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The Design and Synthesis of Nucleoside Triphosphate Isosteres as Potential Inhibitors of HIV Reverse Transcriptase

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Abstract: We describe the synthesis of a variety of lipophilic isosteres of nucleoside triphosphates as potential anti-HIV agents. The citrate molecule proved to be a good mimic of triphosphate by modelling in terms of charge and spatial distribution. Several lipophilc derivatives of citrate were conjugated to the precedented anti-HIV nucleoside d4T *via* ester and amide linkages. A novel synthesis of 5'-amino-d4T is included. Intramolecular rearrangement of several amide-linked isosteres are also reported, along with an alternative synthetic strategy to the desired amide-linked isosteres. © 1997 Elsevier Science Ltd.

INTRODUCTION

The AIDS pandemic is spreading rapidly and cannot, at present, be treated effectively. The most promising licensed anti-viral nucleoside analogues (ddNs) 3'-azido-3'-deoxythymidine (AZT)[1], 2', 3'-dideoxy-2', 3'-didehydrothymidine (d4T)[2], 2',3'-dideoxycytidine (ddC)[3], 2',3'-dideoxyinosine (ddI)[4] and (-)-2', 3'-dideoxy-3'-thiacytidine (3TC)[5] act at HIV-1 reverse transcriptase (RT).

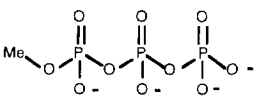
Reverse transcription is essential for virus replication and has no direct counterpart in humans, thus it represents an attractive target[6-10]. The above nucleoside analogues are pro-drugs which are phosphorylated intracellularly by human kinases to nucleoside monophosphates followed by further phosphorylation by other kinases to the active 5'-triphosphates (ddNTP)[11-13]. The ddNTP's may act as competitive inhibitors at RT or as DNA chain-terminators for the newly emerging DNA strands.

Many ddN's however, have a low affinity for host kinases[14-16] and this may explain, in part, the lack of *in vivo* activity. Nucleotides cannot be used directly due to their high polarity and chemical instability. Recent drug delivery advances include the phosphorylation-bypass approach. Masked phosphates of 5'-ddN's have shown promising results with respect to their parent nucleosides[17, 18]. These lipophilic compounds are

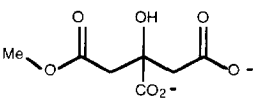
designed to cross the cell membrane and hydrolyse intracellularly to the 5`-monophosphate. This approach is attractive for d4T, whose rate-limiting step for activation is formation of d4T monophosphate[19]. However, the rate-limiting step for a variety of other RT nucleoside inhibitors can vary and is also dependent on cell-type.

The approach that we have adopted is to design and synthesise a chemically and enzymatically stable isostere of a triphosphate and couple it to various anti-HIV nucleosides[20, 21]. This should by-pass all 3 activation steps. The isostere should have the same shape and charge distribution as triphosphate in order to act at RT. A further requirement includes enhanced lipophilicity. increasing cellular uptake of the compounds.

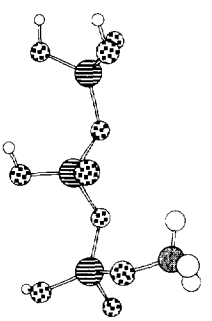
Molecular modelling showed that the citrate molecule should be a good mimic of the triphosphate. The monomethyl esters of both triphosphate and citrate were energy minimised using MM2 calculations and conformational searching was applied to find the global minimum, using the program MACROMODEL. These calculations assumed water as a solvent and the global minima were superimposed. The structures proved almost entirely superimposable, with the carboxylate and hydroxyl group oxygen's of the citrate being in the same position as the phosphate oxygen's (Figure 1). Simple charge calculations also gave good correlation for both the citrate and the triphosphate. It is highly likely that the triphosphate is complexed to magnesium ions in solution *via* the aspartic acid triad (Asp 185, Asp 110 and Asp186) of RT[9]. A citrate - magnesium complex has been observed by solution NMR[22]. indicating further favourable interactions in the



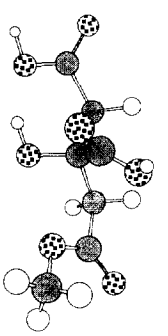
Compound A



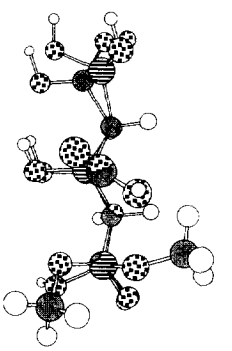
Compound B



Compound A



Compound B

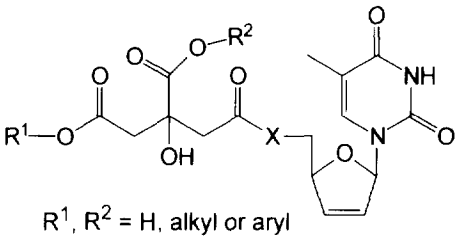


Compounds A and B superimposed

Figure 1

active site. The target molecules are depicted in figure 2. By varying the nature of the substituents it should be possible to optimise the physiochemical properties of the triphosphate isostere.

Owing to the lack of citric acid chemical manipulation in the literature, we felt it timely to publish details of the chemical syntheses involved. We have previously published a communication outlining the biological data of these compounds[20].



R^1, R^2 = H, alkyl or aryl

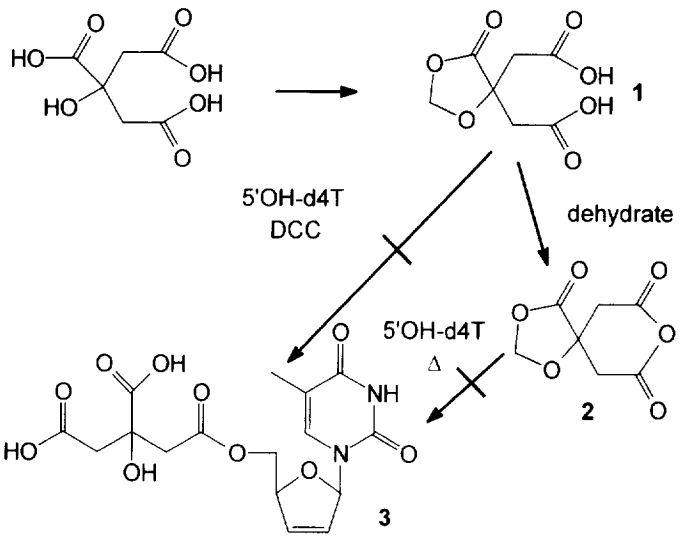
X = O, NH

Figure 2

RESULTS AND DISCUSSION

The initial problem in the chemical manipulation of citric acid arises from its inherent high polarity and low solubility in most organic solvents. Secondly, regioselective derivatisation of this molecule requires selective protection of the two equivalent primary carboxylic acids and a tertiary acid. The formation of a 1, 3-dioxolan-4-one ring in citric acid[23] was considered a key intermediate in the synthesis of a triphosphate isostere. This compound should be readily coupled directly to d4T[24].

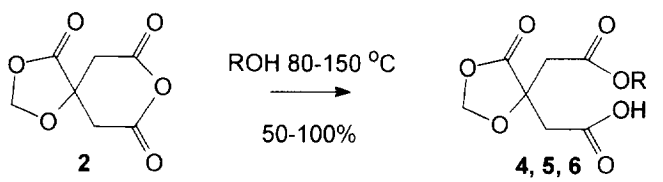
The selectively protected derivative **1** was synthesised by heating citric acid and paraformaldehyde (1:1) in a sealed pressure tube at 145 °C for 4h[25] (scheme 1). The product was obtained by recrystallisation from water in 50% yield. Various attempts of DCC coupling of **1** with d4T failed. In addition, formation of the diacid chloride derivative of **1** with thionyl chloride was unsuccessful. Both these reactions gave intra-molecular ring-closures to form a stable six-membered anhydride **2**. This anhydride was readily synthesised in quantitative yield by refluxing **1** with acetic anhydride for 2h. Attempts to synthesise **2** by alternative methods[23] gave product inferior in both yield and purity. This compound showed remarkable stability. No decomposition was observed after leaving the compound exposed to air for several days. The five-membered ring must impart a great deal of stability to the anhydride as acetonedicarboxylic anhydride, structurally similar to **2**, hydrolyses readily on exposure to air[26]. This surprising stability was confirmed further as reaction of **2** with d4T even at high temperature left unchanged starting materials (scheme 1). However, a trial reaction in refluxing methanol for several hours gave the desired mono-methyl ester (**4**, 100%) cleanly.



Scheme 1

This success prompted another possible route to the required triphosphate isosteres. Mono-alkyl esters of **1**, coupled to d4T under DCC conditions should give a range of protected triphosphate isosteres. A simple alkyl series was considered along with an allyl ester. The allyl group should be selectively cleaved in the presence of the 5'-ester linkage to d4T, thus giving an isostere with a free terminal carboxylic acid group.

Several monoesters were synthesised in high yields by refluxing appropriate dry alcohols with **2** for approximately 5 h (Scheme 2). These reactions proved clean after removal of excess alcohol.



R = Me **4**, Allyl **5**, Benzyl **6**

Scheme 2

Although **4**, **5** and **6** bear resemblance to the triphosphate isostere, the ring-opening of the 1, 3-dioxolan-4-one functionality with an alcohol followed by DCC coupling to d4T would lead to the desired products as protected lipophilic diesters. Ring-opening of a 1, 3-dioxolan-4-one ring by refluxing **2** with an appropriate alcohol with 2 equiv. of triethylamine has been reported[27]. This approach was attempted by reacting **4** with dry methanol plus freshly dried and distilled triethylamine (2 equiv.) at elevated temperature. After work-up, the product **7** was isolated in 54% yield. Similarly, after reacting **5** with allyl alcohol, the diallyl product **8** was isolated in 61% yield.

It was later discovered that the dimethyl product **7** could be synthesised in a one-pot reaction by treating **2** with triethylamine (2 equiv.) in refluxing methanol for 24h. A parallel reaction without triethylamine led to only partial formation of **7** indicating that general base catalysis is required for ring-opening of the 1, 3-dioxolan-4-one following reaction at the cyclic anhydride.

Ester-Linked Nucleoside Triphosphate Isosteres

Coupling the selected citric acid derivatives **4**, **5**, **6** and **8** to d4T with DCC and DMAP in THF or acetonitrile yielded the desired novel protected isosteres **9**, **10**, **11** and **13** in moderate to high yields as a 1:1 mixture of diastereoisomers (scheme 3). The formation of the monoallyl derivative **5** in high yield followed by the successful coupling to d4T provided an efficient route to a protected isostere. The clean deallylation proved possible by reacting **11** with *tris*triphenylphosphine rhodium chloride (Wilkinson's catalyst) in ethanol/water 9:1 at 70 °C for 2.5h[28]. Flash chromatography eluting with a methanol/chloroform gradient provided the desired free acid **12** as a clear oil.

The diallyl-citrate d4T conjugate **13** was successfully deallylated by treatment with Wilkinson's catalyst[28] in aqueous ethanol at 70 °C for 6h. Following flash chromatography, the final target **3** was recovered as a white solid in 32% yield.

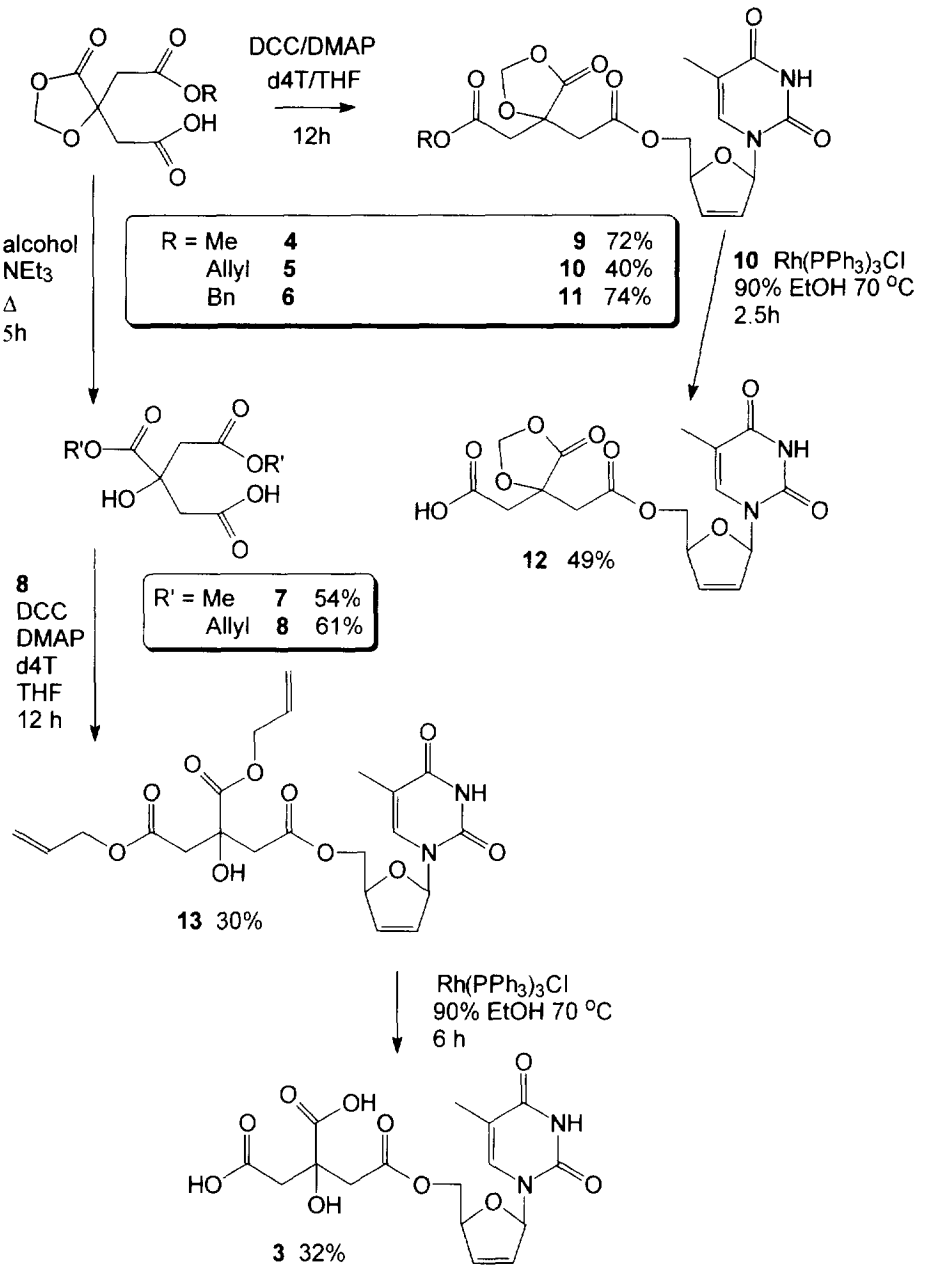
Amide-Linked Nucleoside Triphosphate Isosteres

The presence of an 5'-amide rather than a 5'-ester nucleoside-isostere link was hoped to give more biological stability to the nucleoside-citrate conjugate. Coupling derivatised citric acid compounds to 5'-amino-3'-deoxy-2', 3'-didehydrothymidine (5'-amino-d4T) with DCC and DMAP in a suitable solvent was envisaged. This compound has been previously reported[29] but an alternative strategy was considered.

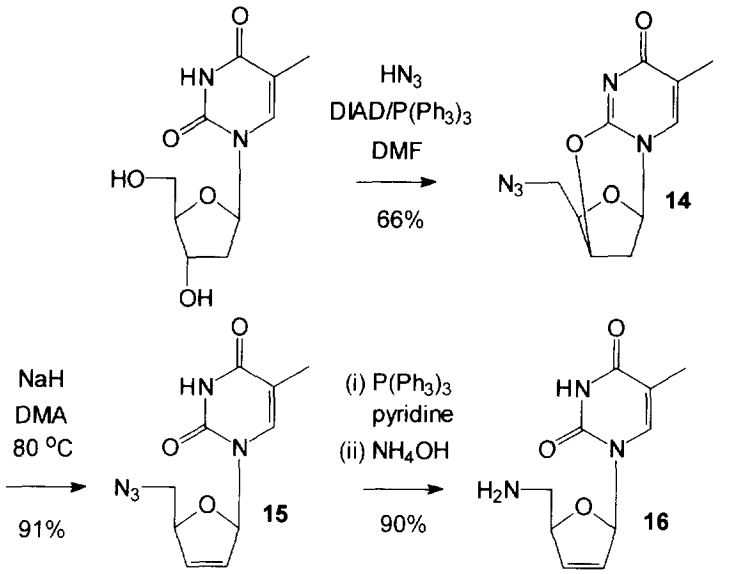
A possible synthesis of 5'-amino-d4T is to convert d4T to 5'-phthaloylamino-3'-deoxy-2', 3'-didehydrothymidine followed by reduction of the phthalimide group to a primary amine. Following a similar synthetic route previously described[30], 5'-phthaloylamino-3'-deoxy-2',3'-didehydrothymidine was prepared, from d4T, in 56% yield using Mitsunobu conditions[31]. However, reduction of this compound and isolation of 5'-amino-d4T proved problematic.

Turner[30] described the synthesis of 5'-azido-3'-deoxy-2', 3'-didehydrothymidine starting from thymidine in 2 steps. Reduction of the azido group should give the required product. An advantage of this route is that use of costly d4T as a starting material is avoided.

A freshly prepared solution of hydrazoic acid was coupled to thymidine with triphenylphosphine and DIAD to form 5'-azidothymidine (scheme 4). A second aliquot of triphenylphosphine/DIAD formed 2, 3'-anhydro-5'-azidothymidine **14** in 66% yield. Reacting **14** with sodium hydride in DMA at 80 °C[32] gave the eliminated product, 5'-azido-3'-deoxy-2', 3'-didehydrothymidine **15** as a pure white solid in 91% yield.


DCC/DMAP
d4T/THF
12h
alcohol
NEt3
Δ
5h
R = Me 4
Allyl 5
Bn 6
9 72%
10 40%
11 74%
10 Rh(PPh3)3Cl
90% EtOH 70 °C
2.5h
12 49%
8
DCC
DMAP
d4T
THF
12 h
R' = Me 7 54%
Allyl 8 61%
13 30%
Rh(PPh3)3Cl
90% EtOH 70 °C
6 h
3 32%

Scheme 3



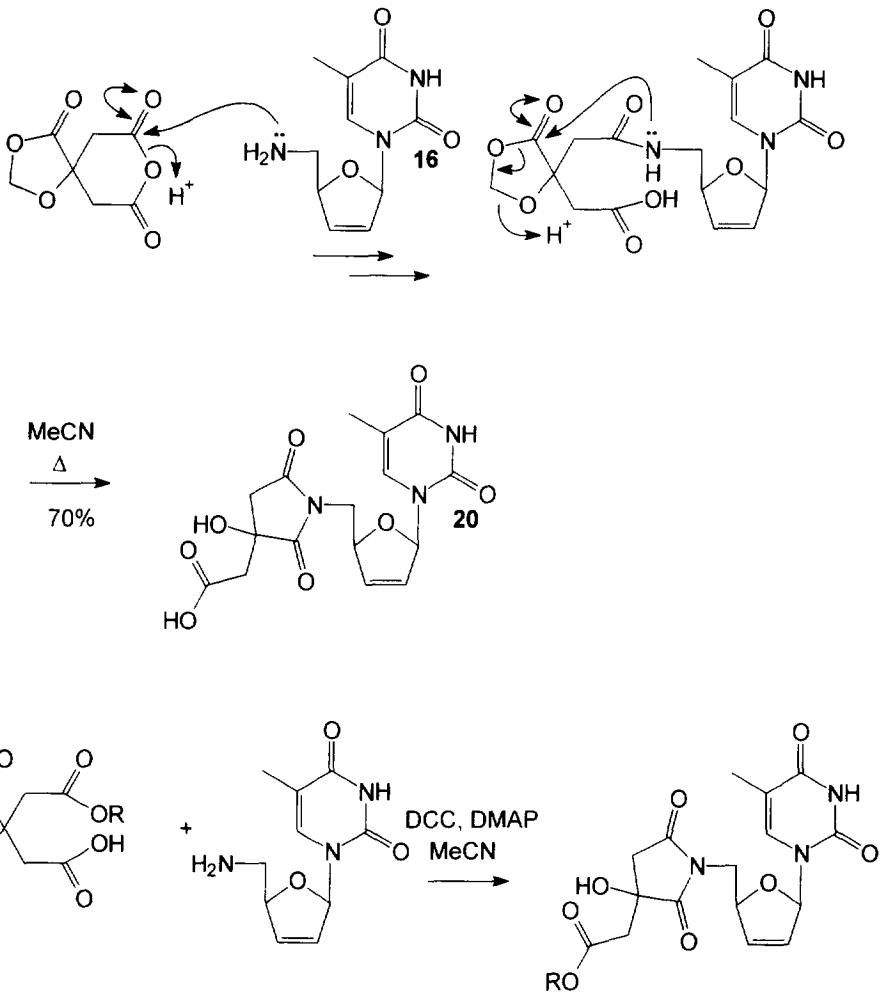
Scheme 4

Reacting **15** with triphenylphosphine, followed by alkaline hydrolysis of the phosphinimine intermediate afforded the desired 5'-amino-3'-deoxy-2', 3'-didehydrothymidine **16** in 90% yield.

DCC type coupling of **16** to the previously synthesised citric acid derivatives should yield novel amide-linked nucleoside triphosphate isosteres. Compounds chosen for coupling were **4**, **5** and **6** in order to compare biological activity of ester against amide linked conjugates. Reacting these compounds with **16** under the same chemical conditions as for d4T gave products in 46-85% yields. However, there was one notable absence in each ^{1}H and ^{13}C NMR *i.e.* a loss of signal at ~ 5.5 ppm and ~95 ppm respectively. These characteristic peaks are due to the methylene peaks (OCH_2O) of the 1, 3-dioxolan-4-one ring. Clearly these compounds had been ring-opened. Initial thoughts were that the lactone had been hydrolysed, with elimination of formaldehyde to give an acid and a tertiary alcohol. However, mass spectra of these compounds in ES +ve ion mode indicated a loss of 18 from the molecular ions of these proposed structures, suggesting an alternative structure.

Nau *et al.*,[23] reported that reacting **2** with aniline gave a surprising structure *i.e.* a cyclic 5 membered ring imide. This reaction proceeds by nucleophilic attack at the cyclic anhydride with aniline to give an amide, followed by intramolecular ring-opening of the 1, 3-dioxolan-4-one ring, with loss of formaldehyde, by the nitrogen of the amide functionality. Similar reactions between **2** and various amines have also been reported[27, 33]. These results could explain the NMR data observed in the coupling between **4**, **5** and **6** to **16** *i.e.* a cyclic imide was isolated in the coupling reaction rather than the expected amide-linked isostere (scheme 5). Further evidence of these newly proposed structures was given by the mass spectra of these compounds. In ES -ve mode, expected molecular ions of the imide structures were observed.

Interestingly, whilst direct treatment of **2** with d4T in a suitable solvent gave no reaction, the corresponding reaction of **2** with 5'-amino-d4T **16** gave a product which proved by NMR and MS to be a cyclic imide **20**. Reaction at the anhydride followed by intramolecular ring-opening of the 1, 3-dioxolan-4-one must have occurred. Clearly, intramolecular rearrangement of these compounds to imides is facilitated by the presence of an activated ester *i.e.* the 1, 3-dioxolan-4-one ring. An alternative synthesis strategy to the desired

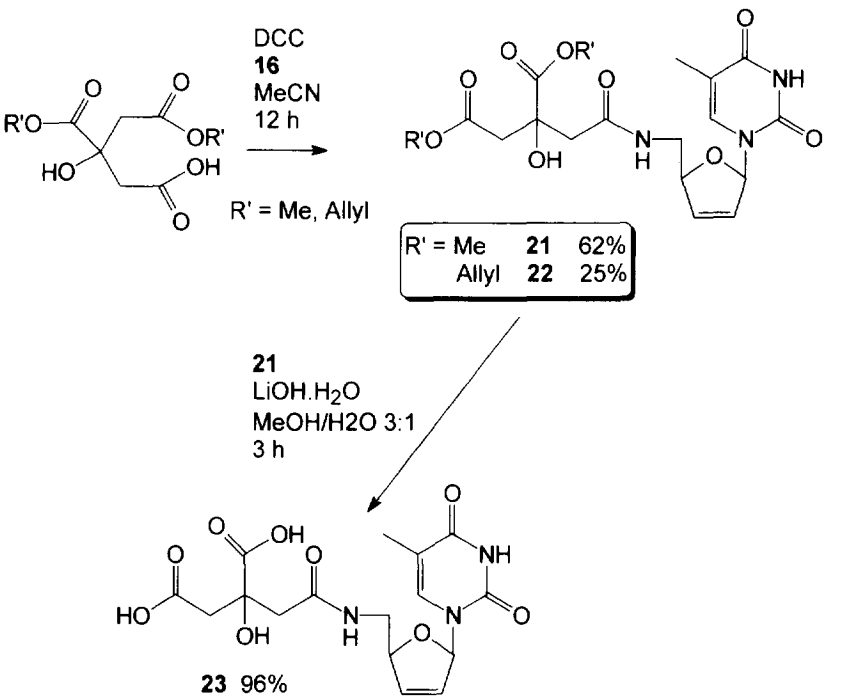


Scheme 5

5-'amide-linked isosteres was required.

Coupling the diesters **7** or **8** to **16** should bypass this problem because these citric acid derivatives lack the 1, 3-dioxolan-4-one ring. Deprotection of these new isostere conjugates could be effected with base hydrolysis and Wilkinson's catalytic deallylation respectively.

Reaction of **7** and **8** with **16** *via* DCC coupling afforded the desired conjugates **21** and **22**, after purification by flash chromatography, in low to moderate yields as a 1:1 mixture of diastereoisomers. It was important not to use DMAP in the coupling as this led to dehydration as shown by NMR and mass spectrometry. This probably occurred by base-induced elimination of water from the citrate isostere. The saponification of **21** was effected by treatment with lithium hydroxide monohydrate in aqueous methanol. Purification of the polar product by flash chromatography gave the final amide-linked deprotected citric acid 5'amino-d4T isostere **23** in 96% yield (scheme 6). **23** failed to give a molecular ion by FAB in either positive or negative ion modes but an $(M-H)^-$ peak was clearly observed by negative ion ESMS. However, as accurate mass was not available for ESMS, **23** was characterised by analytical HPLC (99.2%) and NMR.



Scheme 6

CONCLUSION

Citric acid was chosen as an isosteric replacement for a triphosphate moiety. Following selective protection to a key intermediate **1** containing a 1, 3-dioxolan-4-one ring, the citrate molecule was attached to d4T through an ester linkage *via* DCC coupling. Various ester protected d4T triphosphates were synthesised **4**, **5**, **6**, **7**, **8** and selective allyl ester removal yielded the desired isostere as a free dicarboxylic acid **3**.

Amide-linked isosteres were hoped to give more metabolic stability. A novel high-yielding route to 5-amino-d4T **16** from thymidine was successfully developed. However, following similar DCC coupling to the citrate intermediates, the desired conjugates were not isolated. Instead, an intramolecular rearrangement occurred yielding cyclic imides **17**, **18**, **19**. An alternative synthetic strategy yielded the desired compounds and the final target compound **23** was isolated after ester hydrolysis.

The biological results of all conjugates tested against a variety of HIV infected cell lines are given in a recent publication[20].

Acknowledgements

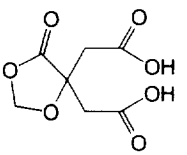
We wish to thank the Medical Research Council for funding (RW), the EPSRC National Mass Spectrometry Service Centre, Swansea, Dr. G. Eaton (mass spectra, University of Leicester), Dr. Jonathan Goodman (University of Cambridge) for help with the molecular modelling and Dr. Steven Turner (University of Wales, Cardiff) for advice on the synthesis of 2, 3'-anhydro-5'-azidothymidine.

EXPERIMENTAL

All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX300 spectrometer at 300 MHz and 75 MHz respectively. Chemical shifts were recorded in parts per million (ppm) downfield from tetramethylsilane. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR with compound either neat on sodium chloride discs or with a diffuse reflectance accessory using a potassium bromide matrix. Elemental analyses were determined on a Perkin-Elmer 240C elemental analyser. Low resolution mass spectra were recorded on a Fisons Instruments VG Platform Electrospray mass spectrometer run in either positive or negative ion mode, with acetonitrile/water as the mobile phase. High resolution mass spectra were determined by the EPSRC Mass Spectrometry Centre, Swansea, UK. Analytical HPLC was determined with an ACS 350/04 HPLC machine using a spherisorb S5ODS2 25 cm × 4.6 mm column. Mobile phase was A = water, B = acetonitrile. Gradient conditions: 82% A 0-10 minutes, then a linear gradient to 20% A 10-30 minutes,

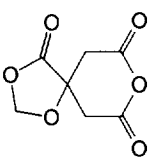
followed by 20% A for a further 30 minutes. Flow rate was set at 1.2 ml min^{-1} and detection was by UV at 256 nm. Solvents were dried and distilled as required using standard conditions, and all experiments using moisture sensitive reagents were performed under a nitrogen atmosphere. Analytical TLC was carried out on silica gel plates (Kieselgel 60 F_{254}, BDH) using UV light and/or 5% molybdophosphoric acid in ethanol for detection. Silica gel for flash chromatography was Sorbsil C60A (40-60 μ). The numbering system used for the drawn structures in the experimental section is to aid NMR interpretation and not to IUPAC nomenclature.

2-[4-(carboxymethyl)-5-oxo-1, 3-dioxolan-4-yl] acetic acid (1)



Citric acid (30 g, 156 mmol) and paraformaldehyde (4.68 g, 156 mmol) were mixed and transferred to a pressure tube which was subsequently sealed. The contents were heated at 145 °C for 4h. The tube was cautiously opened at low temperature and water (100 ml) was added. Sonication led to the precipitation of a white solid which was filtered and dried *in vacuo*. The filtrate was evaporated to dryness and the solid obtained was recrystallised from water to yield more product. Both solids were proved pure by NMR. Total yield **1** (16.0 g, 50%). Elemental analysis: (Found: C, 41.37; H, 4.10. $C_7H_8O_7$ requires C, 41.19; H, 3.95%); IR: ν_{max} /cm $^{-1}$: 2939 m (OH), 1805 s (C=O), 1725 s (C=O), 1440 m (OH bend), 1256 s, 1207 s, 1173 s; δ_H(300 MHz; CD_3OD) 5.53 (2 H, s, $OC\underline{H}_2O$), 2.92 (2 H, d, ^2J 17.05, $C\underline{H}_2COOH$), 2.86 (2 H, d, ^2J 17.05, $C\underline{H}_2COOH$); δ_C(75 MHz; CD_3OD) C: 174.61 (C=O), 171.27 (C=O), 76.30; CH_2: 95.32 ($O\underline{C}H_2O$), 41.03; CI *m/z* 222 (M + NH_4^+, 100%), 178 (8), 148 (22), 120 (21), 52 (7). $C_7H_{12}N_1O_7$ requires 222.06137; found 222.06140

1, 3, 8-trioxaspiro[4.5]decane-4, 7, 9-trione (2)



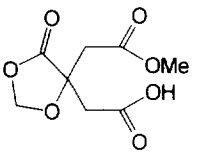
Freshly distilled acetic anhydride (10 ml, 106 mmol) and **1** (4.50 g, 22.1 mmol) were heated at reflux for 2 h. Evaporation of the volatiles left a cream coloured solid **2** (4.10 g, 100%). Elemental analysis: (Found: C, 45.23; H, 3.22. $C_7H_6O_6$ requires C, 45.17; H, 3.25%); IR: ν_{max} /cm $^{-1}$: 2942 w (CH), 1816 s (C=O), 1789 s (C=O), 1229 m, 1155 s, 1060 s; δ_H(300 MHz; d^6 DMSO) 5.65 (2 H, s, $OC\underline{H}_2O$), 3.45 (2 H, d, ^2J 16.5, $C\underline{H}_2COO$), 3.35 (2 H, d, ^2J 16.5, $C\underline{H}_2COO$); δ_C(75 MHz; d^6 DMSO) C: 171.35 (C=O), 165.07 (C=O), 74.10;

CH_2: 94.32 ($O\underline{C}H_2O$), 36.46; CI *m/z* 204 ($M + NH_4^+$, 22%), 74 (56), 69 (58), 46 (100). $C_7H_{10}N_1O_6$ requires 204.05080; found 204.05080

Monoalkyl citrates - general procedure

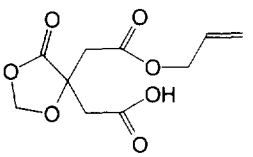
2 and freshly dried alcohol 50-200 equivalents were heated at 80-150 °C for approx. 5h. Evaporation of the excess alcohol gave the desired products in quantitative yield which required no further purification's unless indicated.

2-{4-[(methloxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl} acetic acid (4)



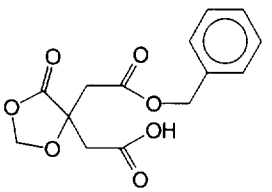
IR: ν_{max} /cm $^{-1}$: 2930 m (OH), 1802 s (C=O), 1736 s (C=O), 1708 s (C=O), 1439 m, 1196 s, 1173 s; δ_H(300 MHz; d^6 DMSO) 5.46 (2 H, s, $OC\underline{H}_2O$), 3.65 (3 H, s, Me), 2.98 (2 H, s, $C\underline{H}_2COOH$), 2.89 (2 H, s, $C\underline{H}_2COOMe$); δ_C(75 MHz; d^6 DMSO) C: 171.90 (C=O), 169.38 (C=O), 168.25 (C=O), 74.49; CH_2: 93.32 ($O\underline{C}H_2O$), 39.54, 39.26; CH_3: 50.74; CI *m/z* 236 ($M + NH_4^+$, 25%), 134 (100). $C_8H_{14}N_1O_7$ requires 236.07702; found 236.07700

2-{4-[(allyloxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl} acetic acid (5)



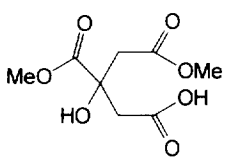
IR: ν_{max} /cm $^{-1}$: 2986 m (OH), 1798 s (C=O), 1738 s (C=O), 1718 s (C=O), 1181 s; δ_H(300 MHz; d^6 DMSO) 5.89 (1 H, m, 8 lines, $\underline{H}C=CH_2$), 5.45 (2 H, 2 × d, ^2J 4.47, $OC\underline{H}_2O$), 5.19-5.38 (2 H, 2 × dd, 4 lines, $HC=C\underline{H}_2$), 4.58 (2 H, d, J 5.28, $C\underline{H}_2HC=CH_2$), 3.02 (2 H, s, CH_2COOR), 2.91 (2 H, s, CH_2COOH); δ_C(75 MHz; d^6 DMSO) C: 173.79, 171.28, 169.36 (C=O), 76.44; CH: 132.98 ($H\underline{C}=CH_2$); CH_2: 119.01 ($HC=\underline{C}H_2$), 95.25 ($O\underline{C}H_2O$), 65.85 ($\underline{C}H_2HC=CH_2$), 41.49, 41.33; CI *m/z* 262 ($M + NH_4^+$, 100%), 232 (30), 188 (14), 160 (55). $C_{10}H_{16}N_1O_7$ requires 262.09266; found 262.09270

2-{4-[(benzyloxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl} acetic acid (6)



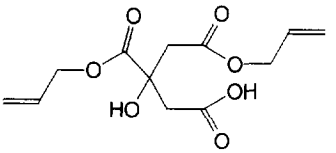
2 (500 mg, 2.67 mmol) and benzyl alcohol (14 ml, 135 mmol) were heated at 150 °C for 2 days. The excess benzyl alcohol was removed *in vacuo* to yield a crude oil which was taken up into near saturated sodium hydrogen carbonate solution (100 ml). The aqueous solution was extracted with chloroform (3 × 25 ml) and carefully acidified with hydrochloric acid. The acidified solution was extracted with ethyl acetate (4 × 25 ml). After drying over magnesium sulphate, filtering and evaporating to dryness a white solid **6** was obtained which was shown to be pure by NMR (419 mg, 53%). IR: ν_{max} /cm $^{-1}$: 2990 m (OH), 1799 s (C=O), 1737 s (C=O), 1705 s (C=O), 1168 s; δ_H(300 MHz; $CDCl_3$) 7.38 (5 H, s, ArH), 5.43 (2 H, d, ^2J 16.42, PhC$\underline{H}_2$), 5.13 (2 H, s, OC$\underline{H}_2$O), 3.05 (2 H, s, C$\underline{H}_2$COOBn), 2.92 (2 H, s, C$\underline{H}_2$COOH); δ_C(75 MHz; $CDCl_3$) C: 175.44 (C=O), 172.94 (C=O), 171.25 (C=O), 138.09 (ArC), 78.11; CH: 130.69; CH_2: 96.87 (O$\underline{C}H_2$O), 68.60 (Ph$\underline{C}H_2$), 43.14, 43.04; CI *m/z* 312 (M + NH_4^+, 100%), 282 (39), 210 (88), 108 (65). $C_{14}H_{18}N_1O_7$ requires 312.10831; found 312.10830

3-hydroxy-3, 4-di(methoxycarbonyl)butyric acid (7)



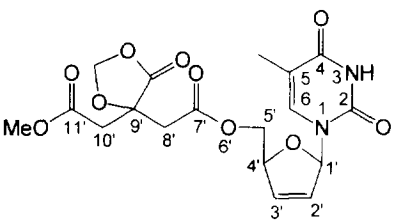
4 (218 mg, 1 mmol) was dissolved in dry methanol (20 ml). Freshly distilled triethylamine (202 mg, 2 mmol) was added and the solution heated at 90 °C for 5 h. After allowing to cool to RT, the black solution was evaporated to dryness. The excess triethylamine was neutralised with 2 M HCl and water was added. The aqueous solution was extracted with ethyl acetate (4 × 15 ml). The organic layer was dried over magnesium sulphate, filtered and evaporated *in vacuo* to yield a brown solid **7** (119 mg, 54%). IR: ν_{max} /cm $^{-1}$: 3398 m (OH), 3022 m (OH), 1740 s (C=O); δ_H(300 MHz; CD_3OD) 3.77 (3 H, s, CH_3), 3.67 (3 H, s, CH_3), 2.95 (2 H, d, C$\underline{H}_2$COOH), 2.80 (2 H, 2 × d, C$\underline{H}_2$COOMe); δ_C(75 MHz; CD_3OD) C: 174.33 (C=O), 172.21 (C=O), 170.77 (C=O), 73.61; CH_2: 43.31, 43.15; CH_3: 52.17, 51.30; CI *m/z* 238 (M + NH_4^+, 100%), 162 (32). $C_8H_{16}N_1O_7$ requires 238.09266; found 238.09270

3, 4-di(allyloxycarbonyl)- 3-hydroxybutyric acid (8)



5 (465 mg, 2.50 mmol) was dissolved in allyl alcohol (25 ml). Freshly distilled triethylamine (253 mg, 2.5 mmol) was added and the solution heated at 100 °C for 5h. The solution was reduced *in vacuo* and co-evaporated from acetone. Water (50 ml) was added and the solution adjusted to pH 2 with 2 M HCl solution. The aqueous solution was extracted with ethyl acetate (3 × 40 ml), dried over magnesium sulphate, filtered and evaporated *in vacuo* to yield a viscous oil **8** (417 mg, 61%). IR: ν_{max} /cm $^{-1}$: 3398 m (OH), 3020 s (OH), 1737 s (C=O), 1442 w, 988 w, 937 w; δ_H(300 MHz; d^6 acetone) 5.97 (2 H, m, 10 lines, $\underline{H}C=CH_2$), 5.38-5.19 (4 H, m, $HC=C\underline{H_2}$), 4.66 (2 H, d, J 5.71, $C\underline{H_2}HC=CH_2$), 4.58 (2 H, d, J 5.58, $C\underline{H_2}HC=CH_2$), 3.32 (4 H, m, $C\underline{H_2}COO$); δ_C(75 MHz; d^6 acetone) 173.06, 171.14, 169.48 (C=O), 73.64; CH: 132.85, 132.69 ($H\underline{C}=CH_2$); CH_2: 117.73, 117.66 ($HC=\underline{C}H_2$), 66.20, 65.19 ($\underline{C}H_2HC=CH_2$), 43.33, 42.82 ($\underline{C}H_2COO$); CI *m/z* 290 (M + NH_4^+, 18%), 160 (19), 102 (100). $C_{12}H_{20}N_1O_7$ requires 290.12396; found 290.12400

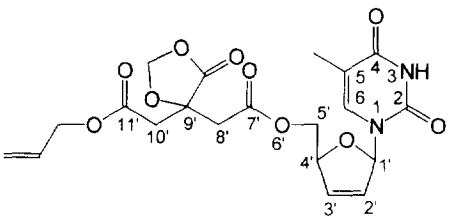
methyl 2-{4-[({[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}oxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl}acetate (9)



4 (146 mg, 0.67 mmol), d4T (50 mg, 0.223 mmol) and DMAP (22 mg, 0.179 mmol) were dissolved in dry THF (2 ml). The solution was cooled to 0 °C and DCC (51 mg, 0.245 mmol) in THF (0.5 ml) was added dropwise. The solution was allowed to warm to room temperature and stirred overnight. The suspension was filtered, evaporated *in vacuo* and subjected to flash chromatography eluting with 75% EtOAc/CH_2Cl_2 to yield a white solid **9** as a 1:1 mixture of diastereoisomers (68 mg, 72 %). Elemental analysis: (Found: C, 50.77; H, 4.90; N, 6.47. $C_{18}H_{20}N_2O_{10}$ requires C, 50.95; H, 4.75; N, 6.60%); IR: ν_{max} /cm $^{-1}$: 1804 m, 1738 s (C=O), 1697 s (C=O), 1682 (C=O), 1178 s, 1080 m; δ_H(300 MHz; $CDCl_3$) 8.65 (1 H, s, NH), 7.17 (1 H, 2 × s, H6'), 7.05 (1 H, m, H1'), 6.33 (1 H, m, H3'), 5.97 (1 H, m, H2'), 5.62 (2 H, s, $OC\underline{H_2}O$), 5.08 (1 H, br m, H4'), 4.29-4.45 (2 H, m, H5'), 2.9-3.0 (4 H, m, H8', H10'), 1.99 (3 H, 2 × d, J 1.1, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 171.94, 171.81 (C=O), 168.00, 167.95 (C=O), 167.53, 167.36 (C=O), 162.61 (C4), 149.83, 149.78 (C2),

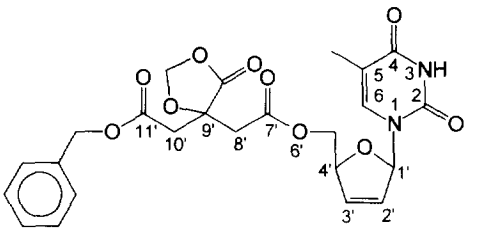
110.41, 110.34 (C5), 74.59, 74.52 (C9'); CH: 134.54, 134.38 (C6), 132.30, 132.25 (C3'), 126.62, 126.59 (C2'), 89.20, 89.12 (C1'), 82.98, 82.90 (C4'); CH_2: 94.32, 94.22 (O<u>C</u>H_2O), 64.84, 64.75 (C5'), 40.28, 40.24, 40.11, 40.06 (C8', C10'); CH_3: 51.54 (CH_3), 11.86 (5CH_3); FAB *m/z* 447 (M + Na^+, 86%), 425 (16), 225 (32), 149 (33), 127 (100). $C_{18}H_{20}N_2O_{10}Na_1$ requires 447.10156; found 447.10034

[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl 2-{4-[(allyloxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl}acetate (10)



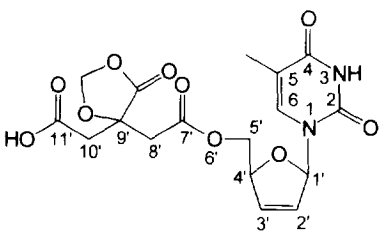
5 (81 mg, 0.350 mmol), d4T (50 mg, 0.223 mmol) and DMAP (22 mg, 0.179 mmol) were dissolved in dry acetonitrile (2 ml) and the solution cooled to 0 °C. DCC (55 mg, 0.268 mmol) in dry acetonitrile (0.5 ml) was added dropwise. The solution was allowed to warm to room temperature and stirred for a further 4 h. The solvent was removed *in vacuo* to leave a crude brown solid. Purification by flash chromatography with an elution gradient, CH_2Cl_2 to 35% EtOAc/CH_2Cl_2 gave a clear viscous oil **10** (40 mg, 40%). IR: ν_{max} /cm $^{-1}$: 1801 m, 1743 s (C=O), 1691 s (C=O), 1184 s, 1083 m; δ_H(300 MHz: $CDCl_3$) 9.25 (1 H, br s, NH), 7.17 (1 H, 2 × s, H6'), 7.03 (1 H, m, H1'), 6.32 (1 H, m, H3'), 5.87-6.00 (2 H, m, H2', <u>H</u>C=CH_2), 5.60 (2 H, m, OC<u>H</u>$_2$O), 5.29-5.39 (2 H, m, HC=C<u>H</u>$_2$), 5.07 (1H, br m, H4'), 4.65 (2 H, d, J 5.86, C<u>H</u>$_2$HC=CH_2), 4.31-4.47 (2 H, m, H5'), 2.92-3.01 (4 H, m, H8', H10'), 1.97 (3 H, 2 × s, 5CH_3); δ_C(75 MHz; $CDCl_3$) C: 173.16, 173.02 (C=O), 168.77, 168.61 (C=O), 168.42 (C=O), 151.23 (C4), 151.23 (C2), 111.64, 111.58 (C5), 75.84, 75.78 (C9'); CH: 135.79, 135.64 (C6), 133.42 (H<u>C</u>=CH_2), 131.68 (C3'), 127.87 (C2'), 90.41, 90.33 (C1'), 84.20, 84.11 (C4'); CH_2: 119.63 (HC=<u>C</u>H_2), 95.54, 95.45 (O<u>C</u>H_2O), 66.47 (<u>C</u>H_2HC=CH_2), 66.07, 66.00 (C5'), 41.65, 41.53, 41.48, 41.38 (C8', C10'); CH_3: 13.07 (5CH_3); FAB *m/z* 473 (M + Na^+, 75%), 451 (47), 127 (100). $C_{20}H_{22}N_2O_{10}Na_1$ requires 473.11721; found 473.11592

[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl 2-{4-[(benzyloxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl}acetate (11)



6 (67 mg, 0.229 mmol), d4T (51 mg, 0.229 mmol) and DMAP (28 mg, 0.229 mmol) were dissolved in dry acetonitrile (10 ml). DCC (66 mg, 0.321 mmol) in dry acetonitrile (2 ml) was added and the solution was stirred at RT overnight. After removing the volatiles *in vacuo* the crude solid was subjected to flash chromatography eluting with 40% EtOAc/ CH_2Cl_2. The product **11** was isolated as an oil as a 1:1 mixture of diastereoisomers (84 mg, 74 %). IR: ν_{max} /cm $^{-1}$: 1799 m, 1738 s (C=O), 1692 s (C=O), 1182 s, 1083 m; δ_H(300 MHz; $CDCl_3$) 9.22 (1 H, br s, NH), 7.39 (5 H, m, ArH), 7.16 (1 H, m, H6), 7.03 (1 H, m, H1'), 6.31 (1 H, m, H3'), 5.96 (1 H, m, H2'), 5.53 (2 H, 2 × s, $OC\underline{H}_2O$), 5.19 (2 H, 2 × s, $PhC\underline{H}_2$), 5.07 (1H, br m, H4'), 4.35 (2 H, m, H5'), 2.89-3.04 (4 H, m, H8', H10'), 1.96 (3 H, 2 × s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 173.49, 173.35 (C=O), 169.08, 168.95 (C=O), 168.98 (C=O), 164.49, 164.46 (C4), 151.58, 151.54 (C2), 136.10 (ArC), 111.97, 111.92 (C5), 76.19, 76.12 (C9'); CH: 135.95, 135.69 (C6), 133.81, 133.75 (C3'), 129.42, 129.38, 128.27, (Ar$\underline{C}$H), 128.19, 128.16 (C2'), 90.72, 90.64 (C1'), 84.51, 84.43 (C4'); CH_2: 95.85, 95.76 ($O\underline{C}H_2O$), 67.99, 67.98 ($Ph\underline{C}H_2$), 66.39, 66.32 (C5'), 42.08, 41.90, 41.71 (C8', C10'); CH_3: 13.42 ($5CH_3$); FAB *m/z* 501 (M + H^+, 19%), 225 (43). $C_{24}H_{25}N_2O_{10}$ requires 501.15092; found 501.15086

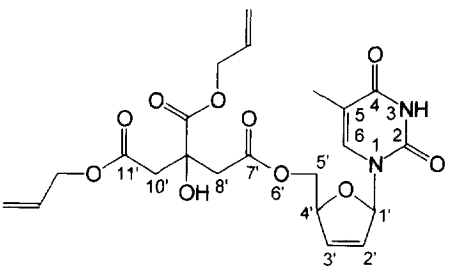
2-{4-[({[5-(5-methyl-2,4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}oxycarbonyl)methyl]-5-oxo-1, 3-dioxolan-4-yl}acetate (12)



10 (35 mg, 0.078 mmol) and *tris*triphenylphosphine rhodium chloride (10 mg, 0.038 mmol) were added to 90% EtOH (3 ml) and heated at 70 °C for 2.5h. Evaporation of the volatiles left a crude red solid. Purification by flash chromatography, eluting with a gradient $CHCl_3$ to 3%MeOH/$CHCl_3$ gave a colourless oil **12**. IR: ν_{max} /cm $^{-1}$: 3407 m (OH), 1797 m, 1700 s (C=O), 1185 s, 1082 m; δ_H(300 MHz; d^4 MeOH) 7.36 (1 H,

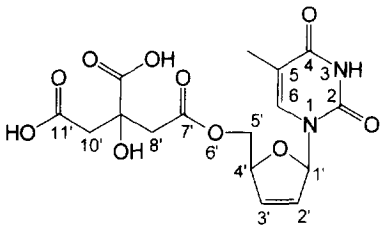
2 × s, H6), 6.88-6.91 (1 H, m, H1'), 6.37-6.41 (1 H, m, H3'), 5.97-6.00 (1 H, m, H2'), 5.50 (2 H, m, OC$\underline{H}_2$O), 5.03 (1 H, br m, H4'), 4.21-4.43 (2 H, m, H5'), 2.87-3.02 (4 H, m, H8', H10'), 1.90 (3 H, s, 5CH_3); δ_C(75 MHz; d^4 MeOH) C: 174.07, 174.00 (C=O), 171.01 (C=O), 169.25, 169.11 (C=O), 165.39 (C4), 151.76 (C2), 110.71 (C5), 76.14 (C9'); CH: 136.79, 136.70 (C6), 133.71 (C3'), 126.79, 126.76 (C2'), 90.40 (C1'), 84.49, 84.35 (C4'); CH_2: 95.27 (O$\underline{C}H_2$O), 65.50, 65.44 (C5'), 41.13, 41.03, 40.90, 40.82 (C8', C10'); CH_3: 11.56 (5CH_3); FAB *m/z* 411 (M + H^+, 70%). $C_{17}H_{19}N_2O_{10}$ requires 411.10397; found 411.10398

1, 2-diallyl 3-{[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl} 2-hydroxy-1, 2, 3-propanetricarboxylate (13)



8 (50 mg, 0.183 mmol), d4T (41 mg, 0.183 mmol) and DMAP (22 mg, 0.183 mmol) were stirred in dry THF (3 ml). DCC (49 mg, 0.239 mmol) in dry THF (2 ml) was added to the above solution and stirred overnight. The suspension was filtered and the filtrate was evaporated to dryness. The crude solid was purified by flash chromatography eluting with a gradient 25% ethyl acetate/dichloromethane to 65% ethyl acetate/dichloromethane to yield a solid **13** (26 mg, 30%). IR: ν_{max} /cm $^{-1}$: 1806 m, 1740 s (C=O), 1690 s (C=O), 1183 s, 1079 m; δ_H(300 MHz; $CDCl_3$) 8.62 (1 H, br s, NH), 7.24 (1 H, 2 × s, H6'), 7.04 (1 H, m, H1'), 6.33 (1 H, m, H3'), 5.87-6.00 (3 H, m, H2', $\underline{H}C{=}CH_2$), 5.43-5.28 (4 H, m, HC=C$\underline{H}_2$), 5.08 (1H, br m, H4'), 4.76 (2 H, m, C$\underline{H}_2$HC=CH_2), 4.63 (2 H, m, C$\underline{H}_2$HC=CH_2), 4.52 (1 H, m, H5'), 4.24 (1 H, m, H5'), 3.02-2.83 (4 H, m, H8', H10'), 1.98 (3 H, 2 × s, 5CH_3); δ_C(75 MHz; $CDCl_3$) C: 172.76 (C=O), 169.33, 169.24 (C=O), 169.13, 169.00 (C=O), 163.35 (C4), 150.54, 150.49 (C2), 111.10, 111.01 (C5), 73.11, 73.08 (C9'); CH: 135.45, 135.23 (C6), 133.28 (H$\underline{C}$=CH_2), 133.09 (H$\underline{C}$=CH_2), 131.36, 131.12 (C3'), 127.14, 127.04 (C2'), 89.73, 89.61 (C1'), 83.82, 83.77 (C4'); CH_2: 119.63 (HC=$\underline{C}H_2$), 118.75 (HC=$\underline{C}H_2$), 67.09, 66.96 ($\underline{C}H_2$HC=CH_2), 65.64 ($\underline{C}H_2$HC=CH_2), 65.08, 64.97 (C5'), 43.18, 43.04, 42.85, 42.70 (C8', C10'); CH_3: 12.47, 12.44 (5CH_3); FAB *m/z* 501 (M + Na^+, 100%), 479 (49). $C_{22}H_{26}N_2O_{10}Na_1$ requires 501.14851; found 501.14852

2-hydroxy-2-[({[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl} oxycarbonyl) methyl]succinic acid (3)



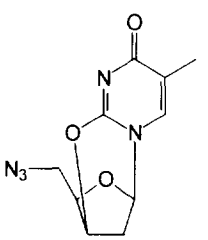
13 (150 mg, 0.314 mmol) and Wilkinson's catalyst (30 mg, 0.0314 mmol) were dissolved in 90% ethanol and heated at 70 °C for 6 h. The solution was evaporated to dryness and the crude red solid was purified by flash chromatography eluting with 7% methanol/chloroform. A pure solid **3** was recovered (39 mg, 32%). IR: ν_{max} /cm $^{-1}$: 3406 m (OH), 1700 s (C=O), 1178 s, 1084 m; δ_H(300 MHz; CD_3OD) 7.35 (1 H, 2 × s, H6), 6.88 (1 H, m, H1'), 6.34 (1 H, m, H3'), 5.94 (1 H, m, H2'), 5.03 (1 H, br m, H4'), 4.11-4.43 (2 H, m, H5'), 2.70-2.95 (4 H, m, H8', H10'), 1.90 (3 H, s, $5CH_3$); δ_C(75 MHz; CD_3OD) C: 175.40 (C=O), 172.28, (C=O), 170.05 (C=O), 165.44 (C4), 151.79 (C2), 110.78 (C5), 73.21, 73.17 (C9'); CH: 136.86 (C6), 134.03 (C3'), 126.63 (C2'), 90.24 (C1'), 84.59, 84.53 (C4'); CH_2: 65.04 (C5'), 42.93, 42.86, 42.79 (C8', C10'); CH_3: 11.60 ($5CH_3$); FAB *m/z* 421 (M + Na^+, 51%), 399 (49). $C_{22}H_{26}N_2O_{10}Na_1$ requires 501.14851; found 501.14852

Hydrazoic acid solution.

Water (5 ml) and sodium azide (7.0 g, 108 mmol) were stirred to form a slurry. Toluene (50 ml) was added and the biphasic mixture was cooled to 0 °C. Sulphuric acid was added cautiously whilst keeping the contents of the flask below 10 °C. The suspension was stirred for 1.5h. The toluene layer was decanted and the salts were washed with toluene (2 × 40 ml). The combined organic washings were dried over sodium sulphate and filtered to give a hydrazoic acid solution 0.59 M (titrated against a standard sodium hydroxide solution 0.1 M)

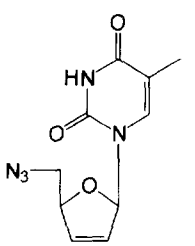
10-(azidomethyl)-4-methyl-8, 11-dioxa-2, 6-diazatricyclo[7.2.1.0^{2,7}]dodec-3-en-5-one

2, 3'-anhydro-5'-azidothymidine (14)[30]



Hydrazoic acid solution (103 ml, 54.5 mmol) was added under nitrogen to a pre-cooled stirred solution of thymidine (12.0 g, 49.5 mmol) and triphenylphosphine (14.29 g, 54.5 mmol) in a mixture of dry DMF (140 ml) and dry toluene (84 ml). DIAD (12.02 g, 59.4 mmol) was then added dropwise, *via* a syringe over 5 minutes. The colourless reaction mixture was warmed to room temperature and stirred for a further 1 h before cooling to 0 °C. A second aliquot of pre-mixed DIAD (12.03 g, 59.5 mmol) and triphenylphosphine (15.6 g, 59.5 mmol) in dry DMF (30 ml) were added dropwise over 5 minutes and the solution was stirred for 2 days. The white precipitate formed was filtered, washed with toluene and dried *in vacuo* to give a pure white solid which was pure by NMR (5.0 g). The crude orange filtrate was concentrated *in vacuo* to give an orange oil. The oil was taken up into chloroform and eluted through a silica gel sinter with 1% methanol/chloroform to 10% methanol/chloroform. Removal of the solvent left a solid which was recrystallised with ethanol yielding more product **14** (3.13 g, total yield 66%). Elemental analysis: (Found: C, 48.19; H, 4.42. $C_{10}H_{11}N_5O_3$ requires C, 48.19; H, 4.45%); δ_H(300 MHz; d^6 DMSO) 7.83 (1 H, s, H6), 6.12 (1 H, m, H1'), 5.50 (1 H, br s, H3'), 4.40 (1 H, m, H4'), 3.70 (2 H, m, H5'), 2.51 (1 H, m, H2'), 1.97 (3 H, s, $5CH_3$); δ_C(75 MHz; d^6 DMSO) C: 171.78 (C2), 154.12 (C4), 116.93 (C5); CH: 137.53 (C6), 87.74 (C4'), 84.06 (C1'), 78.05 (C3'); CH_2: 51.00 (C5'), 33.54 (C2'); CH_3: 13.86 ($5CH_3$)

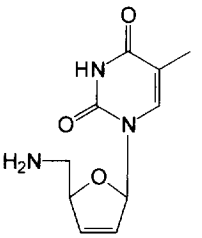
1-[5-(azidomethyl)-2, 5-dihydro-2-furanyl]-5-methyl-1, 2, 3, 4-tetrahydro-2, 4-pyrimidinedione
5'-azido-3'-deoxy-2', 3'-didehydrothymidine (15)[30]



14 (5.0 g, 20.1 mmol) was dissolved in dry DMA (100 ml). Sodium hydride (962.5 mg, 24.1 mmol) was added cautiously and the suspension was stirred at RT for 10 min. After heating the reaction at 80 °C for 1h a dark brown solution developed. The basic solution was neutralised cautiously with hydrochloric acid and the solvent was removed *in vacuo* to give a crude solid. Water (250 ml) and chloroform (125 ml) were added and the biphasic solution was stirred for 5 minutes. The chloroform layer was collected and the aqueous layer was extracted with more chloroform (4 × 50 ml). The combined organic fractions were dried over magnesium sulphate, filtered and evaporated to dryness. The impure solid was dissolved in methanol, adsorbed onto silica gel and purified by flash chromatography eluting with 17% ethyl acetate/diethyl ether to 35% ethyl acetate/diethyl ether to yield a white solid **15** (4.55 g, 91%). δ_H(300 MHz; d^6 $CDCl_3$) 9.26 (1 H, br s, NH), 7.36 (1 H, 2 × s, H6), 7.00 (1 H, m, H1'), 6.26 (1 H, d *pseudo* t, 6 lines, H2'), 5.86 (1 H, m, 4 lines,

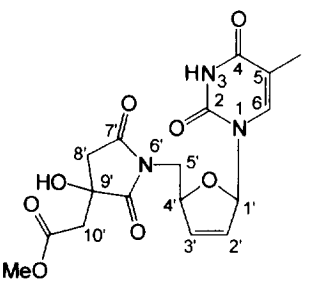
H3'), 4.92 (1 H, m, H4'), 3.60 (2 H, m, H5'), 1.89 (3 H, s, $5CH_3$); δ_C(75 MHz; d^6 $CDCl_3$) C: 164.35 (C2), 151.50 (C4), 111.77 (C5); CH: 136.14 (C6), 134.62 (C3'), 127.67 (C2'), 90.14 (C4'), 84.73 (C1'); CH_2: 54.25 (C5'); CH_3: 12.92 ($5CH_3$)

1-[5-(aminomethyl)-2, 5-dihydro-2-furanyl]-5-methyl-1, 2, 3, 4-tetrahydro-2, 4-pyrimidinedione 5'-amino-3'-deoxy-2', 3'-didehydrothymidine (16)[29]



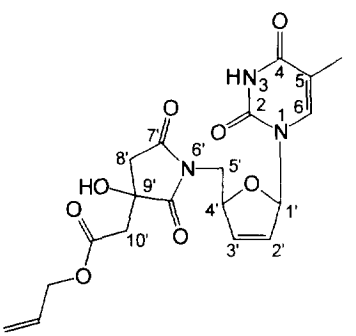
Dry triphenylphosphine (5.05 g, 19.3 mmol) was slowly added to a vented solution of **15** (3.0 g, 12.0 mmol) in pyridine (56 ml) and the resulting solution was stirred at RT for 2 h. Concentrated ammonium hydroxide solution (56 ml) was added and the solution stirred for a further 3h. The reaction vessel was stored in the freezer overnight. Precipitated solid (triphenylphosphine oxide) was filtered and discarded. The solution was evaporated to dryness and purified by flash chromatography eluting with 40% methanol/chloroform to give a white solid **16** (2.42 g, 90%). (Found: C, 53.91; H, 6.01. $C_{10}H_{13}N_3O_3$ requires C, 53.81; H, 5.87%); IR: ν_{max} /cm $^{-1}$: 1694 s (C=O), 1681 s (C=O), 1257 m, 1069 m; δ_H(300 MHz; $CDCl_3$) 9.26 (1 H, br s, NH), 7.42 (1 H, 2 × s, H6), 7.05 (1 H, m, H1'), 6.36 (1 H, d *pseudo* t, 6 lines, H2'), 5.87 (1 H, m, 7 lines, H3'), 4.90 (1 H, m, H4'), 3.74 (2 H, br s, NH_2), 3.00 (2 H, m, H5'), 1.93 (3 H, s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 164.91 (C2), 151.93 (C4), 111.94 (C5); CH: 136.69 (C6), 136.13 (C3'), 127.94 (C2'), 90.42 (C4'), 88.44 (C1'); CH_2: 46.24 (C5'); CH_3: 13.24 ($5CH_3$)

methyl 2-(3-hydroxy-1-{[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}-2, 5-dioxotetrahydro-1*H*-3-pyrrolyl)acetate (17)



4 (50 mg, 0.229 mmol), **16** (51 mg, 0.229 mmol) and DMAP (28 mg, 0.229 mmol) were added to dry acetonitrile (10 ml) to afford a suspension. DCC (66 mg, 0.321 mmol) in acetonitrile (2 ml) was added dropwise to the suspension and left to stir overnight. After filtration, the solution was evaporated to dryness to give a crude solid. Purification by flash chromatography eluting with 4% methanol/chloroform gave a pure solid **17** (56 mg, 62%). IR: ν_{max} /cm $^{-1}$: 3388 m (OH), 1713 s (C=O), 1076 m; δ_H(300 MHz; $CDCl_3$) 9.32 (1 H, br s, NH), 7.26 (1 H, 2 × s, H6), 6.87 (1 H, m, H1'), 6.39 (1 H, m, H3'), 5.96 (1 H, m, H2'), 5.12 (1H, br m, H4'), 4.85 (1 H, br s, OH), 3.83 (2 H, m, H5'), 3.73 (3 H, 2 × s, Me), 2.85-3.08 (4 H, m, H8', H10'), 1.99 (3 H, 2 × s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 179.07, 178.84 (C=O), 174.73, 174.54 (C=O), 171.05, 170.98 (C=O), 164.57, 164.51 (C4), 151.23 (C2), 111.84 (C5), 72.83 (C9'); CH: 136.40, (C6), 134.44, 134.10 (C3'), 127.29 (C2'), 91.35, 90.92 (C1'), 83.45, 83.14 (C4'); CH_2: 42.75, 42.62, 42.37, (C8', C10'), 41.24, 41.07 (C5'); CH_3: 12.79 ($5CH_3$); FAB *m/z* 394 (M + H^+, 12%). $C_{17}H_{20}N_3O_8$ requires 394.12504; found 394.12501

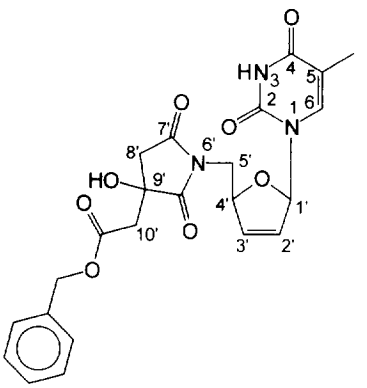
allyl 2-(3-hydroxy-1-{[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}-2, 5-dioxotetrahydro-1*H*-3-pyrrolyl)acetate (18)



5 (100 mg, 0.410 mmol), **16** (50 mg, 0.224 mmol) and DMAP (27 mg, 0.224 mmol) were added to dry acetonitrile (10 ml) to afford a suspension. DCC (65 mg, 0.314 mmol) in acetonitrile (2 ml) was added dropwise to the suspension and left to stir for 3h. After filtration, the solution was evaporated to dryness to give a crude solid. Purification by flash chromatography eluting with 4% methanol/chloroform gave a pure solid **18** (43 mg, 46%). IR: ν_{max} /cm $^{-1}$: 3387 m (OH), 1725 s (C=O), 1714 s (C=O), 1694 s (C=O), 1076 m; δ_H(300 MHz; $CDCl_3$) 9.38 (1 H, br s, NH), 7.24 (1 H, m, H6), 6.86 (1 H, m, H1'), 6.38 (1 H, m, H3'), 5.95 (1 H, m, H2'), 5.87 (1 H, m, $\underline{H}C=CH_2$), 5.33 (2 H, m, $HC=C\underline{H}_2$), 5.11 (1H, br m, H4'), 4.95 (1 H, br s, OH), 4.62 (2 H, d, J 5.83, $C\underline{H}_2HC=CH_2$), 3.83 (2 H, m, H5'), 2.66-3.11 (4 H, m, H8', H10'), 1.97 (3 H, 2 × s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 179.12, 178.83 (C=O), 174.78, 174.58 (C=O), 170.24, 170.16 (C=O), 164.58, 164.53 (C4), 151.23 (C2), 111.85, 111.82 (C5), 72.82, 75.78 (C9'); CH: 136.38, (C6), 134.47, 134.12 ($H\underline{C}=CH_2$), 131.66 (C3'), 127.26 (C2'), 91.29, 90.87 (C1'), 83.44, 83.17 (C4'); CH_2: 119.66, 119.62 ($HC=\underline{C}H_2$), 66.40,

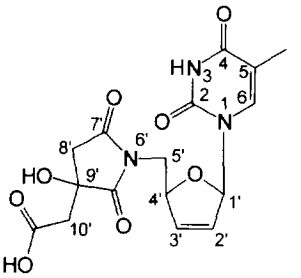
66.35 ($\underline{C}H_2HC=CH_2$), 42.73, 42.58, 42.37, (C8', C10'), 41.41, 41.24 (C5'); CH_3: 12.79, 12.76 ($5CH_3$); FAB *m/z* 420 (M + H^+, 20%). $C_{19}H_{22}N_3O_8$ requires 420.14069; found 420.14073

benzyl 2-(3-hydroxy-1-{[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}-2, 5-dioxotetrahydro-1*H*-3-pyrrolyl)acetate (19)



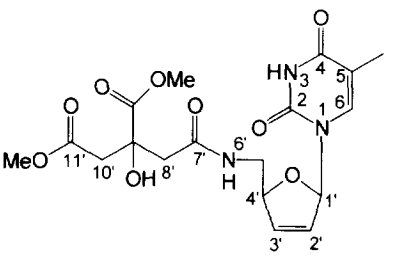
6 (67 mg, 0.229 mmol), **16** (51 mg, 0.229 mmol) and DMAP (28 mg, 0.229 mmol) were added to dry acetonitrile (10 ml) to afford a suspension. DCC (66 mg, 0.321 mmol) in acetonitrile (2 ml) was added dropwise to the suspension and left to stir overnight. After filtration, the solution was evaporated to dryness to give a crude solid. Purification by flash chromatography eluting with 4% methanol/chloroform gave a pure solid **19** (91 mg, 85%). IR: ν_{max} /cm $^{-1}$: 3390 m (OH), 1714 s (C=O), 1076 m; δ_H(300 MHz; $CDCl_3$) 9.26 (1 H, br s, NH), 7.39 (5 H, m, ArH), 7.24 (1 H, 2 × s, H6), 6.85 (1 H, m, H1'), 6.35 (1 H, m, H3'), 5.93 (1 H, m, H2'), 5.15 (2 H, 2 × s, $PhC\underline{H}_2$), 5.09 (1H, br m, H4'), 4.82 (1 H, br s, OH), 3.76 (2 H, m, H5'), 2.84-3.18 (4 H, m, H8', H10'), 1.99 (3 H, 2 × s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 179.77, 179.50 (C=O), 175.41, 174.19 (C=O), 171.01, 170.94 (C=O), 165.24, 164.17 (C4), 151.90 (C2), 136.01 (ArC), 112.58, 112.55 (C5), 73.58, 73.53 (C9'); CH: 137.09, (C6), 135.13, 134.79 (C3'), 129.12, 129.07, 128.87, 128.83 (Ar$\underline{C}$H), 128.01, 127.96 (C2'), 92.04, 91.62 (C1'), 84.14, 83.89 (C4'); CH_2: 67.65, 67.60 ($Ph\underline{C}H_2$), 43.37, 43.21, 43.08 (C8', C10'), 42.24, 42.08 (C5'); CH_3: 13.52, 13.49 ($5CH_3$); FAB *m/z* 470 (M + H^+, 100%). $C_{23}H_{24}N_3O_8$ requires 470.15634; found 470.15634

2-(3-hydroxy-1-{[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}-2, 5-dioxotetrahydro-1*H*-3-pyrrolyl)acetic acid (20)



16 (50 mg, 0.224 mmol) and **5** (42 mg, 0.224 mmol) in dry acetonitrile (5 ml) were heated at reflux for 1 day. The solution was evaporated to dryness and purified by flash chromatography eluting with 15% methanol/dichloromethane. The solid obtained was triturated with isopropanol, filtered and evaporated *in vacuo* to yield a solid **20** (59 mg, 70%). IR: ν_{max} /cm $^{-1}$: 3252 m (OH), 1785 m (C=O), 1707 s (C=O), 1405 m, 1076 m; δ_H(300 MHz; d^6 acetone) 10.13 (1 H, br s, NH), 7.36 (1 H, 2 × s, H6), 6.87 (1 H, m, H1'), 6.43 (1 H, m, H3'), 6.01 (1 H, m, H2'), 5.49 (1H, br s, OH), 4.85 (1 H, m, H4'), 3.75 (2 H, m, H5'), 2.67-3.15 (4 H, m, H8', H10'), 1.87 (3 H, 2 × s, 5CH_3); δ_C(75 MHz; d^6 acetone) C: 178.70 (C=O), 174.82 (C=O), 171.75 (C=O), 164.57 (C4), 151.22 (C2), 110.72, 110.68 (C5), 72.56, 72.48 (C9'); CH: 136.16, (C6), 134.93, 134.88 (C3'), 126.73, 126.67 (C2'), 90.38 (C1'), 83.32, 82.95 (C4'); CH_2: 42.73, 42.42, 42.34, 42.25 (C8', C10'), 40.75, 40.66 (C5'); CH_3: 11.94, 11.91 (5CH_3); FAB *m/z* 380 (M + H$^+$, 14%). $C_{16}H_{18}N_3O_8$ requires 380.10939; found 380.10936

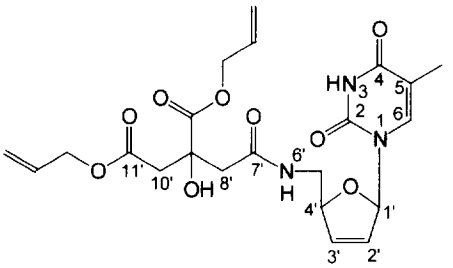
dimethyl 3-hydroxy-3-[({{[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}carbamoyl)methylpentanedioate (21)



7 (61 mg, 0.278 mmol) and **16** (62 mg, 0.278 mmol) were added to acetonitrile (10 ml) to afford a suspension. DCC (74 mg, 0.361 mmol) in acetonitrile (2 ml) was added dropwise. After leaving to stir overnight, the suspension was filtered and the filtrate was evaporated to dryness. The crude solid was purified by flash chromatography eluting with 4.5% methanol/chloroform to give the product as a solid **21** (73 mg, 62 %). IR: ν_{max} /cm $^{-1}$: 3336 m (OH), 3019 s (OH), 1690 s (C=O), 1439 m, 1077 m; δ_H(300 MHz; $CDCl_3$) 7.47 (1

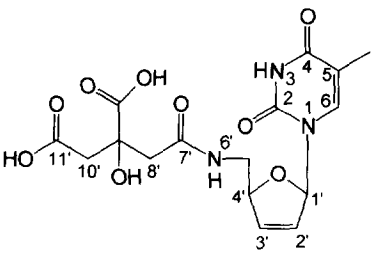
H, br t, NH), 7.19 (1 H, m, H6), 6.97 (1 H, m, H1'), 6.37 (1 H, m, H3'), 5.85 (1 H, m, H2'), 4.94 (1 H, m, H4'), 3.84 (3 H, s, CH_3), 3.71 (3 H, s, CH_3), 3.60 (2 H, m, H5'), 2.97-2.79 (4 H, m, H8', H10'), 1.91 (3 H, s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 174.61, 174.54 (C=O), 170.79, 170.70 (C=O), 170.30 (C=O), 164.58 (C4), 151.21 (C2), 111.63 (C5), 74.47, 72.28 (C9'); CH: 136.60, (C6), 135.67, 135.56 (C3'), 126.40 (C2'), 90.47 (C1'), 85.94 (C4'); CH_2: 44.27, 43.58, (C8', C10'), 43.01 (C5'); CH_3: 53.63, 53.52 (CH_3), 52.47 (CH_3), 12.68 ($5CH_3$); FAB *m/z* 426 (M + H^+, 52%). $C_{18}H_{24}N_3O_9$ requires 426.15125; found 426.15129

diallyl 3-hydroxy-3-[({[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}carbamoyl)methylpentanedioate (22)



8 (91 mg, 0.334 mmol) and DCC (90 mg, 0.435 mmol) were added to acetonitrile (10 ml) to afford a suspension. After 15 minutes, **16** (75 mg, 0.334 mmol) in acetonitrile (2 ml) was added dropwise. The suspension was stirred overnight, filtered and the filtrate was evaporated to dryness. The crude solid was purified by flash chromatography eluting with 7% methanol/chloroform to give the product as a sticky gum **22** (40 mg, 25 %). IR: ν_{max} /cm $^{-1}$: 3320 m (OH), 3019 s (OH), 1690 s (C=O), 1424 m, 1077 m; δ_H(300 MHz; $CDCl_3$) 9.30 (1 H, br s, NH), 7.41 (1 H, m, 4 lines, NH), 7.21 (1 H, m, H6), 6.98 (1 H, m, H1'), 6.38 (1 H, m, H3'), 5.97 (2 H, m, $\underline{H}C=CH_2$), 5.82 (1 H, m, H2'), 5.31 (4 H, m, $HC=C\underline{H}_2$), 4.95 (1 H, m, H4'), 4.74 (2 H, m, $C\underline{H}_2HC=CH_2$), 4.61 (2 H, m, $C\underline{H}_2HC=CH_2$), 3.58 (2 H, m, H5'), 3.03-2.80 (4 H, m, H8', H10'), 1.92 (3 H, s, $5CH_3$); δ_C(75 MHz; $CDCl_3$) C: 173.76, 173.71 (C=O), 170.57 (C=O), 170.03, 169.99 (C=O), 164.49 (C4), 151.15 (C2), 111.65, 111.59 (C5), 74.44, 734.26 (C9'); CH: 135.45, 136.67, 136.58 (C6), 135.71 ($H\underline{C}=CH_2$), 135.57 ($H\underline{C}=CH_2$), 132.03, 131.94 (C3'), 126.40 (C2'), 90.46 (C1'), 85.92 (C4'); CH_2: 119.37 ($HC=\underline{C}H_2$), 118.11 ($HC=\underline{C}H_2$), 67.31 ($\underline{C}H_2HC=CH_2$), 66.08 ($\underline{C}H_2HC=CH_2$), 45.51, 44.94, 44.28, 44.19 (C8', C10'), 43.64 (C5'); CH_3: 12.72, 12.69 ($5CH_3$); FAB *m/z* 478 (M + H^+, 20%). $C_{22}H_{28}N_3O_9$ requires 478.18255; found 478.18255

3-hydroxy-3-[({[5-(5-methyl-2, 4-dioxo-1, 2, 3, 4-tetrahydro-1-pyrimidinyl)-2, 5-dihydro-2-furanyl]methyl}carbamoyl)methylpentanedioic acid (23)



21 (51 mg, 0.120 mmol) and lithium hydroxide monohydrate (44 mg, 5 equiv.) in methanol/water 3:1 (14 ml) were stirred at RT for 3 h after which time no starting material could be detected by TLC (10% methanol/chloroform). The solution was adjusted to pH6 with hydrochloric acid. Removal of the volatiles *in vacuo* left a crude solid which was purified by flash chromatography eluting with chloroform/methanol/water 5:4:1. The deprotected isostere conjugate **23** was isolated (45 mg, 96%). δ_H(300 MHz; D_2O) 7.30 (1 H, m, H6), 6.71 (1 H, m, H1'), 6.32 (1 H, m, H3'), 5.85 (1 H, m, H2'), 4.90 (1 H, m, H4'), 3.32 (2 H, m, H5'), 2.75-2.30 (4 H, m, H8', H10'), 1.78 (3 H, s, $5CH_3$); δ_C(75 MHz; D_2O) C: 180.60 (C=O), 178.88 (C=O), 173.25 (C=O), 166.98 (C4), 152.66 (C2), 111.44 (C5), 75.36 (C9'); CH: 138.04, (C6), 135.76 (C3'), 124.93, 124.89 (C2'), 90.87 (C1'), 85.81, 85.75 (C4'); CH_2: 45.36, 45.12, (C8', C10'), 42.20, 42.15 (C5'); CH_3: 12.00 ($5CH_3$); ES -ve ion *m/z* 396 (M - H^+, 100%); HPLC retention time 3.85 minutes, 99.2%.

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