Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 3903–3909
Bioorganic & Medicinal Chemistry

4′-α-*C*-Branched *N*,*O*-nucleosides: synthesis and biological properties

Ugo Chiacchio,[a] Filippo Genovese,[b] Daniela Iannazzo,[b] Anna Piperno,[b,*] Paolo Quadrelli,[c] Corsaro Antonino,[a] Roberto Romeo,[b] Vincenza Valveri[d] and Antonio Mastino[d]

[a]*Dipartimento Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy*
[b]*Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy*
[c]*Dipartimento di Chimica Organica, Università degli Studi di Pavia V.le Taramelli 10, 27100 Pavia, Italy*
[d]*Dipartimento Scienze Microbiologiche, Genetiche e Molecolari, Università di Messina, Salita Sperone 31, 98100 Messina, Italy*

Received 12 March 2004; revised 22 April 2004; accepted 30 April 2004

Abstract—The synthesis of 4′-α–*C*-branched *N*,*O*-nucleosides has been described, based on the 1,3-dipolar cycloaddition of nitrones with vinyl acetate followed by coupling with silylated nucleobases, The obtained compounds have been evaluated for their activity against HSV-1, HSV-2, HTLV-1. Cytotoxicity and apoptotic activity have been also investigated: compound **10c** shows moderate apoptotic activity in Molt-3 cells.

1. Introduction

Nucleosides play a fundamental role in modern viral chemotherapy as potent inhibitors of HIV, the causative agent of AIDS.[1] After being converted to the triphosphate form in cells by specific kinases,[2] they may either inhibit HIV reverse transcriptase, or be incorporated into the growing DNA chain, resulting in chain termination or disruption.[3] In this context, modifications of the sugar fragment has resulted in the synthesis of interesting nucleoside analogues such as AZT, ddC and ddI,[4] which have shown remarkable activity towards the human immunodeficiency virus.

However, prolonged therapeutic use of this class of compounds resulted in the development of resistant strains and cross-resistance to related nucleosides. Accordingly, considerable efforts have been made to synthesize new modified nucleosides characterized by minor toxicity and improved or more specific biological activity.

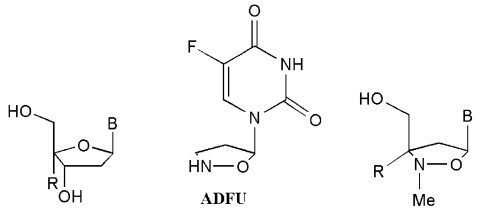
A synthetic strategy is represented by the exploitation of the heteroatom substitution; thus, a series of compounds, where the sugar moiety has been replaced by alternative heterocyclic rings, as 3TC and L-2′,3′-dideoxy-3′-oxacytidine, proved to be effective in the treatment of HIV/HBV infections and different lymphoid and solid tumours, respectively.[5]

The introduction of a side-chain on the sugar led to several branched nucleosides, which have been evaluated as potential antitumoural or antiviral agents;[6] some of them, such as 1-(2-deoxy[1]-2-methylene-β-D-*erythro*-pentafuranosyl)cytosine (DMDC),[7] 1-(2-cyano-2-deoxy-β-D-*arabino*-pentafuranosyl)cytosine(CNDAC),[8] 1-(2-deoxy-2-fluoro-methylene-β-D-*erythro*-pentafuranosyl)-cytosine (FMDC),[9] 1-(3-*C*-ethynyl-β-D-*ribo*-pentafuranosyl)cytosine (ECyd)[10] and its uracil congener (EUrd),[10] have shown potent in vitro and in vivo antitumoural activity (Fig. 1).

4′α-*C*-Branched nucleosides, namely 4′α-*C*-cyano-deoxythymidine **1**, 4′α-*C*-methyl-2′-deoxycytidine **2,** 4′α-*C*-ethynyl-2′-deoxycytidine **3** and 4′α-*C*-hydroxymethyl-deoxythymidine **4** have also been prepared and

Keywords: Modified nucleosides; 1,3-Dipolar cycloaddition; Antiviral activity; Apoptotic activity; *C*-Branched nucleosides.
* Corresponding author. Tel.: +39-090-356230; fax: +39-090-6766562; e-mail: apiperno@pharma.unime.it

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.04.041



1 B = Thymine R = CN
2 B = Cytosine R = CH_3
3 B = Cytosine R = ethynyl
4 B = Thymine R = CH_2OH

Figure 1. Modified nucleosides.

demonstrate to possess strong anti-HIV and anti-leukaemic activities.[11–13]

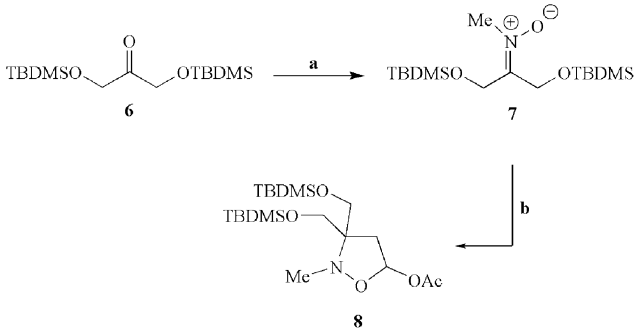
Recently we have reported that *N*,*O*-nucleosides,[14] where the sugar moiety has been replaced by an isoxazolidine ring, exhibit very promising biological properties. In particular, ADFU, while showing low level of cytotoxicity, is a good inductor of apoptosis in lymphoid and monocytoid cells.[15]

On the basis of these findings, we became interested in the synthesis of 4′α-branched-chain *N*,*O*-nucleosides in order to evaluate their biological characteristics; therefore, we designed a series of new 4′-*C*-substituted *N*,*O*-nucleosides **5**. In this communication, we describe the synthesis of these derivatives and their antiviral activities against herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) as well as their apototic activity versus Molt-3 differentiated cells.

2. Chemistry

The synthetic scheme is based on the 1,3-dipolar cycloaddition of *C*-α-disilyloxymethyl-*N*-methyl nitrone **7** with vinyl acetate, followed by nucleosidation performed with silylated purine and pyrimidine bases.[16]

Nitrone **7** has been prepared in good yields starting from 1,3-bis-(-*t*-butyl-dimethyl-silanyloxy)-propan-2-one **6**.[17] The subsequent treatment with *N*-methyl hydroxylamine in toluene, in the presence of triethylamine, gave, after flash chromatography (chloroform/methanol 98:2 as eluant), the expected nitrone **7** (90% yield) (Scheme 1).



Scheme 1. Reagents and conditions: (a) NEt_3, $CH_3NHOH{\cdot}HCl$, Toluene, 2 h, rt. (b) Vinyl acetate, 36 h, rt.

The cycloaddition reaction between **7** and vinyl acetate, carried out at room temperature for 36 h, leads with a complete regioselectivity to isoxazolidine **8** (yield 85%), which has been purified by flash chromatography (cyclohexane/diethyl acetate 95:5 as eluant).

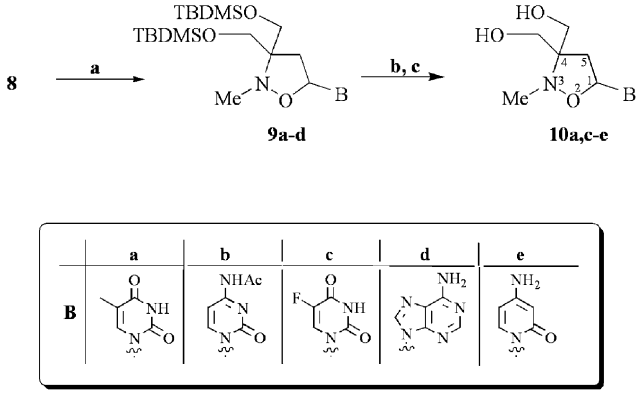
The structure of isoxazolidine **8** has been confirmed by ^1H NMR experiments. Thus, H_4 protons give rise to two doublets of doublets centred at 2.04 and 2.62 ppm; the two methylene groups of the substituents at C_3 resonate as four doublets in the range 3.50–3.80 ppm, while H_5 proton gives rise to a doublet of doublets at 6.23 ppm.

The cycloaddition product has been, then, coupled with silylated nucleobases, according to the Vorbrüggen glycosylation methodology.[16] The condensations of **8** with silylated thymine, *N*-acetylcytosine, 5-fluorouracil and adenine have been performed in acetonitrile, at room temperature, in the presence of 0.15 equiv of TMSOTf as catalyst to give after TBAF treatment the corresponding nucleosides **10a**,**c**–**e** (Scheme 2).

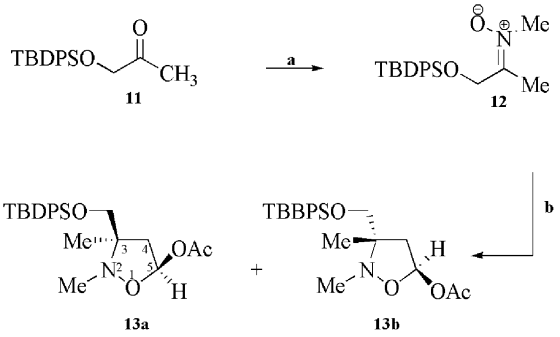
The molecular structure of the reaction products was confirmed on the basis of analytical and spectroscopic data. The ^1H NMR spectra, recorded in D_2O, show diagnostic signals for H_1, which resonate as doublet of doublets in the range 5.92–6.01 ppm, while H_5 protons appear as two doublet of doublets centred at 2.70–2.75 and 2.17–2.30 ppm.

The reaction route has been also addressed towards the formation of 4′-methyl *N*,*O*-nucleosides; thus, treatment of 1-(*tert*-butyl-diphenyl-silanyloxy)propan-2-one[18] **11** with *N*-methyl hydroxylamine afforded nitrone **12** as a *Z*/*E* mixture (2.5:1 ratio). The subsequent cycloaddition reaction with vinyl acetate proceeded without any diastereoselectivity, affording isoxazolidines **13a** and **13b** in the 1:1 relative ratio (Scheme 3).

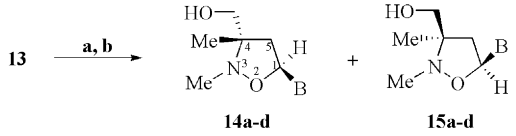
The assignment of *cis*/*trans* configurations has been performed on the basis of NOEDS experiments. For **13a**, irradiation of H-5 proton induced a positive NOE effect on the downfield resonance (H-4a) of methylene protons at C-4 and on the methyl group at C-3; conversely, when H-4b was irradiated, a NOE enhancement

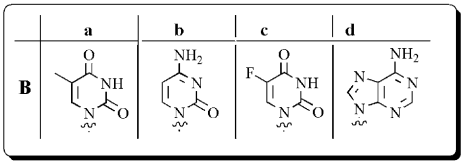


Scheme 2. Reagents and conditions: (a) silylated base/TMSOTf, 12 h, rt; (b) TBAF/THF, 1 h, rt; (c) for **9b**: MeOH, K_2CO_3, 1 h, rt.



Scheme 3. Reagents and conditions: (a) NEt_3, $CH_3NHOH{\cdot}HCl$, Toluene, 2 h, rt. (b) Vinyl acetate, 36 h, rt.





Scheme 4. Reagents and conditions: (a) silylated base/TMSOTf, 12 h, rt; (b) TBAF/THF, 1 h, rt; for **14b** and **15b**: MeOH, K_2CO_3, 1 h, rt.

was observed for the methylene group linked at C-3. These data unambiguously indicate a *cis* relationship between H-5, H-4a and the methyl substituent at C-3. In **13b**, irradiation of H-5 induced a positive NOE effect for the methylene group at C-3.

Coupling of the diastereoisomeric mixture of **13** with silylated bases led to a mixture of α- and β-nucleosides **14** and **15** (40:60 ratio), which have been separated by flash chromatography (Scheme 4).

The stereochemical assignments to the obtained nucleosides have been performed by NOEDS spectroscopy: in α-derivatives **14a**–**d**, irradiation of H-5a, the downfield resonance of methylene protons at C-5, induces a positive NOE effect on H-1 and the CH_2OH group at C-4, so suggesting that these protons are topologically close together. In contrast, in β-derivatives **15a**–**d**, the NOE effect was observed between H-1 and the methyl group at C-4.

3. Biological assays

The synthesized compounds were tested for their antiviral activity against HSV-1, HSV-2 in Vero (African Green Monkey) cells, HTLV-1 in human cells (lymphomonocytes of peripheral blood) and for their toxicity in vitro. None of these derivatives reached the inhibitory concentration 50 at the highest concentrations tested (e.g. 320 μM), indicating a lack of significant antiviral activity. Since the biological activity of this type of inhibitors is often linked to their susceptibility to phosphorylation by kinases, it would be interesting to evaluate the phosphate or phosphonate forms of the synthesized compounds in order to determine whether they could improve the antiviral activity towards retroviruses.

The apoptotic activity was also tested in Molt-3 cells. Only **10c** has shown a moderate activity (32%) at the concentration 500 μM after 48 h. No significant toxicity, when evaluated using the classical trypan blue test, was detected until 3 days of treatment in lymphoid Molt-3 cell line assayed.

4. Experimental

4.1. General

Commercially available chemicals and solvents were reagent grade and used as received. All solvents were dried according to literature methods. Melting points were determined with a Kofler apparatus and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 240B microanalyzer. NMR spectra were recorded on a Varian instrument at 300 or 500 MHz (^1H) and at 75 MHz (^{13}C) using deuterochloroform or deuterated water as solvents; chemical shifts are given in ppm from TMS as internal standard. NOE difference spectra were obtained by subtracting alternative right off-resonance free induction decays (FIDS) from right-on-resonance-induced FIDS. Thin-layer chromatographic separations were achieved through Merck silica gel 60-F_{254} precoated aluminium plates. Preparative separations were carried out by flash chromatography using Merck silica gel 0.035–0.070 mm. The identification of samples from different experiments was secured by mixed mps and superimposable NMR spectra.

4.2. General procedure for the synthesis of the nitrone 7 and 12

A solution of **6** or **11** (19 mmol) *N*-methyl hydroxylamine hydrochloride (19 mmol) and triethylamine (19 mmol) in toluene (70 mL) was left to stir 3 h; after filtration the solvent was evaporated under reduced pressure, and the residue was subjected to flash-chromatography ($CHCl_3/CH_3OH$ 99:1).

4.2.1. [2-{[*tert*-Butyl(dimethyl)silyl]oxy}-1-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)ethylidene](methyl)azane oxide (7). Starting from 1,3-bis-(*t*-butyl-dimethyl-silanyloxy)-propan-2-one, compound **7** was obtained as yellow oil (90%). ^1H NMR ($CDCl_3$): δ 0.15 (s, 12H), 0.95 (s, 18H), 3.81 (s, 3H, *N*–CH_3), 4.45 (s, 2H), 4.65 (s, 2H); ^{13}C NMR ($CDCl_3$): δ 5.62, 18.04, 18.10, 22.41, 22.50, 40.01, 66.47, 67.64, 170.48. HRMS (FAB) calcd for $[M^+]$ $C_{16}H_{37}NO_3Si_2$ 347.6489, found 347.6485. Anal.

Calcd for $C_{16}H_{37}NO_3Si_2$: C, 55.28; H, 10.73%; N, 4.03. Found: C, 55.10; H, 11.02; N, 3.98%.

4.2.2. (*Z*)-(*E*)(2-{[*tert*-Butyl(diphenyl)silyl]oxy}-1-methylethylidene)(methyl)azane oxide (12). Starting from 1-*t*-butyl-diphenyl-silanyloxy)propan-2-one, compound **12** was obtained as inseparable mixture of *E*/*Z* isomers (1:2.5). Yellow oil (82% global yield). *Z*-Isomer ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H), 2.18 (s, 3H), 3.65 (s, 3H, *N*–CH_3), 4.70 (s, 2H), 7.20–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 19.19, 26.63, 36.70 47.58, 62.65, 127.76, 130.56, 132.69, 135.46, 170.48. *E*-Isomer: ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 9H), 1.95 (s, 3H), 3.65 (s, 3H, *N*–CH_3), 4.30 (s, 2H), 7.20–7.40 (m, 10H); ^{13}C NMR ($CDCl_3$): δ 19.19, 26.63, 36.70, 47.77, 62.59, 127.70, 130.72, 132.70, 136.46, 170.40. HRMS (FAB) calcd for [M^+] $C_{20}H_{27}NO_2Si$, 369.6135, found 369.6132.

4.3. General procedure for the synthesis of isoxazolidines 8 and 13a–b

A solution of nitrones **7** or **12** (5.7 mmol) in vinyl acetate (30 mL) was left under stirring for 36 h at room temperature; after this period, the reaction mixture was evaporated under reduced pressure and the residue purified by flash chromatography (hexane/ethyl acetate 98:2).

4.3.1. 3,3-Bis({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-methyl-isoxazolidin-5-yl acetate (8). Starting from nitrone **7**, compound **8** was obtained (85% yield) as a sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.10 (s, 12H), 0.90 (s, 18H), 2.04 (dd, 1H, J = 1.5, 14.1 Hz, H_{4a}), 2.05 (s, 3H), 2.62 (dd, 1H, J = 6.6, 14.1 Hz, H_{4b}), 2.82 (s, 3H, *N*–CH_3), 3.53 (d, 1H, J = 9.6 Hz), 3.68 (d, 1H, J = 9.9 Hz), 3.76 (d, 1H, J = 9.9 Hz), 3.83 (d, 1H, J = 9.6 Hz), 6.23 (dd, 1H, J = 1.5, 6.6 Hz, H_5); ^{13}C NMR (75 MHz, $CDCl_3$): δ 5.96, 18.14, 18.21, 21.50, 23.11, 40.35, 40.91, 62.97, 64.50, 70.05, 95.86, 170.24. HRMS (FAB) calcd for [M^+] $C_{20}H_{43}NO_5Si_2$ 433.7397, found 433.7392. Anal. Calcd for $C_{20}H_{27}NO_3Si_2$: C, 55.38%; H, 9.99%; N, 3.23%. Found: C, 55.10%; H, 9.70%; N, 3.10%.

4.3.2. 3-[(*tert*-Butyl-diphenyl-silyl)oxy-methyl]-2,3-dimethyl-isoxazolidin-5-yl acetate (13a–b). Starting from nitrone 12, isoxazolidines **13a** and **13b** in 1:1 ratio (88% global yield) were obtained and separated by flash chromatography (hexane/ethyl acetate 98:2). The first eluted product was (3*RS*,5*SR*),3-[(*tert*-butyl-diphenyl-silyl)oxy-methyl]-2,3-dimethyl-isoxazolidin-5-yl acetate **13a** as a sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 9H), 1.21 (s, 3H), 2.05 (s, 3H), 2.26 (dd, 1H, J = 3.0, 13.5 Hz, H_{4a}), 2.36 (dd, 1H, J = 6.5, 13.5 H_{4b}), 2.73 (s, 3H, *N*–CH_3), 3.67 (q, 2H, J = 10 Hz), 6.23 (dd, 1H, J = 3.0, 6.5 Hz, H_5), 7.45–7.65 (m 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.20, 18.36, 21.90, 26.90, 39.18, 46.88, 66.41, 68.80, 94.92, 126.93, 129.71, 132.88, 135.23, 170.45. HRMS (FAB) calcd for [M^+] $C_{24}H_{33}NO_4Si$ 427.6183, found 427.6177. Anal. Calcd for $C_{24}H_{33}NO_4Si$: C, 67.41%; H, 7.78%; N, 3.27%. Found: C, 67.15%; H, 7.64%; N, 3.22%. The second eluted product was (3*SR*,5*SR*)-3-[(*tert*-butyl-diphenyl-silyl)-oxy-methyl]-2,3-dimethyl-isoxazolidin-5-yl acetate **13b** as a sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (s, 9H), 1.25 (s, 3H), 2.07 (s, 3H), 2.73 (s, 3H, *N*–CH_3), 2.74 (m, 2H, H_4), 3.62 (d, 1H, J = 10.2 Hz), 3.73 (d, 1H, J = 10.2 Hz), 6.24 (dd, 1H, J = 3.0, 5.0 Hz, H_5), 7.45–7.65 (m 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.25, 18.30, 21.80, 26.90, 39.20, 46.80, 66.41, 67.90, 94.80, 126.63, 129.70, 132.88, 135.20, 170.42. HRMS (FAB) calcd for [M^+] $C_{24}H_{33}NO_4Si$ 427.6183, found 427.6180. Anal. Calcd for $C_{24}H_{33}NO_4Si$: C, 67.41%; H, 7.78%; N, 3.27%. Found: C, 67.10%; H, 7.68%; N, 3.20%.

4.4. General procedure for the synthesis of protected nucleosides 9a,d

A suspension of the nucleobase (0.62 mmol) in dry acetonitrile (3 mL) was treated with bis(trimethylsilyl)acetamide (2.54 mmol), and refluxed for 15 min under stirring. The obtained clear solution was added to a solution of the isoxazolidine **8** (0.52 mmol) in dry acetonitrile (3 mL); trimethylsilyltriflate (0.2 mmol) was added dropwise, and the reaction mixture was left under stirring overnight at rt. After cooling at 0 °C, the solution was neutralized by careful addition of aqueous 5% sodium bicarbonate, and then concentrated in vacuo. After the addition of dichloromethane (8 mL), the organic phase was separated, washed with water (2×10 mL), dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 99:1) to furnish the nucleosides **9a**,**d**.

4.4.1. 1-[3,3-Bis({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-methyl-isoxazolidin-5-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (9a). Starting from **8**, compound **9a** was obtained (60% yield) as a white oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.12 (s, 12H), 0.93 (s, 18H), 1.96 (d, 3H, J = 1.2 Hz), 2.09 (dd, 1H, J = 4.2 and 13.0 Hz, H_4), 2.82 (s, 3H, *N*–CH_3), 2.87 (dd, 1H, J = 7.8 and 13.0 Hz, H_4), 3.60 (d, 1H, J = 9.6 Hz), 3.63 (d, 1H, J = 9.9 Hz), 3.74 (d, 1H, J = 9.9 Hz), 3.89 (d, 1H, J = 9.6 Hz), 6.10 (dd, 1H, J = 4.2 and 7.8 Hz, $H_{5'}$), 7.71 (q, 1H, J = 1.2 Hz, H_6), 8.60 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 5.59, 12.66, 18.32, 18.64, 25.80, 25.84, 38.59, 43.47, 59.36, 63.63, 69.76, 82.14, 110.36, 136.47, 150.36, 163.72. HRMS (FAB) calcd for [M^+] $C_{23}H_{45}N_3O_5Si_2$ 483.8027, found 483.8025. Anal. Calcd for $C_{23}H_{45}N_3O_5Si_2$: C, 57.10%; H 9.38%; N, 13.22%. Found: C, 56.95%; H, 9.08%; N, 12.98%.

4.4.2. *N*-{1-[3,3-Bis({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-methyl-isoxazolidin-5-yl]-2-oxo-1,2-dihydropyrimidin-4-yl}acetamide (9b). Starting from **8**, compound **9b** (64% yield) was obtained as a white oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.35 (s, 12H), 0.95 (s, 18H), 2.00 (dd, 1H, J = 3.0 and 14.4 Hz, H_4), 2.08 (s, 3H), 3.02 (s, 3H, *N*–CH_3), 3.05 (dd, 1H, J = 7.8 and 14.4 Hz, H_4),

3.51 (d, 1H, $J = 9.6$ Hz), 3.57 (d, 1H, $J = 10.2$ Hz), 3.69 (d, 1H, $J = 10.2$ Hz), 3.94 (d, 1H, $J = 9.6$ Hz), 6.02 (dd, 1H, $J = 3.0$ and 7.8 Hz, $H_{5'}$), 7.46 (d, 1H, $J = 7.5$ Hz, H_5), 8.24 (d, 1H, $J = 7.5$ Hz, H_6), 10.21 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.67, 18.04, 18.15, 24.13, 25.78, 25.80, 38.71, 44.28, 59.24, 64.11, 68.29, 84.27, 96.16, 145.06, 155.28, 162.85, 171.50. HRMS (FAB) calcd for [M^+] $C_{24}H_{46}N_4O_5Si_2$ 526.8278, found 526.8275. Anal. Calcd for $C_{24}H_{46}N_4O_5Si_2$: C, 54.71%; H, 8.79%; N, 10.63%. Found: C, 54.62%; H, 8.68%; N, 10.42%.

4.4.3. 1-[3,3-Bis({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-methyl-isoxazolidin-5-yl]-5-fluoropyrimidine-2,4(1*H*,3*H*)-dione (9c). Starting from **8**, compound **9c** (55% yield) was obtained as a white oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.21 (s, 12H), 0.95 (s, 18H), 2.17 (dd, 1H, $J = 3.3$ and 13.2 Hz, H_4), 2.78 (s, 3H, *N*–CH_3), 2.84 (dd, 1H, $J = 8.1$ and 13.2 Hz, H_4), 3.58 (d, 1H, $J = 10.2$ Hz), 3.61 (d, 1H, $J = 9.9$ Hz), 3.72 (d, 1H, $J = 10.2$ Hz), 3.85 (d, 1H, $J = 9.9$ Hz), 6.08 (dd, 1H, $J = 3.3$ and 8.1 Hz, $H_{5'}$), 8.13 (d, 1H, $J = 6.3$ Hz, H_6), 9.00 (br s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.72, 18.36, 18.45, 27.38, 37.48, 41.22, 59.98, 61.04, 69.95, 82.80, 126.50, 142.27, 151.13, 160.37. HRMS (FAB) calcd for [M^+] $C_{22}H_{42}FN_3O_5Si_2$ 503.7655, found 503.7652. Anal. Calcd for $C_{22}H_{42}FN_3O_5Si_2$: C, 48.48%; H, 8.40%; N, 8.34%. Found: C, 48.37%; H, 8.29%; N, 8.27%.

4.4.4. 9-[3,3-Bis({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-methyl-isoxazolidin-5-yl]-9*H*-purin-6-amine (9d). Starting from **8**, compound **9d** (33% yield) was obtained as a white sticky oil. ^{1}H NMR (300 MHz, $CDCl_3$): 0.40 (s, 12H), 0.95 (s, 18H), 2.48 (dd, 1H, $J = 7.2$ and 13.5 Hz, H_4), 2.91 (s, 3H, *N*–CH_3), 2.94 (dd, 1H, $J = 7.4$ and 13.5 Hz, H_4), 3.74 (d, 1H, $J = 10.2$ Hz), 3.80 (d, 1H, $J = 10.5$ Hz), 3.90 (d, 1H, $J = 10.5$ Hz), 4.01 (d, 1H, $J = 10.2$ Hz), 6.25 (dd, 1H, $J = 7.2$ and 7.4 Hz, $H_{5'}$), 7.21 (br s, 2H, NH), 7.98 (s, 1H, H_8), 8.49 (s, 1H, H_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ –5.63, 18.25, 18.36, 26.94, 26.98, 39.18, 41.43, 59.67, 64.13, 71.29, 84.31, 121.43, 144.08, 151.99, 153.68, 162.39. HRMS (FAB) calcd for [M^+] $C_{23}H_{44}N_6O_3Si_2$ 508.8124, found 508.8120. Anal. Calcd for $C_{23}H_{44}N_6O_3Si_2$: C, 54.29%; H, 8.71%; N, 16.52%. Found: C, 54.13%; H, 8.62%; N, 16.44%.

4.5. General procedure for the synthesis of deprotected nucleosides 10a,c–e

A 1 M THF solution of tetrabutyl ammonium fluoride (2.2 mL, 2.2 mmol) was added to a stirred solution of protected nucleosides **9a**,**c**,**d** (1 mmol) in dry THF (10 mL); after 1 h, the solvent was evaporated and the residue was flash-chromatographed, using a $CHCl_3$/MeOH gradient from 98:2 up to 96/4, to furnish the desilylated nucleosides **10a**,**c**,**d**. In the case of compound **9b**, the TBAF treatment afforded a residue which, after solvent evaporation, was dissolved in a mixture of aqueous K_2CO_3 (5%, 5 mL) and methanol (5 mL) and left under stirring for 2 h. The solvent was then evaporated under reduced pressure, and the residue was flash-chromatographed ($CHCl_3$/CH_3OH 95:5) to give **10e**.

4.5.1. 1-[3,3-Bis(hydroxymethyl)-2-methyl-isoxazolidin-5-yl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (10a). Starting from **9a**, compound **10a** was obtained and crystallized from acetone/ether. Yield 85%; white solid, mp 108–110 °C. ^{1}H NMR (300 MHz, D_2O): δ 1.75 (d, 3H, $J = 1.1$ Hz), 2.32 (dd, 1H, $J = 4.2$ and 14.1 Hz, H_4), 2.67 (s, 3H, *N*–CH_3), 2.71 (dd, 1H, $J = 8.1$ and 14.1 Hz, H_4), 3.53 (s, 2H), 3.64 (s, 2H), 5.99 (dd, 1H, $J = 4.2$ and 8.1 Hz, $H_{5'}$), 7.72 (q, 1H, $J = 1.1$ Hz, H_6); ^{13}C NMR (75 MHz, D_2O): δ 14.21, 40.06, 43.02, 62.34, 63.47, 72.60, 85.40, 113.48, 140.29, 154.40, 156.35. HRMS (FAB) calcd for [M^+] ($C_{11}H_{17}N_3O_5$) 271.2741, found 271.2739. Anal. Calcd for ($C_{11}H_{17}N_3O_5$): C, 48.70%; H, 6.32%; N, 15.48%. Found: C, 48.51%; H, 6.25%; N, 15.322%.

4.5.2. 4-Amino-1-[3,3-bis(hydroxymethyl)-2-methyl isoxazolidin-5-yl]pyrimidin-2(1*H*)-one (10b). Starting from **9b**, compound **10e** was obtained and crystallized from acetone/ether. Yield 80%; white solid, mp 115–119 °C. ^{1}H NMR (300 MHz, D_2O): δ 2.20 (dd, 1H, $J = 3.9$ and 14.1 Hz, H_4), 2.67 (s, 3H, *N*–CH_3), 2.75 (dd, 1H, $J = 7.8$ and 14.1 Hz, H_4), 3.48 (s, 2H), 3.65 (s, 2H), 5.86 (d, 1H, $J = 7.5$ Hz, H_5), 5.89 (dd, 1H, $J = 3.9$ and 7.8 Hz, $H_{5'}$), 7.80 (d, 1H, $J = 7.5$ Hz, H_6); ^{13}C NMR (75 MHz, D_2O): δ_C 36.46, 38.54, 49.53, 64.38, 72.9, 100.89, 108.2, 110,9, 118.4, 198.8. HRMS (FAB) calcd for [M^+] $C_{10}H_{16}N_4O_4$ 256.4144, found 256.4141. Anal. Calcd for $C_{10}H_{16}N_4O_4$: C, 46.84%; H, 6.28%; N, 21.84%. Found: C, 46.64%; H, 6.02%; N, 21.74%.

4.5.3. 1-[3,3-Bis(hydroxymethyl)-2-methyl-isoxazolidin-5-yl]-5-fluoropyrimidine-2,4(1*H*,3*H*)-dione (10c). Starting from **9c**, compound **10c** was obtained and crystallized from acetone/ether. Yield 89%; white solid, mp 96–98 °C. ^{1}H NMR (300 MHz, D_2O): 2.28 (dd, 1H, $J = 3.6$ and 13.4 Hz, H_4), 2.64 (s, 3H, *N*–CH_3), 2.69 (dd, 1H, $J = 8.4$ and 13.6 Hz, H_4), 3.45 (m, 2H), 3.62 (m, 2H), 5.96 (dd, 1H, $J = 3.6$ and 8.4 Hz, H5′), 8.06 (d, 1H, $J = 6.5$ Hz, H_6); ^{13}C NMR (75 MHz, D_2O): δ 37.47, 42.10, 59.99, 61.03, 70.00, 83.71, 126.55, 142.30, 151.15, 160.41. HRMS (FAB) calcd for [M^+] $C_{10}H_{14}FN_3O_5$ 275.2375, found 275.2372. Anal. Calcd for $C_{10}H_{14}FN_3O_5$: C, 43.64%; H, 5.12%; N, 15.26%. Found: C, 43.49%; H, 5.02%; N, 15.06%.

4.5.4. 9-[3,3-Bis(hydroxymethyl)-2-methyl-isoxazolidin-5-yl]-9*H*-purin-6-amine (10d). Starting from **9d**, compound **10d** was obtained ad crystallized from acetone/ether. Yield 82%; white solid, mp 112–113 °C. ^{1}H NMR (300 MHz, D_2O): δ 2.6 (dd, 1H, $J = 6.8$ and 13.3 Hz, H_4), 2.71 (s, 3H, *N*–CH_3), 2.82 (dd, 1H, $J = 7.2$ and 13.3 Hz, H_4), 3.61 (m, 2H), 3.80 (m, 2H), 6.25 (dd, 1H, $J = 6.8$, 7.2 Hz, $H_{5'}$), 8.05 (s, 1H, H_8), 8.24 (s, 1H, H_2); ^{13}C NMR (75 MHz, D_2O): δ 18.22, 26.73, 39.08, 41.22, 59.53, 64.09, 71.12, 84.00, 121.13, 143.92, 151.45,

153.36, 162.02. HRMS (FAB) calcd for [M^+]. $C_{11}H_{16}N_6O_3$ 280.2844, found 280.2840. Anal. Calcd for $C_{11}H_{16}N_6O_3$: C, 47.14; H, 5.75; N, 29.98%. Found: C, 47.01; H, 5.62; N, 29.78%.

4.6. General procedure for the synthesis of nucleosides 14a–d and 15a–d

A suspension of nucleobase (2 mmol) in dry acetonitrile (70 mL) was treated with bis(trimethylsilyl) acetamide (3.0 mmol) and stirred at rt until a clear solution was obtained (0.5–6 h). A solution of isoxazolidine **13a–b** (1 mmol) in dry acetonitrile (5 mL) and TMSOTf (0.1 mmol) was added and the resulting mixture was stirred at rt overnight. After this time, the mixture was neutralized by addition of a aqueous 5% $NaHCO_3$ and then concentrated in vacuo. The aqueous layer was extracted with ethyl acetate and combined organic layers were dried over sodium sulfate, filtered and then evaporated to dryness. The residue was then dissolved in THF; TBAF (1.1 mmol) was added and the mixture was stirred at rt for 1.5 h. The solvent was evaporated and the residue was flash-chromatographed, using a $CHCl_3$/MeOH gradient from 98:2 up to 96/4, to furnish the desilylated nucleosides **14a–d** and **15a–d**. For the cytosine derivatives, the desilylation process followed the deacetylation reaction, performed by treatment with a mixture of aqueous K_2CO_3 and methanol as above reported for compound **10e**.

4.6.1. 1-(3-Hydroxymethyl-2,3-dimethyl-isoxazolidin-5-ylmethyl)-1*H*-pyrimidine-2,4-dione (14a and 15a). Starting from **13a–b**, compounds **14a** and **15a** in 40:60 ratio (56% global yield) were obtained and separated by flash chromatography. The first eluted product was (3*SR*,5*SR*)-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-ylmethyl)-1*H*-pyrimidine-2,4-dione **15a**. The second eluted product was (3*RS*,5*SR*)-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-ylmethyl)-1*H*-pyrimidine-2,4-dione[19] **14a**.

4.6.2. 4-Amino-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-yl)1*H*-pyrimidin-2-one (14b and 15b). Starting from **13a–b**,compounds **14b** and **15b** in 40:60 ratio (50% global yield) were obtained and separated by flash chromatography. The first eluted product was (3*RS*,5*SR*)-4-amino-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-yl)1*H*-pyrimidin-2-one **15b** crystallized from acetone/ether. Mp 163–166 °C, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.22 (s, 3H), 2.51 (m, 2H, H_4), 2.52 (s, 3H, *N*–CH_3), 3.72 (s, 2H), 5.70 (d, 1H, $J = 7.5$ Hz), 5.96 (dd, 1H, $J = 5.1$ and 7.3 Hz) 7.65 (d, 1H, $J = 7.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.10, 36.60, 37.20, 51.01, 72.09, 99.90, 108.80, 111.30, 158.20, 164.06. HRMS (FAB) calcd for [M^+] $C_{10}H_{16}N_4O_3$ 240.2630, found 240.2627. Anal. Calcd for $C_{10}H_{16}N_4O_3$: C, 49.99%; H, 6.71%; N, 21.62%. Found: C, 49.89%; H, 6.58%; N, 21.43%. The second eluted product was (3*SR*,5*SR*)-4-amino-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-yl)1*H*-pyrimidin-2-one **14b**, crystallized from acetone/ether. Mp 169–172 °C, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (s, 3H), 2.07 (dd, 1H, $J = 4.5$ and 14.5 Hz, H_{4a}), 2.80 (s, 3H, *N*–CH_3), 3.26 (dd, 1H, $J = 7.5$ and 14.5 Hz, H_{4b}), 3.65 (d, 1H, $J = 11.5$ Hz), 3.74 (d, 1H, $J = 11.5$ Hz), 5.70 (d, 1H, $J = 7.5$ Hz), 6.04 (dd, 1H, $J = 4.5$ and 7.5 Hz) 7.65 (d, 1H, $J = 7.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.20, 36.50, 37.18, 50.06, 70.20, 99.95, 107.80, 112.10, 157.20, 166.08.; HRMS (FAB) calcd for [M^+] $C_{10}H_{16}N_4O_3$ 240.2630, found 240.2626. Anal. Calcd for $C_{10}H_{16}N_4O_3$: C, 49.99; H, 6.71; N, 21.62%. Found: C, 49.85; H, 6.62; N, 21.43%.

4.6.3. 5-Fluoro-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-yl)-1*H*-pyrimidin-2,4-dione (14c and 15c). Starting from **13a–b**, compounds **14c** and **15c** in 40:60 ratio (60% global yield) were obtained and separated by flash chromatography. The first eluted product was (3*RS*,5*SR*)-5-fluoro-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-yl)-1*H*-pyrimidin-2,4-dione **15c**, crystallized from acetone/ether. Mp 103–105 °C, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25 (s, 3H), 2.64 (m, 2H, H_4), 2.65 (s, 3H, *N*–CH_3), 3.65 (m, 2H), 6.04 (dd, 1H, $J = 5.9$ and 6 Hz, H_5), 7.94 (d, 1H $J = 6.5$ Hz) 8.26 (br s, 1H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.0, 37.16, 45.77, 64.44, 67.35, 83.50, 124.77, 125.50, 156.70, 157.60. HRMS (FAB) calcd for [M^+] $C_{10}H_{14}FN_3O_4$ 259.2381, found 259.2376. Anal. Calcd for $C_{10}H_{14}FN_3O_4$: C, 46.33%; H, 5.44%; N, 16.20%. Found: C, 46.28%; H, 5.33%; N, 16.10%. The second eluted product was (3*SR*,5*SR*)-5-fluoro-1-(3-hydroxymethyl-2,3-dimethyl-isoxazolidin-5-yl)-1*H*-pyrimidin-2,4-dione **14c**, crystallized from acetone/ether. Mp 96–98 °C, white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (s, 3H), 1.59 (sb 1H, OH), 2.11 (dd, 1H, $J = 4.5$ and 14 Hz, H_{4a}), 2.77 (s, 3H, *N*–CH_3), 3.10 (dd, 1H, $J = 7.5$ and 14 Hz, H_{4b}), 3.63 (d, 1H, $J = 11$ Hz), 3.77 (d, 1H, $J = 11$ Hz), 6.06 (dd, 1H, $J = 4.5$ and 7.5 Hz, H_5), 7.94 (d, 1H, $J = 6$ Hz), 8.62 (sb, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.10, 37.18, 45.70, 64.60, 68.35, 83.70, 125.10, 125.60, 156.76, 157.58; HRMS (FAB) calcd for [M^+] $C_{10}H_{14}FN_3O_4$ 259.2381, found 259.2378. Anal. Calcd for $C_{10}H_{14}FN_3O_4$: C, 46.33%; H, 5.44%; N, 16.20%. Found: C, 46.13%; H, 5.29%; N, 16.02%.

4.6.4. 9-[3-(Hydroxymethyl)-2,3-dimethylisoxazolidin-5-yl]-9*H*-purin-6-amine (14d and 15d). Starting from **13a–b**, compounds **14d** and **15d** in 40:60 ratio (40% global yield) were obtained and separated by flash chromatography. The first eluted product was (3*RS*,5*SR*)9-[3-(hydroxymethyl)-2,3-dimethyl-isoxazolidin-5-yl]-9*H*-purin-6-amine **15d**, as a sticky oil. ^{1}H NMR (300 MHz, D_2O): δ 1.09 (s, 3H), 2.62 (s, 3H, *N*–CH_3), 2.98 (m, 2H), 3.59 (s, 2H), 6.21 (dd, 1H, $J = 4.2$ and 7.9 Hz), 8.26 (s, 1H), 8.33 (s, 1H); ^{13}C NMR (75 MHz, D_2O): δ 15.30,38.01, 41.30, 62.50, 64.70, 84.30, 120.90, 144.73, 148.90, 153.35, 155.40. HRMS (FAB) calcd for [M^+] $C_{11}H_{16}N_6O_2$ 264.2860, found 264.2855. Anal. Calcd for $C_{11}H_{16}N_6O_2$: C, 49.99%; H, 6.10%; N, 37.80%. Found: C, 49.82%; H, 6.01%; N, 37.72%. The second eluted product was (3*SR*,5*SR*)9-[3-(hydroxymethyl)-2,3-

dimethyl-isoxazolidin-5-yl]-9*H*-purin-6-amine **14d** as a sticky oil. ^{1}H NMR (300 MHz, D_2O): δ ^{1}H NMR (300 MHz, D_2O): δ 1.15 (s, 3H), 2.61 (s, 3H, *N*–CH_3), 2.73 (dd, 1H, $J = 7.9$ and 13.7 Hz H_{4a}), 2.83 (dd, 1H $J = 4.9$ and 13.7 Hz H_{4b}), 3.62 (s, 2H), 6.22 (dd, 1H, $J = 4.9$ and 7.9 Hz), 8.15 (s, 1H), 8.21 (s, 1H); ^{13} C NMR (75 MHz, D_2O: δ; 16.20, 37.98, 41.02, 61.01, 64.55, 84.10,120.66, 144.15, 147.20, 153.12, 155.36. HRMS (FAB) calcd for [M^+] $C_{11}H_{16}N_6O_2$ 264.2860, found 264.2856. Anal. Calcd for $C_{11}H_{16}N_6O_2$: C, 49.99%; H, 6.10%; N, 37.80%. Found: C, 49.87%; H, 5.99%; N, 37.67%.

4.7. Antiviral and cytotoxicity assay for HSV

The newly synthesized nucleosides were evaluated for their activity against HSV-1 and HSV-2 by plaque reduction assay in VERO cells using a methodology reported in literature.[20] Cytotoxicity assays were conducted in rapidly dividing Vero cells, as reported.[20]

4.8. Evaluation of toxicity and apoptosis

Toxicity was evaluated by a standard viability assay, using the trypan blue exclusion test. Normally, apoptosis was evaluated by morphological analysis of the cells, performed following staining with acridine orange as previously described.[21] Briefly, over 600 cells, including those showing typical apoptotic characteristics, were counted using a fluorescence microscope. The identification of apoptotic cells was based on the presence of uniformly stained nuclei showing chromatin condensation and nuclear fragmentation. In some experiments, apoptosis was detected by flow cytometric analysis of isolated nuclei, following staining with propidium iodide, on a Becton Dickinson FAC Scan Analytic Flow Cytometer, as previously described.[22]

Acknowledgements

Financial supports by University of Messina, Catania and MIUR (FIRB and PRIN) are gratefully acknowledged.

References and notes

1. (a) De Clercq, E. New Developments in Anti-HIV Chemotherapy. *Biochim. Biophys. Acta* **2002**, *1587*, 258; (b) De Clercq, E. *Med. Res. Rev.* **2002**, *22*, 531.
2. Lin, T. S.; Guo, J.; Schinazi, R. F.; Chu, C. K.; Xiang, J. N.; Prusoff, W. H. *J. Med. Chem.* **1988**, *31*, 336; De Clercq *Antiviral Res.* **1989**, *12*, 1.
3. (a) Huang, P.; Farquar, D.; Plunkett, W. *J. Biol. Chem.* **1990**, *33*, 37; (b) Brosset, T.; Klein, E.; Créminon, C.; Grassi, J.; Mioskowski, C.; Mioskowski, C.; Lebeau, L. *Tetrahedron* **2001**, *57*, 8129.
4. (a) Mitsuya, H.; Yarchoan, R.; Broder, S. *Science* **1990**, *249*, 153; (b) De Clerq, E.; Van Aerschot, A.; Hederwijn, P.; Baba, M.; Pauwels, R.; Balzarini, J. *Nucleos. Nucleot.* **1989**, *8*, 659.
5. (a) Chang, C. N.; Doong, S. L.; Zhou, J. H.; Beach, J. W.; Jeong, L. S.; Chu, C. K.; Tsai, C. H.; Cheng, Y. C. *J. Biol. Chem.* **1992**, *267*, 13938; (b) Grove, K. L.; Guo, X.; Lui, S. H.; Gao, Z.; Chu, C. K.; Cheng, Y. C. *Cancer Res.* **1995**, *55*, 3008.
6. Nomura, M.; Shuto, S.; Tanaka, M.; Saaki, T.; Shuichi, M.; Shigeta, S.; Matsuda, A. *J. Med. Chem.* **1999**, *42*, 2901.
7. (a) Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamagami, K. *J. Med. Chem.* **1988**, *31*, 1063; (b) Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T. *J. Med. Chem.* **1991**, *34*, 812; (c) Fujii, A.; Yamagami, K.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T.; Sasaki, T. *Cancer Res.* **1991**, *51*, 2319; (d) Lin, T.-S.; Luo, M. Z.; Liu, M. C.; Clarkatzenburg, R. H.; Cheng, Y. C.; Prusoff, W. H.; Mancini, W. R.; Birnbaum, G. I.; Gabe, E.; Giziewicz, J. *J. Med. Chem.* **1991**, *34*, 2607; (e) Baker, C. H.; Banzon, J.; Bollinger, J. M.; Stubbe, J.; Samano, V.; Robins, M. J. *J. Med. Chem.* **1991**, *34*, 1879; (f) Cory, A. H.; Samano, V.; Robins, M. J.; Cory, J. G. *Biochem. Pharmacol.* **1994**, *47*, 365.
8. (a) Matsuda, A.; Nakajima, Y.; Azuma, A.; Tanaka, M.; Sasaki, T. *J. Med. Chem.* **1991**, *34*, 2917; (b) Azuma, A.; Nakajima, Y.; Nishizono, N.; Minakava, N.; Suzuki, M.; Hanaoka, K.; Kobayashi, T.; Sasaki, T.; Marsuda, A. *J. Med. Chem.* **1993**, *36*, 4183; (c) Tanaka, M.; Matsuda, A.; Terao, T.; Sasaki, T. *Cancer Lett.* **1992**, *64*, 67; (d) Azuma, A.; Hanaoka, K.; Kurihara, A.; Kobayashi, T.; Miyauchi, S.; Kamo, N.; Tanaka, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem.* **1995**, *38*, 3391; (e) Matsuda, A.; Azuma, A. *Nucleos. Nucleot.* **1995**, *14*, 461.
9. McCarthy, J. R.; Matthews, D. P.; Stemerick, D. M.; Huber, E. W.; Bey, P.; Lippert, B. J.; Snyder, R. D.; Sunkara, P. S. *J. Am. Chem. Soc.* **1991**, *113*, 7439.
10. Hattori, H.; Tanaka, M.; Fukushima, M.; Sasaki, T.; Matsuda, A. *J. Med. Chem.* **1996**, *39*, 5005.
11. O-Yang, C.; Wu, H. Y.; Fraser-Smith, E. B.; Walker, K. A. M. *Tetrahedron Lett.* **1992**, *33*, 37.
12. O-Yang, C.; Kurz, W.; Eugui, E. M.; McRoberts, M. J.; Verheyden, J. P. H.; Kurz, L. J.; Walker, K. A. M. *Tetrahedron Lett.* **1992**, *33*, 41.
13. Kodama, E. I.; Kohgo, S.; Kitano, K.; Machida, H.; Gatanaga, H.; Shigeta, S.; Matsuoka, M.; Ohrui, H.; Mitsuya, H. *Antimicrob. Agents Chemother.* **2001**, *45*, 1539.
14. (a) Iannazzo, D.; Piperno, A.; Pistarà, V.; Rescifina, A.; Romeo, R. *Tetrahedron* **2002**, *58*, 581; (b) Chiacchio, U.; Borrello, L.; Merino, P.; Iannazzo, D.; Piperno, A.; Rescifina, A.; Richichi, B.; Romeo, G. *Tetrahedron: Asymmetry* **2003**, *14*, 2419.
15. Chiacchio, U.; Corsaro, A.; Iannazzo, D.; Piperno, A.; Pistarà, V.; Rescifina, A.; Romeo, R.; Valveri, V.; Mastino, A.; Romeo, G. *J. Med. Chem.* **2003**, *46*, 3696.
16. Vörbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 12340.
17. Ferraboschi, P.; Reza Elhai, S.; Verza, E.; Meroni Rivolta, F.; Santaniello, E. *Synlett* **1996**, *12*, 1176.
18. (a) Bolm, C.; Felder, M. *Synlett* **1994**, 655–656; (b) Kim, D. K.; Kim, G.; Gam, J.; Cho, Y. *J. Med. Chem.* **1994**, *37*(10), 1471.
19. Chiacchio, U.; Gumina, G.; Rescifina, A.; Romeo, R.; Uccella, N.; Casuscelli, F.; Piperno, A.; Romeo, G. *Tetrahedron* **1996**, *52*, 8889.
20. Schinazi, R. F.; Peters, J.; Williams, C. C.; Chance, D.; Nahmias, A. J. *Antimicrob. Agents Chemother.* **1982**, *22*, 499.
21. Mastino, A.; Sciortino, M. T.; Medici, M. A.; Perri, D.; Ammendolia, M. G.; Grelli, S.; Amici, C.; Pernice, A. *Cell Death Differ.* **1997**, *4*, 649.
22. Matteucci, C.; Grelli, S.; De Smaele, E.; Fontana, C.; Mastino, A. *Cytometry* **1999**, *35*, 145.