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Glycosyl-oxycarbonylaminosulfonyl-2',3'-dideoxynucleoside Derivatives as Lipophilic Nucleotide Mimics. Synthesis and Anti-HIV Activity

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Abstract—Several lipophilic-2',3'-dideoxynucleotide analogues have been synthesized and tested against Human Immunodeficiency Virus (HIV). Glycosyl-oxycarbonylaminosulfonyl-analogues of 3'-deoxythymidine and 2',3'-dideoxyuridine have been synthesized by reaction of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose with chlorosulfonyl isocyanate and the corresponding 2',3'-dideoxynucleoside. Another series of 5'-phosphate-like-3'-deoxythymidine nucleosides (5'-*O*-alkylsulfamoyl- and 5'-*O*-carbamoyl-3'-deoxythymidine) have also been prepared. Both series of compounds can be considered as lipophilic nucleotide mimics.

Introduction

2',3'-Dideoxynucleosides (ddN) are potent and selective inhibitors of Human Immunodeficiency Virus (HIV) replication which act through inhibition of HIV reverse transcriptase (HIV-RT).[1–4] To interact with their target enzyme, pharmacologically effective levels of 2',3'-ddN 5'-triphosphates (ddN-TP) have to be generated. This implies that the candidate 2',3'-ddN has to enter the cell and also has to be transformed to the corresponding 5'-triphosphate by cellular kinases.[5,6] The triphosphate metabolite may act at the RT level as either a competitive inhibitor or an alternate substrate, and thus be incorporated as ddN monophosphate into the viral DNA.[7,8] Since this analogue lacks the 3'-hydroxyl group, its incorporation into DNA will result in chain termination.[4,9]

The efficiency with which ddN are transformed to the 5'-TP derivatives seems to play a role in the differences found in the antiretroviral activity of the ddN.[7,10,11] These differences appear to be related to the different affinities of ddN for the enzyme responsible for their initial phosphorylation to the 5'-monophosphate, which is the limiting step for several ddN, including 3'-deoxythymidine, for their activation to the 5'-TP derivatives.[11,12]

On the other hand, it is known that HIV can replicate in the brain, and the infected brain can serve as a reservoir for the virus.[13] Therefore it is important that anti-HIV agents penetrate the blood-brain barrier and suppress viral replication in the brain.

Different approaches have been used to circumvent the enzymatic monophosphorylation and to facilitate transport through the cell membrane.[14–19] Thus, phosphate derivatives and analogues,[14–17] as well as glycosyl phosphotriesters have been reported by several authors.[19]

Antiherpetic analogues of nucleoside diphosphate hexoses in which the diphosphate bridge is replaced by different isosters have been reported by us.[20–22] Among those, replacement involving the use of an -OCONHSO$_2$–O- group proved to be the most effective. Structure–activity relationship studies on these compounds demonstrated that the phosphate-like 5'-*O*-sulfamoyl moiety is required for activity,[21] while the perbenzoylated hexopyranosyl sugar moiety[22] is important for transport through the cell membrane. However, the only report on the influence of the polyphosphate chain modification on the inhibition of the HIV-RT is that concerning the substitution of the bridging oxygens in this polyphosphate chain of dTTP by NH to provide the corresponding imidotriphosphate analogues[23] and that in which the polyphosphate chain has been replaced by an arylsulfonylurea moiety as a triphosphate mimic.[24]

These facts prompted us to prepare the lipophilic nucleotide mimics **6** and **7** in which the ddN was linked to a 5'-*O*-[[[(2",3",4",6"-tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl moiety and the 5'-mono-phosphate-like compounds **8–12** substituted at the 5'-position with sulfamate or carbamate groups, these mono-phosphate-like compounds have been prepared in order to study whether they could be recognized by cellular kinases and converted, in two steps, to the corresponding 5'-triphosphates. Replacement of the 5'-phosphate group in nucleotides by the non-ionized sulfamate or carbamate groups has been currently used in chemotherapy,[25–30] with the aim of avoiding enzymatic cleavage or failure to cross the cell membrane. The present paper describes the synthesis and anti-HIV activity evaluation of these new lipophilic 2',3'-dideoxynucleotide mimics.

Results and Discussion

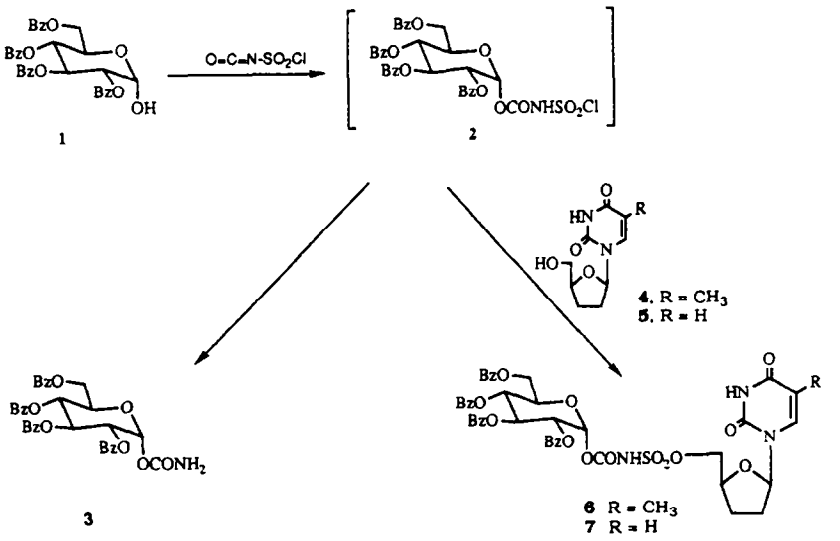
Chemistry

Reaction of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranose (**1**) (Scheme I) with chlorosulfonyl isocyanate in dry methylene chloride at low temperature followed by "*in situ*" reaction of the unstable sugar intermediate **2**, with 3'-deoxythymidine[31] (**4**) or with 2',3'-dideoxyuridine[32] (**5**) gave 5'-*O*-[[[[(2",3",4",6"-tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-deoxythymidine (**6**) and 2',3'-dideoxyuridine derivative (**7**) in 67 and 60% yield, respectively. Compound **2** was formed by reaction of the glucose anomeric hydroxyl group with the isocyanate group of the reagent (chlorosulfonyl isocyanate), more reactive towards nucleophiles than the chlorosulfonyl group. The structures of **6** and **7** were established by analytical and spectroscopic data and by comparison with compounds of similar structures previously synthesized by us.[20,22]

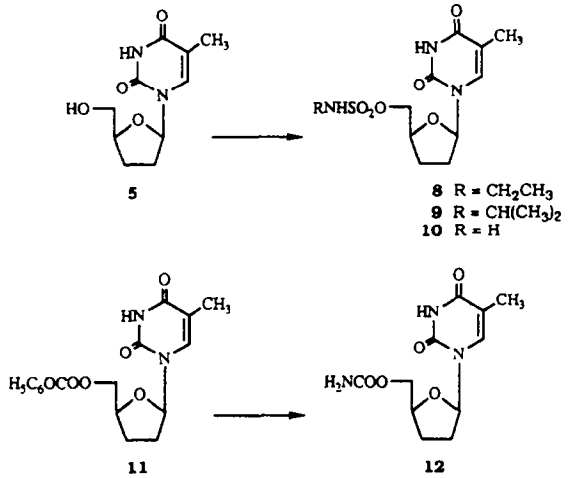
The nature of the indicated five-atom bridge as glucosyl–$OCONHSO_2O$–nucleoside and not the inverse glucosyl–OSO_2NHCOO–nucleoside was confirmed by the formation of the carbamate **3**[20] when the sugar intermediate **2** was left in contact with ambient moisture.

The α-anomeric configuration of the hexose moiety in **6** and **7** was determined from the $J_{1",2"}$=3.7 Hz coupling constant value.[20] The attachment of the [[(glucosyloxy)carbonyl]amino]sulfonyl residue to the 5'-*O*-position of 3'-deoxythymidine (**4**) or 2',3'-dideoxyuridine (**5**) in **6** and **7**, respectively, was demonstrated by the presence in the ^{1}H-NMR spectra of a broad singlet at δ 9.90 for **6** and at δ 9.83 for **7**, corresponding to the 3-NH, proton which disappeared upon D_2O shake, and by the downfield shift of the H-4' and H-5' protons (Δδ=0.2–0.4 ppm) of the ribose moiety with respect to the same protons of the starting compounds **4** or **5**.

5'-*O*-(*N*-Ethylsulfamoyl)- and 5'-*O*-[(*N*-iso-propyl)-sulfamoyl]-3'-deoxythymidine **8** and **9** (Scheme II) were prepared in 62 and 80% yield by reaction of 3'-deoxythymidine[31] with *N*-ethylsulfamoyl chloride[33] and *N*-isopropylsulfamoyl chloride,[33] respectively. Similarly, reaction of **5** with sulfamoyl chloride[34] afforded compound **10** in 82% yield. Finally, 5'-*O*-carbamoyl derivative **12** was prepared in 65% overall yield by reaction of **5** with phenyl chloroformate followed by treatment of the intermediate **11**, thus obtained, with methanolic ammonia.



Scheme I.



Scheme II.

The attachment of the sulfamoyl and carbamoyl moieties to the deoxythymidine 5'-OH group in **8–12** was demonstrated by the presence of the NH-3 signal at (8.91–11.28 ppm) thus indicating that the mentioned moieties were not attached to it, and by the downfield shiftment of the signals corresponding to H-4' ($\Delta\delta$~0.2 ppm) and H-5' ($\Delta\delta$~0.5 ppm) protons, as compared to those of **5**.

Antiviral activity

Compounds **6–10** and compound **12** were evaluated for their anti-HIV-1 activity in MT-4 cells. When ddT or ddU was linked to a 5'-*O*-[[[(2",3",4",6"-tetra-*O*-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl moiety (compounds **6** and **7**, respectively), modest antiviral activity was observed at concentrations that were below the toxicity threshold (EC_{50}: 32 and 38 μg/mL, respectively; CC_{50}: 124 and 91 μg/mL, respectively) (Table 1). Compound **6** was ~20-fold less, and compound **7** was ~7-fold more active than the corresponding parent ddN derivatives, ddT and ddU. Interestingly, compounds **6** and **7** retained their anti-HIV-1 activity in the presence of relatively high concentrations of dThd and dCyd (Table 2). These observations may indicate that both compounds do not release the free ddN but may act on their own right. When the glucosyloxycarbonyl group was split off from the ddT derivative **6** and replaced by either an ethyl (**8**), isopropyl (**9**) or hydrogen (**10**), antiviral activity was completely lost. Also, replacement of the sulfonyl group of compound **10** by a carbonyl moiety (compound **12**) resulted in antivirally inactive compound.

In conclusion, the lipophilic nucleotide mimics **6** and **7** show anti-HIV-1 activity in MT-4 cells at concentrations that are well below the toxicity threshold. The glucosyloxycarbonyl group seems to play an important role in the antiviral activity of these compounds.

Table 1. Anti-HIV-1 and cytotoxic activity of test compounds in human T-lymphocyte (MT-4) cells

Compound	EC_{50}[a] (μg/mL)	CC_{50}[b] (μg/mL)
6	32 ± 8.4	124 ± 28
7	38 ± 14	91 ± 19.5
8	> 40	90 ± 5.8
9	> 200	> 200
10	> 200	> 200
12	> 200	> 200

[a]50% Effective concentration, or compound concentration required to inhibit HIV-induced cytopathicity by 50%.
[b]50% Cytotoxic concentration or compound concentration required to reduce the viability of MT-4 cells by 50%.

Experimental Section

Chemical procedures

Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. ^{1}H-NMR spectra were recorded with a Varian EM-390, a Varian XL-300 and a Bruker AM-200 spectrometer operating at 300 and 200 MHz, and ^{13}C-NMR spectra with a Bruker WP-80-SY, a Bruker AM-200 and a Varian XL-300 spectrometer operating at 20, 50 and 75 MHz, with Me_4Si as internal standard. IR spectra were recorded with a Shimadzu IR-435 spectrometer. UV spectra were taken on a Perkin-Elmer 550 SE spectrophotometer. Analytical TLC was performed on silica gel 60 F_{254} (Merck). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

Table 2. Effect of dThd and dCyd on the anti-HIV-1 activity of compounds **6** and **7** in MT-4 cells[a]

	EC_{50}[b] (μg/mL)			
	upon addition of			
Compound	as such	dThd (25 μM)	dCyd (1000 μM)	dThd (250 μM) + dCyd (1000 μM)
6	32	35	35	34
7	38	35	35	35

[a]The 50% cytotoxic concentration of compounds **6** and **7** did not significantly change upon addition of dThd and/or dCyd.
[b]50% Effective concentration, or compound concentration required to inhibit HIV-1-induced cytopathicity by 50%.

5'-O-[[[[(2",3",4",6"-Tetra-O-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-3'-deoxy-thymidine (6)

A mixture of 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranose (1) (1.8 g, 3 mmol), methylene chloride (9 mL) and 3Å molecular sieves (3 g) was maintained at -30–20 °C, under argon atmosphere for 30 min. Chlorosulfonylisocyanate (0.27 mL, 3 mmol) was added through a septum and the mixture was stirred under the same conditions for 2 h. A solution of 3'-deoxythymidine[24] (0.68 g, 3 mmol) in methylene chloride (8 mL) and pyridine (1 mL) was added. The resulting mixture was allowed to warm up to room temperature. Then it was stirred overnight maintaining the argon atmosphere. The reaction mixture was filtered and the solid was washed with methylene chloride (2 x 8 mL). The filtrate and washings were evaporated to dryness and the residue was purified by column chromatography (EtOAc:hexane, 4:1), to give **6** (1.24 g, 67%) m.p. 164–167 °C (from hexane/EtOAc).

^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ:1.89 (s, 3H, CH_3-5), 1.85–2.25 (m, 4H, H-2', H-3'), 4.18 (m, 2H, H-5'), 4.36 (m, 1H, H-4'), 4.64 (m, 2H, H-6"), 4.80 (m, 1H, H-5"), 5.62 (dd, 1H, $J_{1",2"}$=3.7, $J_{2",3"}$=10.1 Hz, H-2"), 5.97 (t, 1H, H-4"), 6.04 (dd, 1H, $J_{1',2'a}$=4 6, $J_{1',2'b}$=6 4 Hz, H-1'), 6.29 (t, 1H, H-3"), 6.64 (d, 1H, H-1"), 7.29–8.11 (m, 21H, H-6, Ph). ^{13}C NMR [$(CD_3)_2CO$, 20 MHz) δ:12.51 (CH_3-5), 26.31, 32.15 (C-2', C-3'), 63.51 (C-5'), 70.95 (C-6"), 70.12, 70.52, 71.83, 78.94 (C-4', C-2", C-3", C-4", C-5"), 86.98, 91.67 (C-1', C-1"), 111.03 (C-5), 137.24 (C-6), 151.53 (C-2), 158.64 (OCONH), 164.81 (C-4). UV λ_{max} (MeOH) 228 (ε 12,400), 201 nm (ε 23,400). Anal. calcd for $C_{45}H_{41}N_3O_{17}S$: C, 58.25; H, 4.45; N, 4.53; S, 3.45. Found C, 57.90; H, 4.62; N, 4.20; S, 3.43.

5'-O-[[[[(2",3",4",6"-Tetra-O-benzoyl-α-D-glucopyranosyl)oxy]carbonyl]amino]sulfonyl]-2',3'-dideoxyuridine (7)

Following the procedure described for the synthesis of **6**, compound **1** (1.8 g, 3 mmol) was reacted with chlorosulfonylisocyanate (0.27 mL, 3 mmol) and 2',3'-dideoxyuridine[32] (0.64 g, 3 mmol) to give **7** (1.1 g, 60%); m.p. 154–159 °C (from hexane:EtOAc).

^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ: 1.86–2.30 (m, 4H, H-2', H-3'), 4.00–4.27 (m, 3H, H-4', H-5'), 4.61 (m, 2H, H-6"), 4.82 (m, 1H, H-5"), 5.56 (dd, 1H, $J_{1",2"}$=3.7, $J_{2",3"}$=10.2 Hz, H-2"), 5.61 (d, 1H, $J_{5,6}$=8.1 Hz, H-5), 5.98 (t, 1H, H-4"), 5.99 (dd, 1H, H-1'), 6.26 (t, 1H, H-3"), 6.54 (d, 1H, H-1"), 7.31–8.11 (m, 21H, H-6, Ph), 9.83 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2CO$, 50 MHz] δ: 25.91, 32.14 (C-2', C-3'), 63.39 (C-5'), 70.15 (C-6"), 69.95, 70.21, 71.82, 79.32 (C-4', C-2", C-3", C-4", C-5"), 86.96, 91.04 (C-1', C-1"), 102.34 (C-5), 141.55 (C-6), 151.35 (C-2), 158.81 (OCONH), 164.28 (C-4). Anal. calcd for $C_{44}H_{39}N_3O_{17}S$: C, 57.83; H, 4.30; N, 4.59; S, 3.51. Found C, 57.56; H, 4.32; N, 4.25; S, 3.80.

3'-Deoxy-5'-O-[(N-ethyl)sulfamoyl]thymidine (8)

To the mixture of **5** (0.25 g, 1.1 mmol), methylene chloride (75 mL) and pyridine (0.5 mL), a solution of ethylsulfamoyl chloride[33] (0.16 g, 1.1 mmol) in methylene chloride (5 mL) was added. The mixture was stirred at room temperature for 30 min and evaporated to dryness. The residue was purified by column chromatography ($CHCl_3$:MeOH, 20:1) to give **8** (0.23 g, 62%) as a white foam.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.24 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.95 (d, 3H, *J*=1.1 Hz, CH_3-5), 2.10, 2.43 (2m, 4H, H-2', H-3'), 3.21 (m, 2H, CH_2CH_3), 4.24 (dd, 1H, H-5'*a*), 4.35 (m, 1H, H-4'), 4.41 (dd, 1H, H-5'*b*), 5.36 (t, 1H, *J*=6.0 Hz, $NHCH_2$), 6.12 (dd, 1H, $J_{1',2'a}$=4.0 $J_{1',2'b}$=6.4 Hz, H-1'), 7.48 (q, 1H, H-6), 9.44 (bs, 1H, NH-3). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 12.21, 14.90 (CH_3-5, CH_3CH_2), 25.70, 31.60 (C-2', C-3'), 38.84 (CH_3CH_2), 70.23 (C-5'), 77.74 (C-4'), 86.30 (C-1'), 110.96 (C-5), 135.84 (C-6), 150.63 (C-2), 164.13 (C-4). UV λ_{max} (MeOH) 264 (ε 8100), 206 nm (ε 8100). Anal. calcd for $C_{12}H_{19}N_3O_6S$: C, 43.23; H, 5.74; N, 12.60; S, 9.61. Found C, 42.63 H, 5.86 N, 12.49 S, 9.43.

3'-Deoxy-5'-O-[(N-isopropyl)sulfamoyl]thymidine (9)

Compound **5** (0.25 g, 1.1 mmol) was reacted with *N*-isopropylsulfamoyl chloride[33] (0.17 g, 1.1 mmol) and was worked-up as described before for the preparation of **8**, to give **9** (0.307 g, 80%) as a white foam.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.24, 1.26 [2d, 6H, *J* = 6.5 Hz, $CH(CH_3)_2$], 1.95 (d, 3H, *J* = 0.9 Hz, CH_3-5), 2.10, 2.42 (2m, 4H, H-2', H-3'), 3.65 [m, 1H, $CH(CH_3)_2$], 4.22 (dd, 1H, H-5'*a*), 4.34 (m, 1H, H-4'), 4.40 (dd, 1H, H-5'*b*), 4.80 (d, 1H, *J* = 7.3 Hz, NHCH), 6.12 (dd, 1H, $J_{1',2'a}$=4.2, $J_{1',2'b}$=6.6 Hz, H-1'), 7.46 (q, 1H, H-6), 8.91 (bs, 1H, NH-3). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 12.25, (CH_3-5), 23.27 [$(CH_3)_2CH$], 25.74, 31.65 (C-2', C-3'). 47.20 [$(CH_3)_2CH$], 70.16 (C-5'), 77.69 (C-4'), 86.24 (C-1'), 111.02 (C-5), 135.68 (C-6), 150.52 (C-2), 163.90 (C-4). UV λ_{max} (MeOH) 263 (ε 14,000), 205 nm (ε 14,300). Anal. calcd for $C_{13}H_{21}N_3O_6S$: C, 44.95; H, 6.09; N, 12.09; S, 9.23. Found: C, 44.70; H, 6.20; N, 11.79; S, 8.90.

3'-Deoxy-5'-O-sulfamoylthymidine (10)

A mixture of **5** (0.2 g, 0.88 mmol), dioxane (20 mL), 4-dimethylaminopyridine (0.216 g, 1.76 mmol) and sulfamoyl chloride[34] (0.406 g, 3.52 mmol) was stirred at room temperature for 5 days, and concentrated to dryness. The residue was purified by column chromatography. By eluting with $CHCl_3$:MeOH (25:1) the starting

compound **5** (0.02 g) was recovered. By eluting with $CHCl_3$:MeOH (10:1) compound **10** (0.2 g, 82%) was obtained: m.p. 191–193 °C (dec.) (from $CHCl_3$:MeOH).

^1H NMR [$(CD_3)_2SO$, 300 MHz] δ: 1.78 (d, 3H, J = 1.1 Hz, CH_3-5), 1.83–2.34 (m, 4H, H-2', H-3'), 4.12–4.27 (m, 3H, H-4', H-5'), 6.02 (dd, 1H, $J_{1',2'a}$=4.3, $J_{1',2'b}$=6.8 Hz, H-1'), 7.50 (q, 1H, H-6), 7.60 (bs, 2H, NH_2), 11.28 (bs, 1H, NH-3). UV λ_{max} (MeOH) 265 (ε 8800), 206 nm (ε 8300). Anal. calcd for $C_{10}H_{15}N_3O_6S$: C, 39.34; H, 4.95; N, 13.76; S, 10.50. Found: C, 39.33; H, 4.82; N, 13.41; S, 10.70.

3'-Deoxy-5'-O-(phenyloxycarbonyl)thymidine (11)

To a stirred solution (ice bath) of **5** (0.3 g, 1.32 mmol) in pyridine (4 mL), phenyl chloroformate (0.35 mL, 2.6 mmol) was added dropwise. The mixture was allowed to reach room temperature and concentrated to dryness. The residue was treated with water (10 mL) and extracted with chloroform (2 x 25 mL). The organic layer was dried (Na_2SO_4), filtered and evaporated to dryness. The crude residue was used as starting material for the next step. A pure sample was obtained by column chromatography ($CHCl_3$:$(CH_3)_2CO$, 8:1) to give **11** as a white foam.

^1H NMR ($CDCl_3$, 90 MHz) δ:1.91 (d, 3H, J = 0.9 Hz, CH_3-5), 1.88–2.62 (m, 4H, H-2', H-3'), 4.30–4.70 (m, 3H, H-4', H-5'), 6.17 (dd, 1H, H-1'), 7.11–7.52 (m, 5H, Ph), 7.55 (q, 1H, H-6), 9.36 (bs, 1H, NH-3). Anal. calcd for $C_{17}H_{18}N_2O_6$: C, 58.95; H, 5.24: N, 8.08. Found: C, 58.78; H, 5.34; N, 7.95.

5'-O-Carbamoyl-3'-deoxythymidine (12)

A mixture of the crude **11** residue, methanol (3 mL), and NH_4OH (5 mL) was stirred at room temperature for 1.5 h and evaporated to dryness. The residue was purified by column chromatography ($CHCl_3$:$(CH_3)_2CO$, 3:1) to give **12** (0.231 g, 65%): m.p. 177–179 °C (from $CHCl_3$). IR (KBr) υ (cm^{-1}): 3500, 3450 (NH_2).

^1H NMR [$(CD_3)_2SO$, 200 MHz] δ: 1.79 (s, 3H, CH_3-5), 1.98, 2.21 (2m, 4H, H-2', H-3'), 3.96–4.19 (m, 3H, H-4', H-5'), 5.98 (dd, 1H, $J_{1',2'a}$ = 4.5, $J_{1',2'b}$ = 6.7 Hz, H-1'), 6.57 (bs. 2H, NH_2), 7.44 (s, 1H, H-6), 11.23 (bs, 1H, NH-3). ^{13}C NMR [$(CD_3)_2SO$, 50 MHz] δ: 12.17 (CH_3-5), 25.87, 30.43 (C-2', C-3'), 64.99 (C-5'), 77.79 (C-4'), 84.62 (C-1'), 109.44 (C-5), 135.87 (C-6), 150.45 (C-2), 156.57 ($OCONH_2$), 163.80 (C-4). Anal. calcd for $C_{11}H_{15}N_3O_5$: C, 49.07; H, 5.61; N, 15.60. Found: C, 49.05; H, 5.62; N, 15.91.

Antiretrovirus activity

HIV-1 was originally obtained from the culture supernatant of the persistently HIV-infected H9 cell line (H9 / HTLV-III_B),[35] which was kindly provided by R. C. Gallo and M. Popovic (National Institutes of Health, Bethesda, MD). Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells.

The methodology of the anti-HIV assays has been described previously.[36,37] Briefly, MT-4 cells (5 x 10^5 cells/mL) were suspended in fresh culture medium and infected with HIV-1 at 100 times the 50% cell culture infective dose ($CCID_{50}$) per mL of cell suspension. Then 100 μM of infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of test compounds. After 5 days, the number of viable cells for both virus-infected and mock-infected cell cultures was determined by trypan blue staining. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were defined as the compound concentrations required to reduce by 50% the number of viable cells in the virus-infected and mock-infected cell cultures, respectively.

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