Design, Synthesis, Conformational Analysis and Biological Activities of Purine-Based 1,2-Di-substituted Carbocyclic Nucleosides

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New 1,2-di-substituted carbocyclic nucleosides with 6-chloropurine, adenine and hypoxanthine bases were synthesized by construction of purine on the primary amino group of (±)-*trans*-2-aminocyclopentylmethanol. AM1 calculations showed close correspondence between the positions of the heteroatoms in the adenine derivative and dideoxyadenosine. The most active of the new compounds in antiviral assays and antitumoral assays against L1210/0, MOLT4/C8 and CEM/0 cells was the 6-chloropurine derivative.

Key words carbonucleosides; purine derivative; AM1 semiempirical method; antiviral agent; antitumor agent

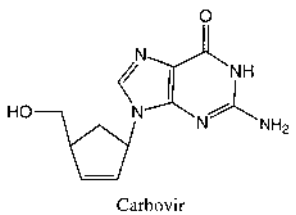
In the past decade, a large number of nucleoside analogues with antiviral and/or antitumoral properties have been successfully designed and synthesized.[1—3] Some 2′,3′-dideoxynucleosides are currently the drugs of choice for the treatment of certain viral infections (including human immunodeficiency virus (HIV) infection), these work by blocking viral reproduction, thus inhibiting reverse transcriptase.[4] Another successful modification has been the replacement of the endocyclic oxygen atom of the nucleoside sugar ring with a methylene group,[5] which reduces phosphorylase- and hydrolase-catalyzed reactivity (thereby increasing the *in vivo* half life)[6] and increases lipophilicity (thus favoring absorption and penetration of the cell membrane). The potent HIV-1 inhibitor carbovir combines both these structural modifications.[7]

We have recently investigated the properties of 1,2-disubstituted carbocyclic nucleosides (OTCs), in which the hydroxymethyl group of a carbocyclic sugar analogue is substituted at a position adjacent to the nitrogenated base.[8] Molecular modeling of the *cis* isomers of cyclopentane-based OTCs has shown that in the most stable conformers the glycoside linkage is *anti* ($\chi = -90°$, where for purine aglycons χ=C4–N9–C1′–C2′) and γ (C1′–C2′–C1″–O2″) can, as in most active nucleosides,[9,10] be $-60°$ (*gg*), $+60°$ (*gt*) or 180° (*tg*).[11]

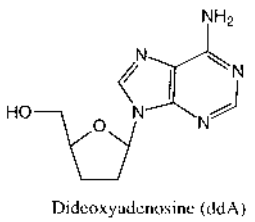
In this paper we report the synthesis, theoretical conformational analysis and preliminary antiviral and antitumoral activities of a new series of cyclopentane-based OTCs in which the hydroxymethyl group is *trans* to a purine base (adenine, 6-chloropurine or hypoxanthine).

Results and Discussion

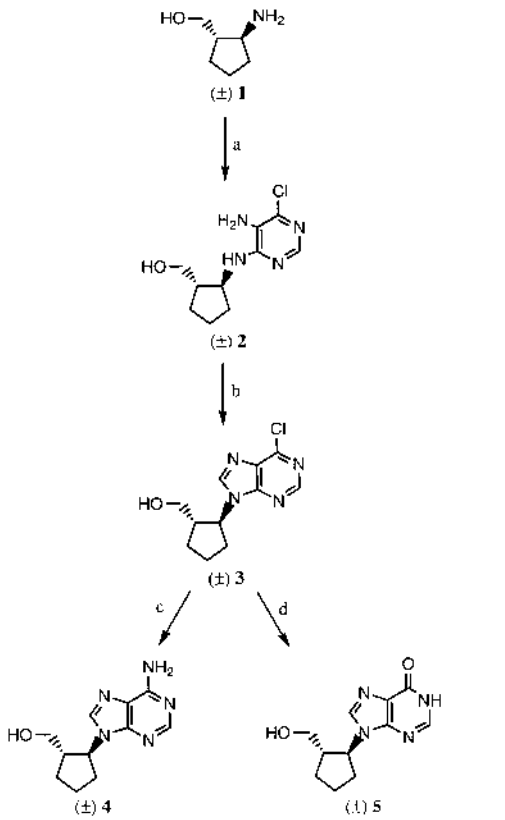
As shown in Chart 1, racemic compounds **3**, **4** and **5** were synthesized starting from (±)-*trans*-2-aminocyclopentylmethanol (**1**), which was separated from a mixture of *cis* and *trans* isomers obtained in two steps from commercially available ethyl 2-oxocyclopentylcarboxylate (overall yield 84%).[12] The amine **1** was condensed with 5-amino-4,6-dichloropyrimidine in refluxing *n*-butanol containing triethylamine, affording compound **2** in 71% yield. Ring closure with triethyl orthoformate in an acidic medium then gave an 80% yield of the 6-chloropurine **3**, the *trans* stereochemistry of which was shown by a proton nuclear Overhauser effect (NOE) experiment.[13] Compound **3** was treated with a solution of ammonia in methanol to obtain the adenine derivative



Carbovir



Dideoxyadenosine (ddA)



Reagents: a) 5-Amino-4,6-dichloropyrimidine, *n*-BuOH, Et_3N, reflux; b) $CH(OEt)_3$, HCl, r.t.; c) NH_3, MeOH, reflux; d) 0.5 M NaOH, reflux.

Chart 1

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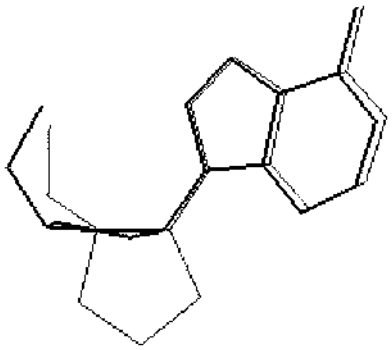

Fig. 1. Superimposition of the Stable Conformers of Compound **4** (non bold) and ddA (bold)

4 in 98% yield, and with hot sodium hydroxide to obtain the hypoxanthine derivative **5** in 94% yield.

Conformational analysis of compounds **3**—**5** was performed by means of AM1 calculations in which the parameters varied were those with the most influence on the relative positions of the base and the hydroxyl group on C1″: the dihedral angles χ and γ, and the pucker of the cyclopentane ring. Great conformational freedom was shown by the finding that the conformers detected for any given compound differed in energy by no more than about 4 kcal/mol. However, in keeping with published data for similar compounds,[9,10] the most stable had *anti* glycoside linkages ($\chi=-90°$ or $-115°$) and hydroxymethyl side chains with *gg*, *gt* or *tg* conformations ($\gamma=-60°$, $+60°$ or $180°$). In the conformer with $\chi=-90°$ and $\gamma=+60°$, the cyclopentane ring adopted an N conformation and the distances of the OH group from the nitrogen atoms of the base were almost identical to those found in ddA (2′,3′-dideoxyadenosine) and in most other active nucleosides. This similarity is illustrated by the root mean square (RMS) distances between corresponding heteroatoms being just 0.2—0.35 Å when this conformer was superimposed on the active conformer of ddA ($\chi=-90°$, $\gamma=-60°$) in such a way as to minimize this RMS, although the attainment of this minimum requires the cyclopentane ring to lie perpendicular to the ddA sugar ring; see Fig. 1.

Compounds **3**—**5** achieved no significant inhibition of replication of the following viruses in assays carried out in the following cell cultures: HIV-1 and HIV-2 in human T-lymphocyte (CEM) cells; Vesicular stomatitis, Coxsackie B4 and Polio-1 in human epithelial (HeLa) cells; HSV-1 (KOS), HSV-2 (G), HSV-1 TK$^-$ (B2006), HSV-2 TK$^-$ (VMW1837), Vaccinia and Vesicular stomatitis in human embryonic skin–muscle fibroblast (E6SM) cells, or Parainfluenza-3, Reovirus-1, Sindbis, Coxsackie B4 and Semliki forest in African green monkey kidney (Vero) cells. Assays comparing compounds **3**—**5** with Ara A showed compound **3** to cause a 50% reduction in cell proliferation at concentrations of $56.1\pm3.0\,\mu$M for murine leukemia cells L1210 (Ara A: $14.2\pm6.4\,\mu$M), $39.2\pm8.0\,\mu$M for Molt4/C8 human T-lymphocytes (Ara A: $11.9\pm7.3\,\mu$M) and $19.0\pm4.2\,\mu$M for CEM/0 T cells (Ara A: $24.8\pm1.9\,\mu$M).

Experimental

Chemistry Melting points were determined in a Reichert Kofler thermopan apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a Perkin-Elmer 1640FT spectrometer (ν in cm^{-1}). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX 300 NMR spectrometer, using tetramethylsilane (TMS) as an internal standard (δ in ppm, J in Hz). Mass spectrometry was carried out in a Hewlett Packard 5988A spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer. Flash chromatography (FC) was performed on silica gel (Merck 60, 230—400 mesh).

(±)-*trans*-5-Amino-6-chloro-4-[2-(hydroxymethyl)cyclopentylamino]-pyrimidine (2) A mixture of the aminoalcohol **1**[12] (1 g, 8.70 mmol), 5-amino-4,6-dichloropyrimidine (1.48 g, 9.02 mmol), Et_3N (5 ml) and *n*-BuOH (30 ml) was refluxed for 1 h under Ar. The solvent was then evaporated under a vacuum, and the solid residue was redissolved in ethyl acetate by stirring with IRA-420 (OH) until all turbidity had disappeared. The resin was filtered out, the solvent was evaporated under a vacuum, and the residue was purified by FC using 98 : 2 CH_2Cl_2/MeOH as an eluent, which gave pure **2** (1.5 g, 71%). IR (KBr) cm^{-1}: 3250, 2923, 1650, 1581, 1484, 1475, 1444, 1012. ^{1}H-NMR (DMSO-d_6) δ: 1.32—1.98 (m, 7H, (–CH_2–)$_3$, –CH–C–O), 3.42 (m, 2H, –CH_2–O), 4.07 (q, J=6.76, 1H, –CH–N), 4.59 (t, J=5.14, 1H, aliphatic –OH), 5.07 (br s, 2H, –NH_2), 6.63 (d, J=6.86, 1H, NH–), 7.69 (s, 1H, H-2). ^{13}C-NMR (DMSO-d_6) δ: 22.5 (4′), 27.7 (3′), 32.3 (5′), 45.1 (2′), 54.5 (1′), 61.6 (6′), 124.0, 137.4, 146.3, 152.3. MS *m/z* (%): 244 ([M+2]$^+$, 11), 242 (M$^+$, 32), 221 (37), 169 (15), 146 ([M+2]$^+-C_6H_{10}O$, 29), 144 (M$^+-C_6H_{10}O$, 100), 117 (13), 67 (12). *Anal.* Calcd for $C_{10}H_{15}ClN_4O$: C, 49.49; H, 6.23; N, 23.08. Found: C, 49.56; H, 6.30; N, 23.28.

(±)-*trans*-6-Chloro-9-[2-(hydroxymethyl)cyclopentyl]-9*H*-purine (3) A mixture of compound **2** (150 mg; 0.62 mmol), $CH(OEt)_3$ (3.4 ml; 0.02 mol) and conc. HCl (0.04 ml) was stirred for 12 h at room temperature. The solvent was then evaporated under a vacuum and the solid residue was redissolved in tetrahydrofuran (THF) (10 ml) and 0.5 M HCl (13 ml). After 2 h of stirring at room temperature, the mixture was neutralized with 0.5 M NaOH, the solvent was evaporated under vacuum (forming an azeotropic mixture with ethanol–toluene) and the solid residue was purified by FC using 99 : 1 CH_2Cl_2/CH_3OH as an eluent, which gave pure **3** (125 mg, 80%), mp 108—110 °C. IR (KBr) cm^{-1}: 3292, 3065, 2953, 1593, 1565, 1394, 1337, 1220, 1063, 952, 634. ^{1}H-NMR ($CDCl_3$) δ: 1.64—2.51 (m, 7H, (–CH_2–)$_3$, –CH–C–O), 2.77 (m, 1H, aliphatic –OH), 3.62 (m, 2H, –CH_2–O), 4.85 (q, J=8.18, 1H, –CH–N), 8.21 (s, 1H, H-8), 8.73 (s, 1H, H-2). ^{13}C-NMR ($CDCl_3$) δ: 23.4 (4′), 27.6 (3′), 32.4 (5′), 48.7 (2′), 59.1 (1′), 63.5 (6′), 132.4 (5), 144.4 (8), 151.5 (4), 151.9 (2), 152.1 (6). MS *m/z* (%): 254 ([M+2]$^+$, 4), 252 (M$^+$, 11), 181 (12), 157 ([M+2]$^+-C_6H_9O$, 33), 155 (M$^+-C_6H_9O$, 100), 119 (12), 80 (6), 67 (11). *Anal.* Calcd for $C_{11}H_{13}ClN_4O$: C, 52.28; H, 5.19; N, 22.17. Found: C, 52.31; H, 5.17; N, 22.09.

(±)-*trans*-9-[2-(Hydroxymethyl)cyclopentyl]adenine (4) Gaseous ammonia was bubbled for 1 h through a solution of **3** (100 mg, 0.39 mmol) in CH_3OH (20 ml) in a steel reactor at −80 °C. The reactor was closed and heated for 20 h in an oven at 60 °C, the solvent was evaporated under a vacuum, and the solid residue was purified by FC using 98 : 2 CH_2Cl_2/CH_3OH as an eluent, which gave pure **4** (90 mg, 98%), mp 153—155 °C; IR (KBr) cm^{-1}: 3300, 2950, 2871, 1687, 1610, 1567, 1477, 1419, 1303, 651. ^{1}H-NMR ($CDCl_3$) δ: 1.48—2.13 (m, 6H, (–CH_2–)$_3$), 2.50 (m, 1H, –CH–C–O), 3.36 (m, 2H, –CH_2–O), 4.58 (m, 2H, –CH–N, aliphatic –OH), 7.14 (br s, 2H, –NH_2), 8.11, 8.17 (each s, each 1H, H-2, H-8). ^{13}C-NMR ($CDCl_3$) δ: 23.0 (4′), 27.7 (3′), 32.5 (5′), 47.1 (2′), 57.9 (1′), 62.6 (6′), 119.6 (5), 140.3 (8), 149.8 (4), 152.4 (2), 156.3 (6). MS *m/z* (%): 233 (M$^+$, 23), 216 (M$^+-NH_3$, 24), 162 (27), 136 (70), 135 (M$^+-C_6H_{10}O$, 100), 108 (31), 81 (6), 67 (9). *Anal.* Calcd for $C_{11}H_{15}N_5O$: C, 56.64; H, 6.48; N, 30.02. Found: C, 56.49; H, 6.60; N, 29.68.

(±)-*trans*-9-[2-(Hydroxymethyl)cyclopentyl]hypoxanthine (5) A mixture of **3** (100 mg, 0.39 mmol) and 0.5 M NaOH (5 ml) was refluxed for 5 h. The solvent was then evaporated under a vacuum and the solid residue was purified by FC using 95 : 5 CH_2Cl_2/CH_3OH as an eluent, which gave pure **5** (87 mg, 94%), mp 237—238 °C. IR (KBr) cm^{-1}: 3350, 3337, 2863, 1702, 1593, 1545, 1414, 1132, 643. ^{1}H-NMR (DMSO-d_6) δ: 1.46—2.17 (m, 6H, (–CH_2–)$_3$), 2.47 (m, 1H, –CH–C–O), 3.33 (m, 2H, CH_2–O), 4.59 (q, J=8.10, 2H, –CH–N), 8.01, 8.15 (each s, each 1H, H-2, H-8), 12.26 (br s, 1H, aromatic –OH). ^{13}C-NMR (DMSO-d_6) δ: 23.0 (4′), 27.7 (3′), 32.9 (5′), 47.5 (2′), 58.1 (1′), 62.5 (6′), 124.7 (5), 139.6 (8), 145.5 (4), 148.6 (2), 157.1 (6). MS *m/z* (%): 234 (M$^+$, 25), 204 (M$^+-CH_2$, 9), 164 (13), 163 (12), 137 (M$^+-C_6H_9O$, 100) , 136 (M$^+-C_6H_{10}O$, 60), 109 (22), 81 (14), 67 (14). *Anal.* Calcd for $C_{11}H_{14}N_4O_2$: C, 56.40; H, 6.02; N, 23.92. Found: C, 56.45; H, 5.83; N, 23.84.

Computational Methods Optimization of theoretical molecular geometries was carried by the AM1 semiempirical quantum mechanical method[14] using the program AMPAC,[15] which was run on an SGI work station. The geometry was optimized by varying the torsion angles χ [C4–N9–C1′–C2′] and γ [C1′–C2′–C10′–O2″] between 0° and 360° in 10° increments.

Biological Activity Assays Assays of antiviral activity and cytotoxicity were carried out in accordance with established procedures.[16)]

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References

1) Hobbs J. B., "Purine and Pyrimidine Targets," in Comprehensive Medicinal Chemistry, Vol. 2, ed. by Hansch C., Pergamon Press, Ch., 1990, pp. 299—332.
2) De Clercq E., *J. Med. Virol*., **5**, 149—164 (1995).
3) Mansour T. S., Storer R., *Current Pharmaceutical Design*, **3**, 227—264 (1997).
4) *a*) De Clercq E., *Clinical Microbiology Reviews*, **10**, 674—693 (1997); *b*) *Idem*, *The Medical Letter*, **1998**, 5—10.
5) Marquez V. E., *Advances in Antiviral Drug Design*, **2**, 89—146 (1996).
6) Shealy Y. F., Clayton J. D., *J. Am. Chem. Soc*., **88**, 3885—3887 (1966).
7) Vince R., Hua M., *J. Med. Chem*., **33**, 17—21, (1990).
8) Santana L., Teijeira M., Uriarte E., De Clercq E., Balzarini J., *Nucleosides & Nucleotides*, **14**, 521—523, (1995); Santana L., Teijeira M., Uriarte E., Fadda A., Podda G., Catinella S., Traldi P., *Rapid Commun. Mass Spectrom.*, **1996**, 1316—1319.
9) Saenger W., "Principles of Nucleic Acid Structure," Springer-Verlag, New York, Inc., New York, 1984.
10) Herdewyn P., Balzarini J., De Clercq E., "Advances in Antiviral Drug Design," Vol. 1, ed. by De Clercq E., JAI Press, Inc., Greenwich, CT, 1993, pp. 233—318.
11) Santana L., Teijeira M., Terán C., Uriarte E., Casselato U., Graziani R., *Nucleosides & Nucleotides*, **15**, 1179—1187 (1996).
12) Teijeira M., Doctoral Thesis, University of Santiago de Compostela, Spain, 1996.
13) Teijeira M., Santana L., Uriarte E., *Magn. Reson. Chem*., **35**, 806—807 (1997).
14) Dewar M. J. S., Zoebisch E. G., Healy E. F., Stewart J. J. P., *J. Am. Chem. Soc.*, **107**, 3902—3909 (1985).
15) BIOSYM Technologies, Inc., 10065 Barnes Canyon Road, San Diego, CA 92121.
16) De Clercq E., "*In vitro* and *ex vivo* test systems to rationalize drug design and delivery," ed. by Crommelin D., Couvreur P., Duchene D., Editions de Santé, Paris, 1994, pp. 108—125.