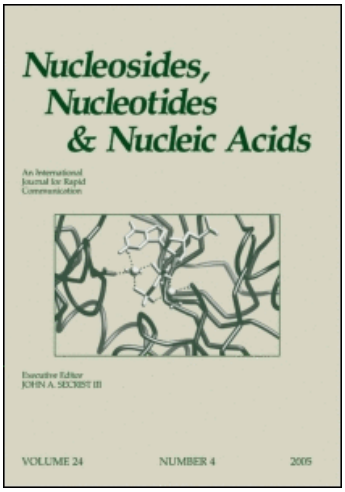

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Synthesis of Mimics to Thymidine Triphosphate and 3'-Deoxy-3'-fluorothymidine Triphosphate

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SYNTHESIS OF MIMICS TO THYMIDINE TRIPHOSPHATE AND 3´-DEOXY-3´-FLUOROTHYMIDINE TRIPHOSPHATE.

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Abstract: *Nucleoside triphosphate isosteres 5´-deoxy-5´-[[[[(4-methylphenyl)sulfonyl]amino]carbonyl]amino]thymidine (**1**), 5´-deoxy-5´-[[[[(4-chlorophenyl)sulfonyl]amino]carbonyl]amino]thymidine (**2**), 5´-deoxy-5´-[[[[(3-methylbenzyl)sulfonyl]amino]carbonyl]amino]thymidine (**7**), 3´,5´-dideoxy-3´-fluoro-5´-[[[[(4-methylphenyl)sulfonyl]amino]carbonyl]amino]thymidine (**9**) and 3´-deoxy-3´-fluoro-5´-O-[[[(4-methylphenyl)sulfonyl]amino]carbonyl]thymidine (**10**) have been synthesized from 5´-deoxy-5´-aminothymidine and 3´-deoxy-3´-fluorothymidine.*

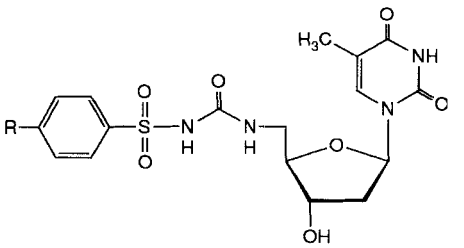
The nucleoside analogues 3´-azido-3´-deoxythymidine (AZT), 2´,3´-dideoxycytidine (DDC) and 2´,3´-dideoxyinosine (DDI) are so far the only drugs approved in single or combination therapy for the treatment of HIV infection. Nucleoside analogues like AZT are generally activated *in vivo* by cellular kinases, in three enzymatical steps, to form the corresponding nucleoside triphosphates which are believed to be recognised as substrates for HIV-1 reverse transcriptase resulting in DNA incorporation and termination of chain elongation.

Previouosly the synthesis of a lipophilic triphosphate isostere, mimicking the tertiary structure of an ATP-Mg^{2+} complex has been reported, but the *in vitro* biological activities were not presented.[1] Another attempt to construct a triphosphate mimic was

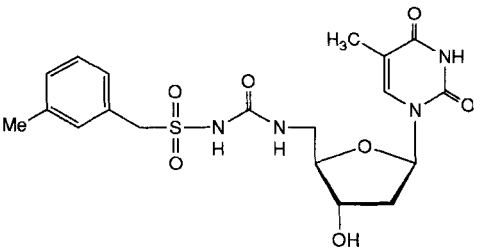
reported where the oxygen binding the α and β phosphates of both thymidine triphosphate and 3′-azido-3′-deoxythymidine triphosphate was replaced by a nitrogen giving compounds that were not substrates but competitive inhibitors of HIV-1 RT. When the oxygen binding the β and γ phosphates also was replaced by nitrogen a 10-fold decrease in this activity was observed.[2]

In this paper we describe several noncharged, lipophilic, nucleoside triphosphate mimics as potential inhibitors of HIV-1 RT. A noncharged lipophilic mimic was selected to enhance the possibility of oral bioavailability and cell wall penetration of the infected cells. Recently a model[3] as well as the crystal structure[4] of HIV-1 RT has been published. Using this structural information it is possible to view a lipophilic interaction from Tyr-188 or Val-108 that extends to the space of a lipophilic replacement of the γ phosphate in the nucleoside triphosphate.[5]

In designing the triphosphate mimics, the pyrophosphate containing the α and β phosphates was replaced by the readily available sulfonylurea group, and the γ phosphate group was replaced by an aryl group. Arylsulfonylureas are today used as oral hypoglycemic agents where their biological receptor appears to be the ATP-sensitive K^+ channels of the pancreatic β-cells.[6]



1 R=Me
2 R=Cl



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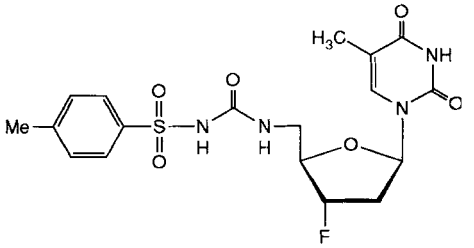

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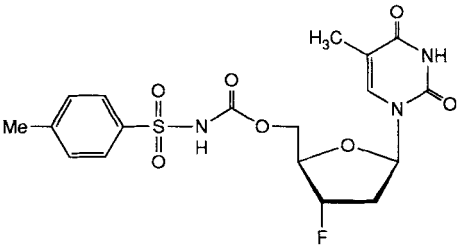

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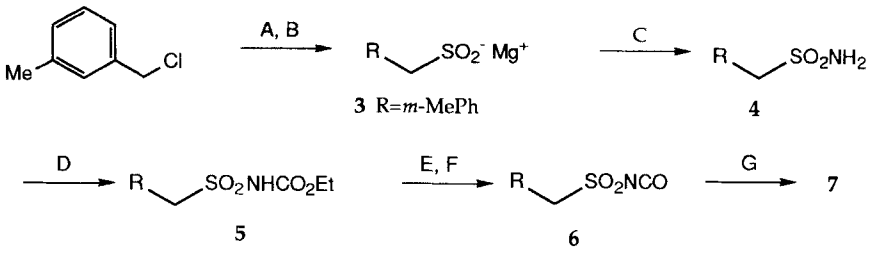
Results and discussion

5′-Deoxy-5′-[[[[(4-methylphenyl)sulfonyl]amino]carbonyl]amino]thymidine (**1**) and 5′-deoxy-5′-[[[[(4-chlorophenyl)sulfonyl]amino]carbonyl]amino]thymidine (**2**) were



A; Mg, diethyl ether **B**; SO_2 (g) **C**; NaOAc, H_2NOSO_3H **D**; K_2CO_3, ethyl chloroformate **E**; chlorotrimethylsilane, Et_3N, toluene **F**; Δ, acetonitrile **G**; 5′-amino-5′-deoxythymidine, dioxane

Scheme 1

prepared by reacting 5′-amino-5′-deoxythymidine[7] with the corresponding arylsulfonylisocyanate[8] to give **1** and **2** in 51% and 57% yield respectively. 5′-Deoxy-5′-[[[[(3-methylbenzyl)sulfonyl]amino]carbonyl]amino]thymidine (**7**) was prepared in 59% yield by reacting 3-methylbenzylsulfonylisocyanate (**6**) with 5′-amino-5′-deoxythymidine. The isocyanate **6** (scheme 1) was prepared by reacting 3-methylbenzyl magnesium chloride with gaseous sulfurdioxide to give the sulfinate salt **3** in 98% yield. Compound **3** was dissolved in water to which sodium acetate and hydroxylamine-*O*-sulfonic acid was added giving 3-methylbenzylsulfonamide (**4**) in 85% yield.[9] Reacting **4** with ethyl chloroformate following the procedure described by Cassady et. al.[10] gave ethyl *N*-(3-methylbenzylsulfonyl)carbamate (**5**) in 40% yield. Carbamate **5** was then reacted instantly[11] with chlorotrimethylsilane followed by heating to give the very unstable isocyanate **6** which was used directly.[12]

Compounds **9** and **10** were prepared in an analogous manner in 86% and 96% yield respectively.

5′-Amino-3′,5′-dideoxy-3′-fluorothymidine (**8**), used for the preparation of **9**, was prepared in 82% yield by reacting 3′-deoxy-3′-fluorothymidine[13] with triphenylphosphine, carbon tetrabromide and lithium azide[14] followed by catalytic hydrogenation.

It was possible to prepare **1**, **2** and **7** without protection of the the 3′-hydroxyl group of the nucleoside prior to reaction with the isocyanate.

Compounds **1**, **2**, **7**, **9** and **10** were tested in an assay for HIV-1 RT inhibition[15] and in a soluble-formazan assay for HIV-1 and cytopathic effects.[16] All compounds were inactive in these *in vitro* assays.

Experimental

General methods: All solvents were distilled prior to use. Thin layer chromatography was performed using silica gel 60 F-254 (Merck) plates with detection by UV and/or charring with 8% sulphuric acid. Column chromatography was performed on silica gel (Matrix Silica Si 60A, 35-70m, Amicon). Organic phases were dried over anhydrous magnesium sulphate. Concentrations were performed under reduced pressure. Optical rotations were recorded on a Perkin Elmer 241 polarimeter. IR-spectra were recorded on a Perkin Elmer 257 IR-Spectrophotometer. NMR spectra were recorded on a JEOL GSX-270 instrument, shifts are given in ppm downfield from tetramethylsilane in deuterated DMSO, 70 °C, unless otherwise stated. Elemental analyses of the compounds were performed by combustion and/or by accurate mass,[17, 18] positive ionization FAB-mode.[19] A JEOL SX102 mass-spectrometer with a xenon gun with nitrobenzyl alcohol as matrix were used. A standard TLC-probe equiped with a dual target was used and spectra were accumulated by altering every second scan between the standard and the sample to be detrmined.[20] In all experiments the mass obtained deviated < 5 mmu. When more than one possible composition was suggested, the incorrect alternatives were excluded by NMR data.

5′-Deoxy-5′-[[[[(4-methylphenyl)sulfonyl]amino]carbonyl]amino]-thymidine (1). To a stirred solution of 5′-deoxy-5′-aminothymidine (37 mg, 0.153 mmol) in dry dioxane (5 mL) under nitrogen was added *p*-toluenesulfonyl isocyanate (30 mg, 0.152 mmol). The reaction mixture was stirred for 30 min when TLC ($CHCl_3$-MeOH, 9:1) indicated complete reaction. Concentration of the reaction mixture followed by silica gel column chromatography ($CHCl_3$-MeOH, 9:1) gave pure **1** as a white solid in 60% yield (0.040 g, 0.095 mmol). $[\alpha]_D^{22}$ +34.9° (*c* 0.96, DMSO); Mass deviation found: 3.0 mmu; ^{13}C NMR δ 12.2 (CH_3, thymine), 21.4 (CH_3, Ar), 39.0 (C-2′), 41.9 (C-5′), 71.5 (C-3′), 84.9 (C-1′), 85.0 (C-4′),110.7 (C-5), 127.5-129.9 (C-Ar), 136.7 (C-6), 150.9 (C-4), 164.6 (C-2). Anal. Calcd for $C_{18}H_{22}N_4O_7S \cdot 2\ H_2O$: C, 45.56; H, 5.52; N, 11.81 Found: C, 46.08; H, 5.24; N, 11.18 .

5′-Deoxy-5′-[[[[(4-chlorophenyl)sulfonyl]amino]carbonyl]amino]-thymidine (2). Using the same procedure as for the preparation of **1** with 4-chlorophenylsulfonyl isocyanate gave **2** in 57 % yield. $[\alpha]_D^{22}$ +20.0° (*c* 0.73, DMSO); ^{13}C NMR δ 12.2 (CH_3, thymine), 21.4 (CH_3, Ar), 39.0 (C-2′), 41.9 (C-5′), 71.5 (C-3′), 84.9 (C-1′), 85.3 (C-4′), 110.7 (C-5), 128.0-129.1 (C-Ar), 136.7 (C-6), 150.9 (C-4), 164.6 (C-2). Anal. Calcd for $C_{17}H_{19}ClN_4O_7S \cdot 1\ H_2O$: C, 42.73; H, 4.43; N, 11.72. Found: C, 42.48; H, 4.10; N, 11.60.

3-Methylbenzylsulfonamide (4). 3-Methylbenzylmagnesium chloride was prepared from 3-methylbenzylchloride (3.93 g, 28 mmol, 4.05 mL) and magnesium turnings (0.73 g, 30 mmol) in dry diethyl ether (50 mL). After refluxing for 2 h dry diethylether (100 mL) was added and the solution was cooled to -50 °C. Sulfur dioxide gas was passed through the solution for 45 min. After stirring for an additional 2 h, nitrogen gas was passed through the solution for 16 h. Concentration and drying of the residue in vacuum gave 3-methylbenzylsulfinate (**3**). This salt was dissolved in water (125 mL) to which NaOAc (2.52 g, 30.8 mmol) was added followed by the addition of hydroxylamine-*O*-sulfonic acid (3.48 g, 30.8 mmol). After stirring for 20 h the solid was isolated by filtration and dried in vacuum to give the title compound **4** in 83% yield (3.98 g, 23.3 mmol). ^{1}H NMR (DMSO, 30°C) δ 2.32 (s, 3H, CH_3), 4.21 (s, 2H, Ar-CH_2), 6.80 (s, 2H, NH_2), 7.14-7.27 (m, 4H, Ar). Anal. Calcd for $C_8H_{11}NO_2S$: C, 51.87; H, 5.99; N, 7.56. Found: C, 51.65; H, 5.85; N, 7.53.

Ethyl *N*-(3-methylbenzylsulfonyl)carbamate (5). To a stirred solution of 3-methylbenzylsulfonamide (**4**) (1.0 g, 5.37 mmol) in dry acetone (30 mL) was added finely powdered K_2CO_3 (0.97 g, 7.0 mmol). The reaction mixture was heated to reflux and ethyl chloroformate (0.76 g, 7.0 mmol) was added dropwise over a period of 1 h. After 15 h the mixture was cooled and filtered. The solid was dissolved in a minimum amount of water and acidified with aqueous HCl (conc.). Extraction with diethyl ether (2 x 25 mL), drying and concentration gave **5** in 40% yield (0.542 g, 2.1 mmol). ^{1}H NMR (DMSO, 30°C) δ 1.31 (t, 3H, CH_3), 2.36 (s, 3H, Ar-CH_3), 4.27 (m, 2H, CH_2), 4.61 (s, 2H, Ar-CH_2), 7.05 (s, 1H, N-H), 7.13-7.40 (m, 4H, Ar).

3-Methylbenzylsulfonylisocyanate (6). To a stirred solution of ethyl *N*-(3-methylbenzylsulfonyl)carbamate (**5**) (0.386 g, 1.5 mmol) in dry toluene (5 mL) was added chlorotrimethylsilane (0.489 g, 4.5 mmol). The reaction mixture was cooled to 0°C and triethylamine (0.151 g, 1.5 mmol) in dry toluene (1 mL) was slowly added. The reaction mixture was stirred for 2 h at 0°C. After evaporation off the excess chlorotrimethylsilane and 25% of the toluene, the solution was filtered under nitrogen and the residual solvent evaporated. The remaining oil was dissolved in dry acetonitrile (5 mL) and refluxed for 12 h under nitrogen. Concentration gave **6** as a viscous oil (IR-analysis; 2250 cm^{-1}, -SO_2NCO) which was used in the next step without further purification due to its instability.

5′-Deoxy-5′-[[[[(3-methylbenzyl)sulfonyl]amino]carbonyl]amino]-thymidine (7). To a stirred solution of 5′-amino-5′-deoxythymidine (80 mg, 0.33

mmol) in dry dioxane (10 mL), unpurified 3-methylbenzylsulfonylisocyanate from the previous step in dioxane (2 mL) was added. The reaction mixture was stirred for 1 h and then heated to 80°C for 72 h. Concentration followed by silica gel column chromatography ($CHCl_3$-MeOH, 9:1) gave **7** in 66% yield (100 mg, 22 mmol). $[\alpha]_D^{22}$ +56.85 (*c* 0.76, DMSO); ^1H NMR (DMSO, 30°C) δ 1.81 (s, 3H, CH_3, thymine), 2.07 (m, 2H, H-2′), 2.28 (s, 3H, Ar-CH_3), 3.31 (m, 2H, H-5′), 3.81 (m, 1H, H-3′), 4.14 (m, 1H, H-4′), 4.62 (s, 2H, Ar-CH_2), 5.37 (d, 1H, N-H), 6.22 (t, 1H ,H-1′), 6.46 (t, 1H, N-H), 7.09-7.28 (m, 4H, Ar), 7.53 (s, 1H, H6). Anal. Calcd for $C_{19}H_{24}N_4O_7S$: C, 50.43; H, 5.35; N, 12.38. Found: C, 50.38; H, 5.22; N, 12.34.

5′-Amino-3′,5′-dideoxy-3′-fluorothymidine (8) To a vigorously stirred mixture of 3′,5′-dideoxy-3′-fluorothymidine (427 mg, 1.75 mmol), triphenylphosphine (467 mg, 1.78 mmol) and lithium azide (428 mg, 8.75 mmol) in dry dimethyl formamide (5 mL) was added carbon tetrabromide (590 mg, 1.78 mmol) at room temperature. The solution was stirred for 20 h after which methanol (1 mL) was added. Concentration followed by filtration through silica gel with EtOAc as eluent gave, after further concentration, a white solid. The residue dissolved in ethanol (20 mL) was hydrogenated over 10% palladium on charcoal (50 mg) for 5 h to give **8** in 82% yield (347 mg, 1.43 mmol). Mass deviation found: 1.8 mmu; ^1H NMR (DMSO, 50°C) δ 1.82 (s, 3H, CH_3, thymine), 2.40 (m, 2H, H-2′), 2.75 (m, 2H, H-5′), 4.00 (m, 1H, H-4′), 5.32 (m, 1H, H-3′), 6.16 (t, 1H, H-1′), 7.67 (s, 1H, H-6). Anal. Calcd for $C_{10}H_{14}N_3O_3F$: C, 49.38%; H, 5.80%; N, 17.28%. Found: C, 49.22%; H, 5.98%; N, 16.61%

3′,5′-Dideoxy-3′-fluoro-5′-[[[[(4-methylphenyl)sulfonyl]amino]-carbonyl]amino]thymidine (9). To a stirred solution of 5′-amino-3′,5′-dideoxy-3′-fluorothymidine (100 mg, 0.41 mmol) in dry dioxane (2 mL) under nitrogen atmosphere was added a solution of *p*-toluenesulfonyl isocyanate (88 mg, 69 μl, 0.43 mmol) in dioxane (2 mL). The reaction mixture was stirred for 30 min when TLC ($CHCl_3$-MeOH, 9:1) indicated complete reaction. Concentration of the reaction mixture followed by silica gel column chromatography ($CHCl_3$-MeOH, 9:1) gave **9** in 86% yield (155 mg, 0.35 mmol). Mass deviation found: 2.3 mmu; ^{13}C NMR (DMSO, 50°C) δ 11.85 (Thy-CH_3), 21.91 (Ar-CH_3), 35.48 and 35.80 (C-2′, $^2J_{C\text{-}F}$ 22 Hz), 79.08 (C-5′), 82.29 and 82.64 (C-4′, $^2J_{C\text{-}F}$ 8.2 Hz), 84.23 (C-1′), 92.76 and 95.35 (C-3′, $^1J_{C\text{-}F}$ 176 Hz), 109.89 (C-6), 125.56-127.05 (C-Ar), 129.29 (C-5), 137.44 (C-Ar), 143.53 (C-Ar), 150.34 (Thy-CO), 151.79 (CO), 163.53 (Thy-CO)

3′-Deoxy-3′-fluoro-5′-*O*-[[[(4-methylphenyl)sulfonyl]amino]-carbonyl]-thymidine (10). The same procedure as for the preparation of **1** but using

3′-deoxy-3′-fluorothymidine (95 mg, 0.39 mmol) gave 96 % (0.37 mmol) pure **10**. $[\alpha]_D^{22}$ +26.4° (*c* 0.91, DMSO); Mass deviation found: 2.9 mmu; ^{1}H NMR δ 1.71 (s, 3H, CH_3, thymine), 2.08 (m, 2H, H-2′), 2.40 (s, 3H, CH_3, Ar), 4.19 (m, 2H, H-5′), 4.31 (m, 1H, H-4′), 5.29 (m, 1H, H-3′), 6.22 (dd, 1H, H-1′), 7.4-7.8 (m, 5H, Ar-H,H-6). Anal. Calcd for $C_{18}H_{24}FN_4O_7S$: C, 48.98; H, 4.57; N, 9.52. Found: C,49.49; H, 4.90; N, 10.01.

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