 ± 2.4
Abacavir	2.3 ± 1.0	4.2 ± 1.7

[a] 50% Effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%.

Table 2 Inhibitory effects on the proliferation of murine leukemia cells (L1210/0) and human T-lymphocyte cells (Molt4/C8, CEM/0)

Compound	IC_{50}[a]/μg mL^{-1} L1210	Molt4/C8	CEM
(±)-Norcarbovir	>200	>200	>200
(±)-Norabacavir	45 ± 4	42 ± 18	19 ± 1
Carbovir	—	—	157 ± 12
Abacavir	—	—	97 ± 34

[a] 50% inhibitory concentration.

(Tables 1, 2). Norabacavir showed some anti-HIV activity but at a concentration rather close to its toxicity threshold. The EC_{50} value was about 5.0 μg mL^{-1}, which is comparable to that of abacavir,[4] although norabacavir was more toxic than abacavir. However, for norcarbovir, no anti-HIV activity was detected, even at concentrations up to 200 μg mL^{-1}. The reasoning for this absence of activity may be that the hydroxyl group at the 4′-position of norcarbovir is not available for phosphorylation, which is critical for the anti-HIV activity of carbovir.[3,11] Another possible reason is that the triphosphorylated derivative of norcarbovir may not be well recognized by HIV RT. Since the enzymes that convert carbovir and abacavir to their 5′-monophosphates are different,[11,12] it is also possible that norabacavir could be phosphorylated while norcarbovir could not. Alternatively, norabacavir may not need to be phosphorylated, but act, as such, as an AdoHcy hydrolase inhibitor. This may explain why its selectivity index (ratio of IC_{50} to EC_{50}, both measured in proliferating cells) was rather low.

However, norcarbovir and norabacavir were also tested against a variety of other DNA and RNA viruses including herpes simplex virus type 1 and type 2, vaccinia virus and vesicular stomatitis virus (VSV) in HEL cell cultures, VSV, Coxsackie virus B4 and respiratory syncytial virus in HeLa cell cultures, and parainfluenza virus-3, reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures, but they proved unable to prevent virus-induced cytopathicity at subtoxic concentrations (≥400 μg mL^{-1} for norcarbovir and >40 μg mL^{-1} for noracabavir). Since several viruses such as vaccinia virus and VSV are known to be sensitive to AdoHcy hydrolase inhibitors, these inactivity data do not point to an efficient inhibition of AdoHcy hydrolase as a target for the test compounds.

Conclusions

Racemic norcarbovir and norabacavir were efficiently synthesized in only three steps from commercially available starting materials cyclopentadiene and 2-amino-6-chloro-9*H*-purine. This is the first reported synthesis of norabacavir, which showed anti-HIV potency comparable to that of abacavir, while its congener, norcarbovir did not show any anti-HIV activity. The explanation to their biological activity was proposed and remains to be further elucidated.

Experimental

General comments

Tetrahydrofuran (THF) was distilled from sodium metal–benzophenone ketyl. All other solvent and chemicals were purchased from Acros and used as is. Silica gel flash column chromatography was performed using silica gel 60 (30–70 μm irregular particles). Melting points are uncorrected. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on Varian UnityPlus 300 at 300 MHz and 75 MHz, respectively. High resolution mass spectra were recorded on a JEOL JMS-AX505 HA double sector mass spectrometer.

cis-(±)-4-(2-Amino-6-chloro-9*H*-purin-9-yl)-2-cyclopenten-1-ol (4)

To a stirred solution of 2-amino-6-chloro-9*H*-purine (500 mg, 2.95 mmol) in dry dimethyl sulfoxide (6 mL) at room temperature under N_2, was added palladium(0) prepared by mixing palladium acetate (20 mg, 0.086 mmol) and triphenylphosphine (139 mg, 0.53 mmol) in dry THF (2 mL), and the mixture was stirred for 2 min. The solution was cooled to 0 °C, and a solution of **3** (266 mg, 3.25 mmol) in dry THF (1 mL) was added over 15 min. The resulting solution was allowed to warm to room temperature over 3 h and stirred overnight. The yellow solution was evaporated to a viscous oil that was taken up in methylene chloride (20 mL) and filtered through a small pad of Celite. The solvent was removed and the residue was purified by silica gel chromatography using (i) ethyl acetate followed by (ii) ethyl acetate–methanol (10 : 1) as the eluent to give 377 mg (51%, yield was improved to 81% when this reaction was performed at gram scale) of the title compound as a light yellow solid. Mp 158–160 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03 (s, 1 H), 6.93 (s, 2 H), 6.19 (m, 1 H), 5.99 (m, 1 H), 5.29 (m, 1 H), 4.71 (br s, 1 H), 2.85 (dt, $J_1 = 14$ Hz, $J_2 = 7.5$ Hz, 1 H), 1.67 (dt, $J_1 = 14$ Hz, $J_2 = 4.2$ Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.62, 153.44, 149.28, 141.16, 139.66, 130.41, 123.39, 73.51, 56.75, 41.11; HRMS (FAB) calcd for $C_{10}H_{11}ClN_5O$ (MH^+) 252.0652, found 252.0675.

(±)–Norcarbovir (1)

A mixture of **4** (325 mg, 1.29 mmol) and 0.33 N NaOH (40 mL) was refluxed for 5 h, where upon the solvent was removed by azeotroping with ethanol *in vacuo.* The residue was adsorbed onto silica gel which was packed onto a column and eluted with chloroform–methanol (3 : 1), giving 176 mg (59%, after recrystallization from CH_3OH–H_2O) of the title compound as white powder. Mp >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.64 (s, 1 H), 7.63 (s, 1 H), 6.48 (br s, 2 H), 6.16 (dt, $J_1 = 5$ Hz, $J_2 = 2$ Hz, 1 H), 5.96 (d, $J = 5.4$ Hz, 1 H), 5.27 (d, $J = 6.2$ Hz, 1 H), 5.21 (m, 1 H), 4.71 (br m, 1 H), 2.84 (dt, $J_1 = 14$ Hz, $J_2 = 8$ Hz, 1 H), 1.62 (dt, $J_1 = 14$ Hz, $J_2 = 4.4$ Hz, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.59, 154.22, 151.33, 140.02, 136.06, 131.55, 117.28, 74.35, 57.09, 42.28; HRMS (FAB) calcd for $C_{10}H_{12}N_5O_2$ (MH^+) 234.0991, found 234.1006.

(±)–Norabacavir (2)

A solution of **4** (200 mg, 0.80 mmol) and cyclopropylamine in ethanol (16 mL) was refluxed under N_2 overnight, and then cooled to room temperature. The solution was adsorbed onto silica gel which was packed onto a column and eluted with ethyl acetate followed by EtOAc–MeOH (5:1), giving 197 mg (91%) of the title compound as slightly yellow solid (recrystallized from CH_3OH–EtOAc). Mp 191–193 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (s, 1 H), 7.36 (br d, 1 H), 6.15 (m, 1 H), 5.94 (dd, $J_1 = 4.4$ Hz, $J_2 = 1.2$ Hz, 1 H), 5.87 (br s, 2 H), 5.47 (br s, 1 H), 5.26 (br m, 1 H), 4.70 (br s, 1 H), 3.04 (br s, 1 H), 2.84 (dt, $J_1 = 14$ Hz, $J_2 = 8$ Hz, 1 H), 1.64 (dt, $J_1 = 14$ Hz, $J_2 = 4$ Hz, 1 H), 0.63 (m, 4 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.73, 156.65, 151.38, 139.71, 136.05, 131.78, 114.32, 74.42, 56.89, 42.09, 24.54, 7.14; HRMS (FAB) calcd for $C_{13}H_{17}N_6O$ (MH^+) 273.1464, found 273.1441. The structure was also confirmed by X-ray.†

Antiviral and cytostatic activity assays

The activity of the test compounds against HIV-1(III_B) and HIV-2(ROD)-induced cytopathicity was examined in human lymphocytic CEM cell cultures at day 4–5 post infection, and the antiviral activity was estimated by microscopical examination of virus-induced giant cell formation. HIV-1 and HIV-2 were added at ~100 $CCID_{50}$ (cell culture infective dose-50) to the cell cultures.

The antiviral assays, other than anti-HIV assays, were based on inhibition of virus-induced cytopathicity in either HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus and vesicular stomatitis virus], Vero (parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, Punta Toro virus), or HeLa (vesicular stomatitis virus, Coxsackie virus B4, respiratory syncytial virus) cell cultures. Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8 μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

To determine the cytostatic activity of the test compounds, murine leukemia L1210, human lymphocytic Molt4/C8 and CEM cells were seeded in 96-well microtiter plates at ~5×10^4 cells per 200 μl well in RPMI-1640 cell culture medium in the presence of various concentrations (200, 40, 8, 1.6, 0.32 μg ml^{-1}) of the test compounds. The cell cultures were cultivated for 48 h (L1210) or 72 h (Molt4/C8, CEM) at 37 °C in a CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (Analis, Gent, Belgium).

Acknowledgements

This work was supported by grants from the National Institutes of Health (GM68012) and from the European Commission (HPAW-CT-2002-90001 and QLRT-2000-00291). We gratefully acknowledge the Lizzadro Magnetic Resonance Research Center at Notre Dame for NMR facilities, Nonka Sevova for mass spectrometry facilities and Dr A. Beatty for X-ray crystal determination of compound **2**. MJM gratefully acknowledges the kind hospitality of the HKI and the University of Notre Dame for a sabbatical opportunity.

Notes and references

† CCDC reference number 633037. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b700321h

1 For pertinent reviews, see: (*a*) M. Ferrero and V. Gotor, *Chem. Rev.*, 2000, **100**, 4319; (*b*) D. M. Huryn and M. Okabe, *Chem. Rev.*, 1992, **92**, 1745.
2 L. L. Bennett, W. M. Shannon, P. W. Allan and G. Arnett, *Ann. N. Y. Acad. Sci.*, 1975, **255**, 342.
3 R. Vince and M. Hua, *J. Med. Chem.*, 1990, **33**, 17.
4 (*a*) S. M. Daluge, US Pat., 5034394, 1991; (*b*) S. S. Good, S. M. Daluge, S. V. Ching, K. M. Ayers, W. B. Mahony, M. B. Faletto, B. A. Domin, B. S. Owens, R. E. Dornsife, J. A. McDowell, S. W. LaFon and W. T. Symonds, *Antiviral Res.*, 1995, **26**, A229; (*c*) S. M. Daluge, S. S. Good, M. B. Faletto, W. H. Miller, M. H. St. Clair, L. R. Boone, M. Tisdale, N. R. Parry, J. E. Reardon, R. E. Dornsfife, D. R. Averett and T. A. Krenitsky, *Antimicrob. Agents Chemother.*, 1997, **41**, 1082; (*d*) S. M. Daluge, M. T. Martin, B. R. Sickles and D. A. Livingston, *Nucleosides, Nucleotides Nucleic Acids*, 2000, **19**, 297.
5 For a review see: M. T. Crimmins, *Tetrahedron*, 1998, **54**, 9229.
6 (*a*) E. De Clercq, *Biochem. Pharmacol.*, 1987, **36**, 2567; (*b*) M. Cools and E. De Clercq, *Biochem. Pharmacol.*, 1989, **38**, 1061.
7 (*a*) S. M. Siddiqi, X. Chen, S. W. Schneller, S. Ikeda, R. Snoeck, G. Andrei, J. Balzarini and E. De Clercq, *J. Med. Chem.*, 1994, **37**, 551; (*b*) S. D. Patil and S. W. Schneller, *J. Med. Chem.*, 1992, **35**, 3372.
8 D. M. Coe, S. M. Roberts and R. Storer, *J. Chem. Soc., Perkin Trans. 1*, 1992, 2695.
9 S. K. Basra, M. G. B. Drew, J. Mann and P. D. Kane, *J. Chem. Soc., Perkin Trans. 1*, 2000, 3592.
10 M. J. Konkel and R. Vince, *Tetrahedron*, 1996, **52**, 799.
11 L. L. Bondoc, W. M. Shannon, J. A. Secrist, R. Vince and A. Fridland, *Biochemistry*, 1990, **29**, 9839.
12 (*a*) M. B. Faletto, W. H. Miller, E. P. Garvey, M. H. St. Clair, S. M. Daluge and S. S. Good, *Antimicrob. Agents Chemother.*, 1997, **41**, 1099; (*b*) W. B. Parker, S. C. Shaddix, B. J. Bowdon, L. M. Rose, R. Vince, W. M. Shannon and L. L. Bennett, Jr., *Antimicrob. Agents Chemother.*, 1993, **37**, 1004.

