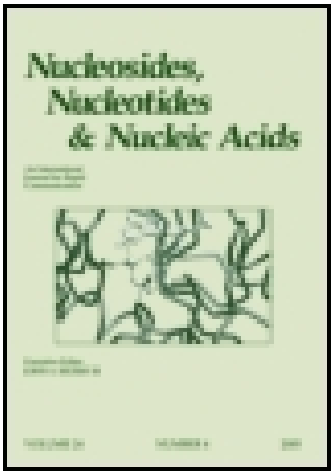

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Synthesis of Mimics to Thymidine and 5-(2″-Thienyl)-2′-Deoxyuridine Triphosphates

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SYNTHESIS OF MIMICS TO THYMIDINE AND 5-(2´´-THIENYL)-2´-DEOXYURIDINE TRIPHOSPHATES

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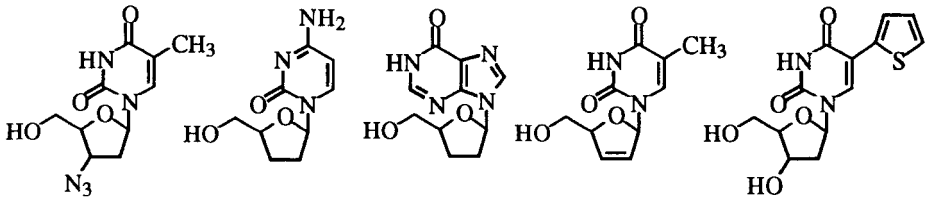
Abstract : Dicarboxylic acid 5´-monoesters of thymidine and 5-(2-thienyl)-2´´-deoxyuridine have been synthesised and evaluated as triphosphate mimics. Glutaric (**25, 29**), adipic (**26, 30**), pimelic (**27, 31**) and *trans*-1,4-cyclohexane dicarboxylic acids (**28, 32**) were employed to vary the distance between two carboxylate functions and were structurally compared to the triphosphate moiety by molecular modelling. The glutarate and adipate derivatives can assume conformations fitting the triphosphate, and the thienyl compounds **29** and **30** were the ones having overall best inhibitory activities against DNA pol α and HIV-1 RT.

The unnatural nucleosides 3´-azido-3´-deoxythymidine (AZT), 2´,3´-dideoxycytidine (DDC), 2´,3´-dideoxyinosine (DDI) and 2´,3´-didehydro- 2´,3´-dideoxythymidine (D4T) are so far the only drugs approved in therapy for the treatment of human immunodeficiency virus (HIV) infection. The activation (phosphorylation) of these nucleosides is generally accomplished by cellular kinases, but in many cases the nucleoside analogues have a poor affinity for nucleoside kinases[1-5].



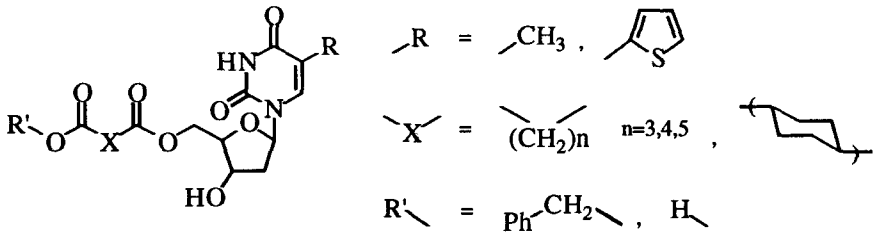
AZT DDC DDI D4T **1**

We have previously found that 5-(2´´-thienyl)-2´-β-deoxyuridine triphosphate (**1-TP**) has an activity comparable with that of 3´-azidothymidine (AZT) triphosphate against reverse transcriptase (RT) of human immunodeficiency virus type 1 (HIV-1)[6,7]. The nucleoside 5-(2´´-thienyl)-2´-β-deoxyuridine (**1**) was inactive against HIV-1 in cell culture assays[6], most probably due to the phosphorylating inefficiency of the cellular thymidine kinase[8]. Other 5-(2´´-thienyl)-β-nucleoside triphosphates prepared by us all have lower antiviral activity compared to **1-TP**[7].

To serve as an inhibitor for the HIV-RT, **1** must be transformed into nucleoside triphosphate through the action of cellular nucleoside kinases. However, this transformation is apparantly not very efficient[1,2,3,5,9]. The fact that triphosphates are unable to penetrate cell membranes due to high charge has led to different strategies for introduction of drugs and prodrugs into the cell. Nucleoside triphosphates have been made more lipophilic by complexation with nitrogen containing carriers[10,11]. These carriers do not mediate specific nucleotide transport across liposom walls but rather act as detergents to break the liposomal structure[11]. Nucleoside monophosphate prodrugs have been made lipophilic by preparation of various 5´-*O*-phosphonates[12-16], 5´-*O*-phosphoramidates[17,18] and aryl bis(nucleosid-5´-yl) phosphates[19]. Prodrugs have also been prepared of antivirally active nucleoside phosphonate compounds[20-22] and nucleoside prodrugs have been prepared by 5´-*O*-esterification[23,24]. Several observations support the idea that substrates with a low charge will have affinity for phosphate binding sites[25,26]. There are less ionic groups for phosphate binding in catalytic sites than in regulatory sites[27] and lipophilic triphosphate mimics have been prepared with the objective of functioning as an enzyme bound nucleotide-metal complex[28] or as HIV-RT inhibitors[29].

HIV-1 RT is composed of two subunits, p66 and p51[30,31] and the crystal structure of the HIV-1 RT heterodimer has been determined[32,33]. The polymerase active site of this enzyme has considerable structural similarity to the polymerase active site of the Klenow fragment of *Escherichia coli* DNA polymeras 1[32,34,35], and contains three aspartic acids positioned for catalytic binding to Mg^{2+} and the nucleoside triphosphate substrate. Recently this has been shown to hold also for rat DNA polymerase β[36,37], and two divalent metal ions may be involved in the catalytic binding[36,37,38,39]

With these results in mind, we decided to synthesise some nucleoside derivatives with 5´-*O*-substituents which presumably could function as simple triphosphate mimics by incorporating into the substituents structural elements which could interact with a divalent metal ion and aspartic acids at the catalytic site. Such compounds would be independant of cellular kinases[4,5,8,40] and additionally have a lipophilic character which



SCHEME 1

would enable them to penetrate into the cells. In this paper we describe several 5′-*O*-esters of thymidine and 5-(2′′-thienyl)-2′-deoxyuridine (SCHEME 1) synthesised from 3′-*O*-protected and -unprotected nucleosides (SCHEME 5). We have also synthesised some 5′-deoxy-5′-amidoderivatives of 5-(2′′-thienyl)-2′,3′-dideoxyuridine[41].

In designing the triphosphate mimics we replaced the α phosphate with an ester group in order to maintain some of the hydrophilic interaction of the original phosphate group. We also decided to change the lipophilic chain length to another carbonyl function, in the form of an ester or carboxylic acid, which could give some hydrophilic interaction at the site for the γ phosphate in the enzyme. Glutaric, adipic and pimelic acid as well as 1,4-*trans*-cyclohexane dicarboxylic acid, in the form of their monomethyl esters, were structually compared to a triphosphate monomethyl ester using the Sybyl program.

Results and discussion

Because of the above mentioned structural similarities in the polymerase active site of different polymerases, we decided to use the triphosphate conformation in rat DNA polymerase β presented by Pelletier *et al.*[42] for comparison with our triphosphate mimics. The triphosphate and the four diacids were compared to each other in the form of their monomethyl esters, the methyl representing the 5′-CH_2 of the nucleoside. This was done presuming that the nucleoside conformation essentially is the same when bound to the polymerase active site. The bond between the α-phosphorous and the oxygen of the pyrophosphate moiety was elongated by 25% (from 1.6 to 2.0 Å) in order to get a triphosphate conformation that would resemble the transition state. The following conclusions can be drawn from the modelling: 1) The glutaric and adipic acids can adopt conformations which fit nicely to the one of the triphosphate. The two carbonylic oxygens of the two diacids can be located at the same positions and have the same

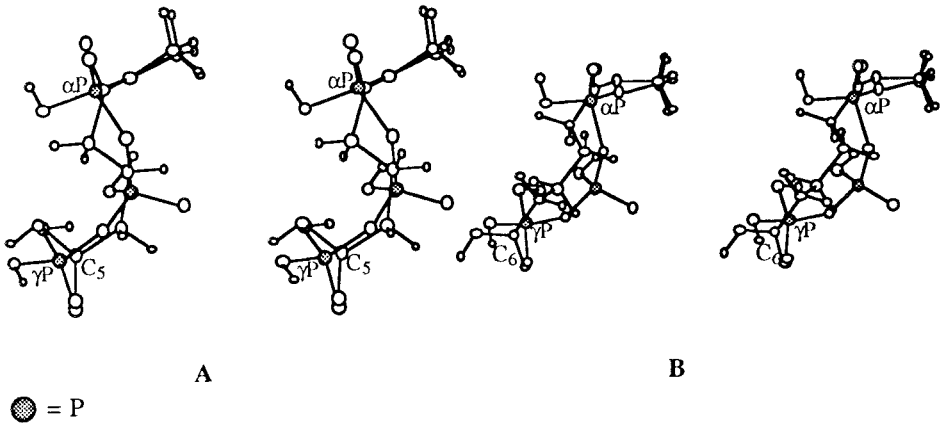
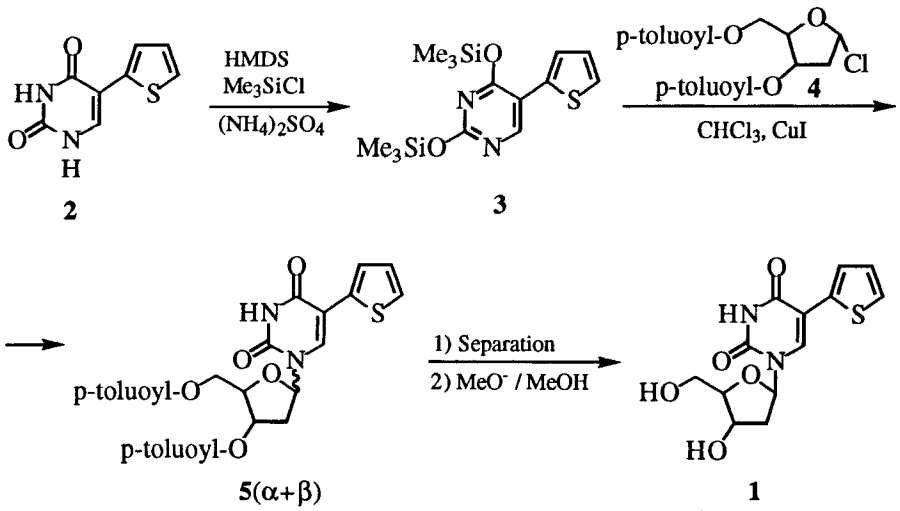


FIGURE 1. Stereodiagrams of the overlapping of glutaric acid (**A**) and adipic acid (**B**) monomethyl esters with the triphosphate monomethyl ester.

directions in space as the α- and γ-phosphorous doubly bound oxygens (FIGURE 1). Compared to the energy minimised conformations of the two diacids, the conformations that can mimic the triphosphate are about 15 kcal/mol higher in energy in both cases. 2) Pimelic acid is too long to have a low energy conformation that can mimic the triphosphate satisfactory. 3) 1,4-*Trans*-cyclohexane dicarboxylic acid is too rigid to mimic the triphosphate.

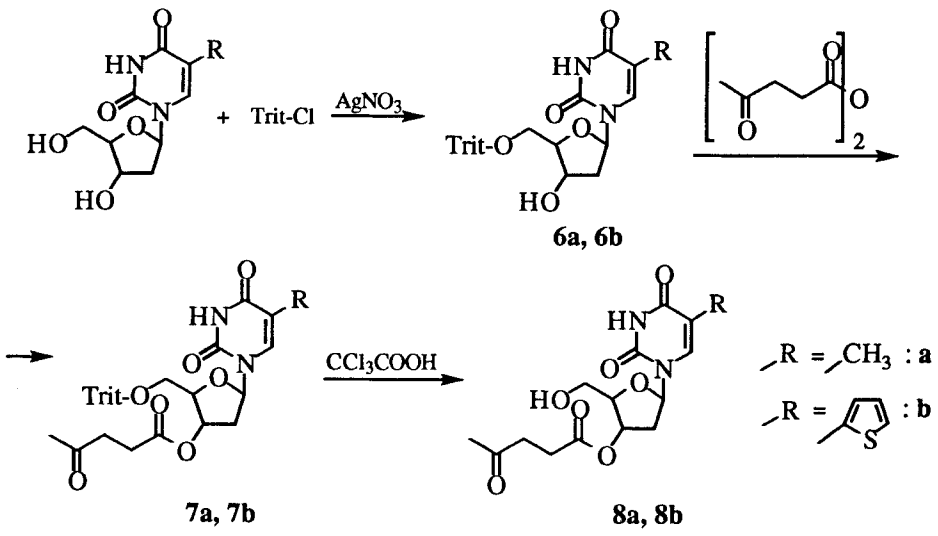
5-(2′′-Thienyl)-2′-deoxyuridine (**1**) was prepared by converting 5-(2′-thienyl)uracil (**2**)[43] to 2,4-di-(trimetylsilyloxy)-5-(2′-thienyl)-pyrimidine (**3**)[44] followed by coupling with α-2-deoxy-3,5-di-*O*-toluoylribofuranosyl chloride (**4**)[45] in chloroform[46]. CuI-catalysis was used in order to obtain as high β/α ratio as possible[47] (SCHEME 2). The 3′-*O*-levulinoyl-protected nucleosides **8a** and **b** were prepared from the corresponding nucleosides by protection of the 5′-hydroxyl group by tritylation with 4,4′-dimethoxytrityl chloride[48] followed by esterification of the 3′-hydroxyl group with levulinic acid using levulinic acid anhydride[49] This method of protection has been extensively used in polynucleotide syntheses[50-53]. The trityl group was removed by reaction with trichloroacetic acid (SCHEME 3).

The glutaric, adipic and pimelic acid monobenzyl esters (**9-11**, SCHEME 4) were prepared as described by English *et al.*[54] The mono-benzyl esters were used without purification and transformed to the acid chlorides (**13-15**, SCHEME 4) by reaction with oxalyl chloride at room temperature. *Trans*-cyclohexane-1,4-dicarboxylic acid

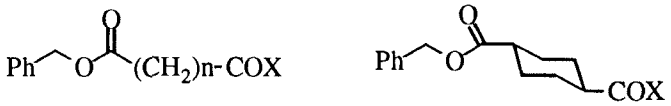


HMDS = 1,1,1,3,3,3-hexamethyldisilazane
p-toluoyl- = p-CH_3-C_6H_4-C(=O)-

SCHEME 2



SCHEME 3



X=OH, n=3 : **9**, n=4 : **10**, n=5 : **11**
X=Cl, n=3 : **13**, n=4 : **14**, n=5 : **15**

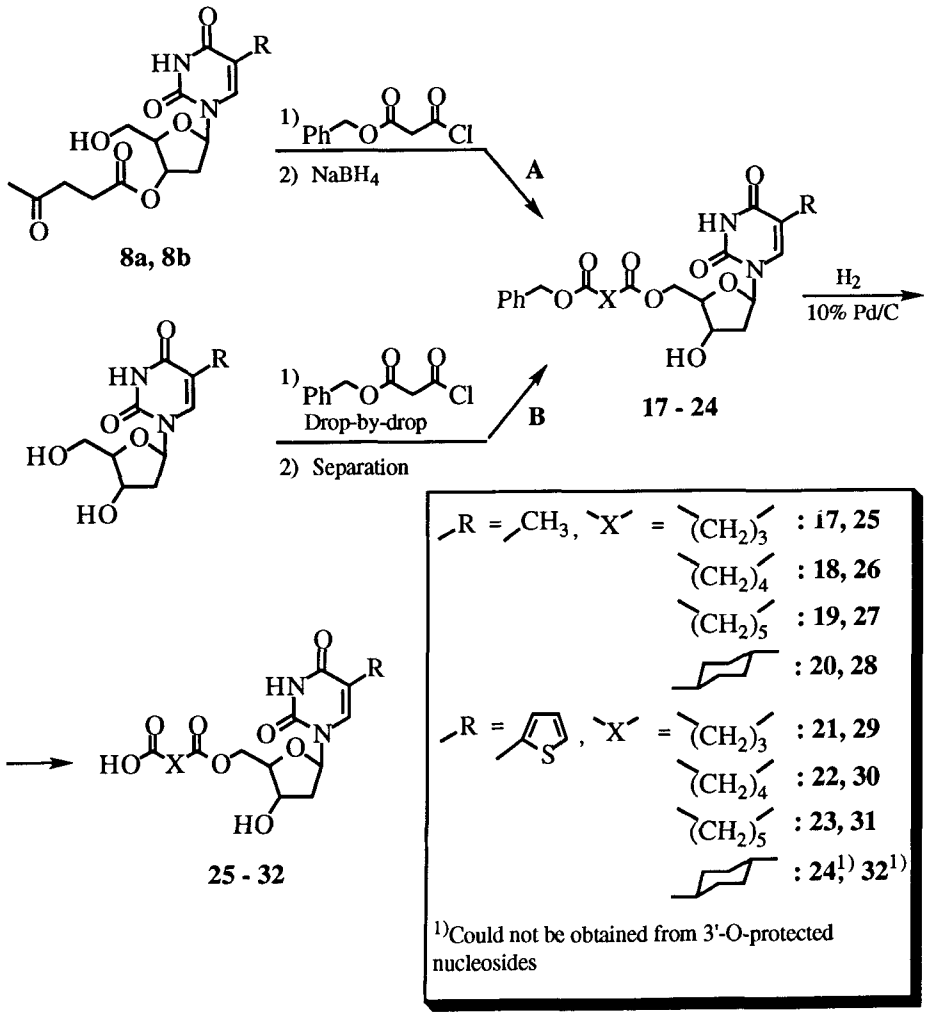
X=OH : **12**
X=Cl : **16**

SCHEME 4

monobenzyl ester (**12**, SCHEME 4) could not be prepared by the same methodology due to partial isomerisation when subjected to acid and heat, giving a mixture of *cis* and *trans* products. Instead, dicyclohexyl carbodiimide (DCC) with 4-dimethylamino-pyridine (DMAP) as catalyst was used at room temperature in *N,N*-dimethylformamide (DMF) for the preparation of the monobenzyl ester **12**[55]. The acid chloride (**16**, SCHEME 4) was prepared in the same way as described above, but could not be distilled due to isomerisation. It was purified by washing the ether solution rapidly with ice-cold water.

The mixed diesters **17-24** were prepared according to two routes. Method A consisted of reaction between the 3′-*O*-levulinoyl-protected nucleosides **8a** or **b** and diacid chloride monobenzyl esters **13-16** followed by reduction of the levulinates with sodium borohydride to remove the levulinic acid protecting group[49] (SCHEME 5). This did, however, not work in the cases of the *trans*-1,4-cyclohexane dicarboxylic acid derivative **24** due to isomerisation. Method B consisted of dropwise addition of a dilute solution of the diacid chloride monobenzyl esters **13-16** to a solution of **1** or thymidine in pyridine. By this procedure Nishino *et al.* obtained 5′-*O*-aroyl-2′-deoxyribonucleosides in 84-93% yield[56]. We found that this method could be used successfully with our aliphatic benzyl ester acid chlorides, if they were dissolved in dichloromethane instead of pyridine. This gave direct access to the mixed diesters **17-24** (SCHEME 5) in reasonable yields. The drawback of this method was that the 3′-*O*- and 5′-*O*-esterified products had to be separated using HPLC, but the higher total yields compared to the more tedious method A compensated for this.

Compounds **25-32** were obtained by removing the benzylic function from the corresponding compounds **17-24** by hydrogenation over Pd on carbon. However, larger amounts of Pd-catalyst and a higher hydrogen pressure had to be used with the 5-(2′′-thienyl) substituted derivatives **17-20** compared to the thymidine derivatives **21-24** (SCHEME 5).



SCHEME 5

All new compounds were characterised by high-resolution mass spectroscopy and ^{1}H NMR spectra. ^{1}H NMR were run in CD_3OD except for compounds **29** and **30** which had to be run in CD_3CN/D_2O 7/3 due to low solubility in methanol. The ^{1}H NMR data for the thymidine derivatives **17-20** and **25-28** are almost identical except for the 5′-substituents, and that is also the case for the 5-(2′′-thienyl)-2′-deoxyuridine derivatives **21-24, 31** and **32**. The ^{1}H-NMR data for the 5-(2′′-thienyl)-2′-deoxyuridine derivatives **29** and **30** deviate due to different solvent. ^{1}H NMR data are only given for compounds

TABLE 1

Compound	IC_{50} (μg/ml) DNA pol α (Act. CT DNA)	IC_{50} (μg/ml) HIV-1 RT (Act. CT DNA)
25	70	180
26	>200	80
27	>200	>200
28	105	140
29	26	33
30	24	60
31	190	>200
32	41	140
1	10	>300
1-TP	0.6	0.13

17, **21** and **29** since deviation in chemical shifts and coupling constants within the three groups are less than 0.05 ppm and 0.4 Hz, respectively.

Compounds **25-32** were tested for inhibition of HIV-1 in cell culture as well as of cellular (calf thymus, CT) DNA polymerase α and HIV-1 RT. Inhibition of HIV-1 in cell culture was performed as XTT assay in MT4 cells (human T cell line). The effect on cell growth was determined as an XTT assay on non-confluent HEL cells without presence of any virus[57]. None of the compounds **25-32** were active against HIV-1 in cell culture at a concentration of 100μg/ml. The HIV-1 RT assay was performed as descibed previously[58]. With activated calf thymus DNA as a template primer, dATP, dTTp and dCTP (17μM each) were used as substrates, together with 3H-dGTP at a concentration close to the Km value. All assays were performed in duplicates. Compound **1** and its triphosphate were included for comparison and the results are summarised in TABLE 1.

The glutaric and adipic esters had shown the best fit in the modelling analysis, and the thienyl derivatives **29** and **30** are the best inhibitors of both DNA pol α and HIV-1 RT. In the thymidine series the glutarate compound **25** has a low activity against DNA pol α, while the adipate compound **26** is not inhibitory. Against HIV-1 RT the activity pattern is reversed. The pimelic acid derivatives **27** and **31** were inactive and the cyclohexane derivatives **28** and **32** had weak activities, against both enzymes, with the exception of a higher activity of the thienyl derivative **32** against DNA pol α.

The above results demostrate that dicarboxylic acid derivatives can be used as simple models for 2′-deoxyribofuranosyl nucleoside triphosphates. In modelling the dicarboxylic acid monoester derivatives to the triphosphate monoester, the contribution of the nucleoside portion was assumed to be constant. Obviously, this is an oversimplification. The overall higher activities of the 5-thienyl derivatives, reflects the additional binding contribution of the heteroaryl moiety to the two enzymes used in the assay. Against HIV-1 RT there is a significant increase in inhibition by the triphosphate mimics **29** and **30** compared to the parent compound **1**. Against DNA pol α the inhibition by compounds **29** and **30** is lower than it is by compound **1**. To ascertain the significance especially of the DNA pol α results, a kinetic analysis comparing the mode of action of the glutarate and adipate derivatives versus the parent compound **1** remains to be done.

Experimental

Molecular mechanics modelling and calculations were performed using the Sybyl program. The triphosphate conformation used was the one presented by Pelletier *et. al*[42] with an elongated (2.0 Å) bond between the α-phosphorous and the oxygen of the pyrophosphate moiety. The monomethyl esters of the four diacids were first energy minimised and the resulting conformations were used to create conformations that could mimic the triphosphate. Finally, the energies of the mimicing conformations were calculated and compared to the minimised energies.

Melting points were recorded on a Gallenkamp MFB-595-010M melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer. The mass spectra were recorded on a JEOL JMS - SX 102 spectrometer.

*Preparation of diacid monobenzyl esters (**9-12**) and diacid chloride monobenzyl esters (**13-16**),* SCHEME 4.

The glutaric, adipic and pimelic acid monobenzyl esters (**9-11**) were prepared as described by English *et al.*[54]. The monoesters were used without further purification: they were dissolved in 10 equivalents of oxalyl chloride at 0°C, and the reaction mixtures were then allowed to reach room temperature. After 120 h the excess oxalyl chloride was removed by evaporation, and distillation in vacuum of the crude product yielded the diacid chloride monobenzyl esters (**13-15**).

*Glutaric acid chloride monobenzyl ester (**13**)* . Yield (from glutaric acid) : 19%, bp 130-132°C/0.4 mm Hg.

*Adipic acid chloride monobenzyl ester (**14**)* . Yield (from adipic acid) : 35%, bp 147-150°C/0.5 mm Hg.

Pimelic acid chloride monobenzyl ester (15) . Yield (from pimelic acid) : 23%, bp 156-160°C/0.3 mm Hg.

Trans-*1,4-cyclohexanedicarboxylic acid monobenzyl ester (12).*

Trans-1,4-cyclohexanedicarboxylic acid (20.0 g, 11.6 mmol), DMAP (1.5 g, 1.2 mmol) and benzyl alcohol (18.0 ml, 17.4 mmol) were dissolved in 100 ml of anhydrous DMF. DCC (39.0 g, 19.2 mmol) was added at 0°C and the reaction mixture was allowed to stand at room temperature for 24 h. The reaction mixture was filtered through Celite and treated in the same way as the monoesters **10** and **11**[54]. Yield : 23%.

Trans-*1,4-cyclohexanedicarboxylic acid chloride monobenzyl ester (16)* was prepared from **12** in the same way as the diacid chloride monobenzyl esters **13-15**. The product was dissolved in ether and quickly washed with ice water. Yield (from monoester) : 74%.

β-5-(2´´-Thienyl)-2´-deoxyuridine (1), SCHEME 2.

1 was prepared according to Hubbard *et al.*[46] by condensation between 2,4-ditrimethylsilyloxy-5-(2´-thienyl)-pyrimidine (**3**) and α-2-deoxy-3,5-di-*O*-p-toluoylribofuranosyl chloride (**4**)[45] in chloroform with one equivalent of copper(I) iodide[47]. Separation of the α and β anomers was carried out on silica gel 60 with dichloromethane/methanol 9/1, giving a total yield of 69% β-5-(2´´-thienyl)-2´-deoxy-3´,5´-di-*O*-toluoyluridine (**3**). The p-toluoyl protecting groups were removed by adding 730 ml of 0.2 M sodium methoxide in methanol dropwise to a solution of 26.6 g (48.8 mmol) of **3** in 4900 ml of methanol. The reaction mixture was stirred at room temperature for 24 h and filtered through silica gel 60. The silica was washed with methanol and the solvent removed by evaporation *in vacuo*. Chromatography on silica 60 with dichloromethane/methanol 9/1 gave β-5-(2´´-thienyl)-2´-deoxyuridine (**1**) in 95% yield.

General procedure for the preparation of 3´-O-levulinoyl-2´-deoxy-ribonucleosides (8a and b), SCHEME 3.

The 5´-*O*-dimethoxytritylated 2´-deoxy-ribonucleosides **6a** and **b** were prepared according to Hakimelahi *et al.*[48]. The reaction mixture was stirred at room temperature for 24 h (thymidine) or 168 h (5-(2´´-thienyl)-2´-deoxyuridine). **6a** and **b** were purified by chromatography using silica gel 60 and dichloromethane/methanol 9/1. **6a** was obtained in 80% yield as reported[48] and **6b** was obtained in 43% yield. **6a** and **b** were converted to their corresponding 3´-*O*-levulinoyl derivatives, **7a** and **b,** by the procedure of Hassner *et al.*[49] Instead of crystallisation[49], the reaction mixture was poured onto ice water and extracted three times with dichloromethane. The combined organic phase was washed once with 2 N sulfuric acid, saturated sodium bicarbonate solution (until

neutral), and twice with water. The organic phase was dried over magnesium sulphate, filtered and the solvent removed by evaporation *in vacuo* to give a crude product (**7a** or **b**) that was used without further purification. 5′-*O*-Dimethoxytritylated 3′-*O*-levulinoyl-2′-deoxy-ribonucleoside (60 mmol) was dissolved in 1000 ml of 0.1M trichloroacetic acid in dichloromethane/ethanol 97/3 and was stirred at room temperature for 2 h. 10 g of solid sodium bicarbonate was added, and after 15 minutes the reaction mixture was filtered through silica gel 60. The silica was washed with methanol and the filtrate concentrated by evaporation *in vacuo*. The crude product was applied to a column of silica 60 and chromatographed with gradient.

3′-O-Levulinoyl-thymidine (8a) . Yield (from **6a**) 85%, eluent chromatography dichloromethane/methanol (99/1 → 93/7)

5-(2′′-Thienyl)-3′-O-levulinoyl-2′-deoxyuridine (8b) . Yield (from **6b**) 91%, eluent chromatography dichloromethane/methanol (97/3 → 95/5)

General procedure for preparation of benzyl [1′,2′-dideoxy-1′-β-(1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5′-yl] diesters. (17-24)

Method A, SCHEME 5.

The diacid chloride monobenzyl ester (3.74 mmol) was added dropwise to a stirred solution of 2.95 mmol of 3′-*O*-levulinoyl-2′-deoxy-ribonucleoside in 3 ml of anhydrous pyridine in a 10 ml pressure bottle at 0°C. The reaction mixture was allowed to reach room temperature and was stirred for 24 h. After the addition of 5 ml of methanol, the solvent was removed by evaporation in vacuo. The crude product was dissolved in a mixture of 15 ml of tetrahydrofuran (THF) and 2 ml of water. Sodium borohydride (0.45 g, 12 mmol) dissolved in 2 ml of water was added dropwise and the reaction mixture was stirred at room temperature[1,2] for 15 minutes[49] (longer reaction times give basic hydrolysis of the 5′-ester bond, yielding predominantly the 2′-deoxyribonucleoside). The reaction mixture was filtered through silica gel 60, the silica was washed with methanol and the filtrate concentrated by evaporation *in vacuo*. The crude product was purified by column chromatography using silica gel 60 (see below).

Method B[56], SCHEME 5.

The diacid chloride monobenzyl ester (1.42 mmol) dissolved in 3 ml of anhydrous dichloromethane, was added dropwise under nitrogen during 2 h to a stirred solution of

1. In the case of **20** → **28** : 15 minutes at 0°C to avoid isomerisation.
2. In the case of X=cyclohexyl and R=thienyl, reaction at room temperature gives isomerisation and no reaction at all takes place at 0°C.

1.29 mmol 2´-deoxy-ribonucleoside in 5 ml of anhydrous pyridine. The reaction mixture was stirred for 0.5 h under nitrogen. After the addition of 5 ml of methanol the solvent was removed by evaporation in vacuo and the crude product was purified by column chromatography using silica gel 60 (see below). Further purification was carried out by HPLC using a Polygosil Silica 250x20 mm column with chloroform/methanol as eluent (see below).

*Benzyl [1´,2´-dideoxy-1´-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5´-yl]glutarate (**17**).* Yield : 62% (method A, from **8a**), 41% (method B, from thymidine), eluent column chromatography dichloromethane/methanol (96/4) (method A and B), eluent HPLC chloroform/methanol (98/2) (method B), mp 88-91°C. ^{1}H NMR (CD_3OD) δ 1.87 (d, 1H, CH_3, J=1.2 Hz), 1.94 (m, 2H, CH_2-CH_2-CH_2), 2.19 (ddd, 1H, H-2´a, J=6.4 Hz, J=7.4 Hz, J=13.8 Hz), 2.28 (ddd, 1H, H-2´b, J=3.6 Hz, J=6.3 Hz, J=13.8 Hz), 2.44 (t, 4H, CH_2-CH_2-CH_2, J=7,2 Hz), 4.06 (dt, 1H, H-4´, J=3.5 Hz, J=5.5 Hz), 4.24 (dd, 1H, H-5´a, J=3.5 Hz, J=12.0 Hz), 4.33 (m, 1H, H-3´), 4.38 (dd, 1H, H-5´b, J=5.5 Hz, J=12.0 Hz), 5.11 (s, 2H, Ph-CH_2) 6.25 (t, 1H, H-1´, J=6.4 Hz), 7.27-7.45 (m, 5H, Ph), 7.47 (q, 1H, H-6, J=1.2 Hz). Anal. calcd. for $C_{22}H_{27}O_8N_2$: MW 446.1690. Found M+H 447.1759.

*Benzyl [1´,2´-dideoxy-1´-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5´-yl]adipate (**18**).* Yield : 71% (method A, from **8a**), 45% (method B, from thymidine), eluent column chromatography dichloromethane/methanol (96/4) (method A and B), eluent HPLC chloroform/methanol (98/2) (method B), mp 88-91°C. Anal. calcd. for $C_{23}H_{29}O_8N_2$: MW 460.1846. Found M+H 461.1935.

*Benzyl [1´,2´-dideoxy-1´-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5´-yl]pimelate (**19**).* Yield : 62% (method A, from **8a**), 47% (method B, from thymidine), eluent column chromatography dichloromethane/methanol (96/4) (method A and B), eluent HPLC chloroform/methanol (98/2) (method B), mp 88-92°C. Anal. calcd. for $C_{24}H_{31}O_8N_2$: MW 474.2003. Found M+H 475.2082.

*Benzyl [1´,2´-dideoxy-1´-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5´-yl]*trans-*1,4-cyclohexanedicarboxylate (**20**).* Yield : 26% (method A, from **8a**), 37% (method B, from thymidine), eluent column chromatography dichloromethane/methanol (96/4) (method A and B), eluent HPLC chloroform/methanol (98/2) (method B), mp 156-162°C. Anal. calcd. for $C_{25}H_{31}O_8N_2$: MW 486.2003. Found M+H 487.2082.

*Benzyl [1´,2´-dideoxy-1´-β-(5-(2´´-thienyl)-1,2,3,4-tetrahydro-2,4-di-oxopyrimidine-1-yl)-D-ribofuranos-5´-yl]glutarate (**21**).* Yield : 13% (method A, from **8b**), 54%

(method B, from **1**), eluent column chromatography dichloromethane/methanol (96/4) (method A and B), eluent HPLC chloroform/methanol (98/2) (method B), mp 141-143°C. ^{1}H NMR (CD_3OD) δ 1.81 (m, 2H, CH_2-*CH_2*-CH_2), 2.28 (t, 4H, *CH_2*-CH_2-*CH_2*, J=7,2 Hz), 2.30 (m, 1H, H-2'a), 2.39 (ddd, 1H, H-2'b, J=3.5 Hz, J=6.3 Hz, J=13.8 Hz), 4.16 (dt, 1H, H-4', J=3.5 Hz, J=5.5 Hz), 4.31 (dd, 1H, H-5'a, J=3.5 Hz, J=12.0 Hz), 4.36 (m, 1H, H-3'), 4.42 (dd, 1H, H-5'b, J=5.5 Hz, J=12.0 Hz), 5.06 (s, 2H, Ph-*CH_2*) 6.27 (t, 1H, H-1', J=6.4 Hz), 7.02 (dd, 1H, H-4'', J=3.7 Hz, J= 5.2 Hz), 7.34 (dd, 1H, H-5'', J=1.2 Hz, J=5.2 Hz), 7.39 (dd, 1H, H-3'', J=1.2 Hz, J=3.7 Hz), 7.31-7.34 (m, 5H, Ph), 8.00 (s, 1H, H-6). Anal. calcd. for $C_{25}H_{27}O_8N_2S$: MW 514.1410. Found M+H 515.1488.

*Benzyl [1',2'-dideoxy-1'-β-(5-(2''-thienyl)-1,2,3,4-tetrahydro-2,4-di-oxopyrimidine-1-yl)-D-ribofuranos-5'-yl]adipate (**22**).* Yield : 10% (method A, from **8b**), 50% (method B, from **1**), eluent column chromatography dichloromethane/methanol (96/4) (method A and B), eluent HPLC chloroform/methanol (98/2) (method B), mp 140-144°C. Anal. calcd. for $C_{26}H_{29}O_8N_2S$: MW 528.1567. Found M+H 529.1649.

*Benzyl [1',2'-dideoxy-1'-β-(5-(2''-thienyl)-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5'-yl]pimelate (**23**).* Yield : 26% (method A, from **8b**), 50% (method B, from **1**), eluent column chromatography dichloromethane/methanol (95/5) (method A and B), eluent HPLC chloroform/methanol (97/3) (method B), mp 139-141°C. Anal. calcd. for $C_{27}H_{31}O_8N_2S$: MW 542.1723. Found M+H 543.1810.

*Benzyl [1',2'-dideoxy-1'-β-(5-(2''-thienyl)-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5'-yl]trans-1,4-cyclohexanedicarboxylate (**24**).* Yield : 22% (method B, from **1**), eluent column chromatography dichloromethane/ methanol (98/2) (method B), eluent HPLC n-heptane/ethylacetate/i-propanol (60/30/10) (method B), mp 156-160°C. Anal. calcd. for $C_{28}H_{31}O_8N_2$: MW 554.1723. Found M+H 555.1801.

*General procedure for preparation of [1',2'-dideoxy-1'-β-(1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5'-yl] esters. (**25-32**)*

Method A, B, scheme 5

The benzyl [1',2'-dideoxy-1'-β-(1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5'-yl] diester (0.45 mmol) was dissolved in a mixture of 8 ml of 99.5% ethanol and 2 ml of glacial acetic acid. 20 mg (thymidines) or 400 mg (5-(2''-thienyl)-2'-deoxyuridines) of 10% Pd-on-charcoal was added. The reaction mixture was stirred at room temperature for 24 h under 1 atm of hydrogen (thymidines) or for 72 h under 5 atm of hydrogen (5-(2''-thienyl)-2'-deoxyuridines). The reaction mixture was filtered through Celite and the solvent removed by evaporation *in vacuo*. The product was purified by HPLC using a RP C_{18} Dynamax 250x20 mm column.

*[1′,2′-Dideoxy-1′-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5′-yl]glutarate (**25**).* Yield : 48% (syrup), eluent HPLC acetonitrile/water (80/20). Anal. calcd. for $C_{15}H_{21}O_8N_2$: MW 356.1220. Found M+H 357.1299.

*[1′,2′-Dideoxy-1′-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5′-yl]adipate (**26**).* Yield : 43% (syrup), eluent HPLC acetonitrile/water (80/20). Anal. calcd. for $C_{16}H_{23}O_8N_2$: MW 370.1377. Found M+H 371.1454.

*[1′,2′-Dideoxy-1′-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5′-yl]pimelate (**27**).* Yield : 45%, eluent HPLC acetonitrile/water (80/20), mp 136-139°C. Anal. calcd. for $C_{17}H_{25}O_8N_2$: MW 384.1533. Found M+H 385.1615.

*[1′,2′-Dideoxy-1′-β-(5-methyl-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-1-yl)-D-ribofuranos-5′-yl]*trans-*1,4-cyclohexane dicarboxylate (**28**).* Yield : 82%, eluent HPLC acetonitrile/water (80/20), mp 154-159°C. Anal. calcd. for $C_{18}H_{25}O_8N_2$: MW 396.1533. Found M+H 397.1614.

*[1′,2′-Dideoxy-1′-β-(5-(2′′-thienyl)-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5′-yl]glutarate (**29**).* Yield : 12%, eluent HPLC acetonitrile/water/acetic acid (20/80/2), mp 170-173°C. ^{1}H NMR (CD_3CN/D_2O 7/3) δ 1.69 (m, 2H, CH_2-*CH_2*-CH_2), 2.22 (t, 4H, *CH_2*-CH_2-*CH_2*, J=7,2 Hz), 2.24 (ddd, 1H, H-2′a, J=3.8 Hz, J=6,7 Hz, J=14.1 Hz), 2.37 (ddd, 1H, H-2′b, J=6.5 Hz, J=6.7 Hz, J=14.1 Hz), 4.13 (dt, 1H, H-4′, J=3.6 Hz, J=4.0 Hz), 4.26 (dd, 1H, H-5′a, J=3.3 Hz, J=12.3 Hz), 4.30 (dd, 1H, H-5′b, J=4.0 Hz, J=12.3 Hz), 4.35 (m, 1H, H-3′), 6.17 (t, 1H, H-1′, J=6.7 Hz), 7.05 (dd, 1H, H-4′′, J=3.7 Hz, J= 5.1 Hz), 7.32 (dd, 1H, H-3′′, J=1.1 Hz, J=3.7 Hz), 7.38 (dd, 1H, H-5′′, J=1.1 Hz, J=5.1 Hz), 7.91 (s, 1H, H-6). Anal. calcd. for $C_{18}H_{21}O_8N_2S$: MW 424.0941. Found M+H 425.1040.

*[1′,2′-Dideoxy-1′-β-(5-(2′′-thienyl)-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5′-yl]adipate (**30**).* Yield : 13%, eluent HPLC acetonitrile/water/acetic acid (30/70/5), mp 167-170°C. Anal. calcd. for $C_{19}H_{23}O_8N_2S$: MW 438.1097. Found M+H 439.1177.

*[1′,2′-Dideoxy-1′-β-(5-(2′′-thienyl)-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5′-yl]pimelate (**31**).* Yield : 10%, eluent HPLC acetonitrile/water (20/80), mp 117-120°C. Anal. calcd. for $C_{20}H_{25}O_8N_2S$: MW 452.1254. Found M+H 453.1320.

*[1′,2′-Dideoxy-1′-β-(5-(2′′-thienyl)-1,2,3,4-tetrahydro-2,4-dioxo-pyrimidine-1-yl)-D-ribofuranos-5′-yl]*trans-*1,4-cyclohexanedicarboxylate (**32**).* Yield : 15%, eluent HPLC acetonitrile/water (20/80), mp 153-158°C. Anal. calcd. for $C_{21}H_{25}O_8N_2S$: MW 464.1254. Found M+H 465.1338.

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