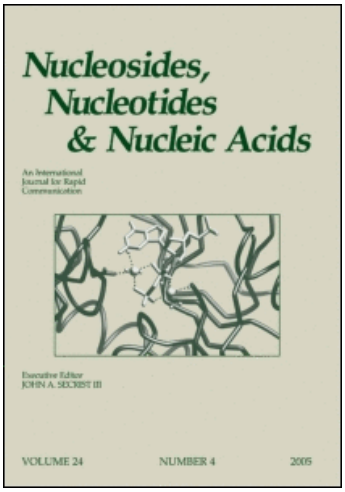


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Some Hydroxamic Acids Related to Uridine: Potential Inhibitors of Ribonucleoside Diphosphate Reductase

Peter Currid[a]; Richard H. Wightman[a]
[a] Department of Chemistry, Heriot-Watt University, Edinburgh, UK

To cite this Article Currid, Peter and Wightman, Richard H.(1997) 'Synthesis of Some Hydroxamic Acids Related to Uridine: Potential Inhibitors of Ribonucleoside Diphosphate Reductase', Nucleosides, Nucleotides and Nucleic Acids, 16: 1, 115 — 128
To link to this Article: DOI: 10.1080/07328319708002527
URL: http://dx.doi.org/10.1080/07328319708002527

NUCLEOSIDES & NUCLEOTIDES, 16(1&2), 115-128 (1997)

SYNTHESIS OF SOME HYDROXAMIC ACIDS RELATED TO URIDINE: POTENTIAL INHIBITORS OF RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE

Peter Currid and Richard H. Wightman*

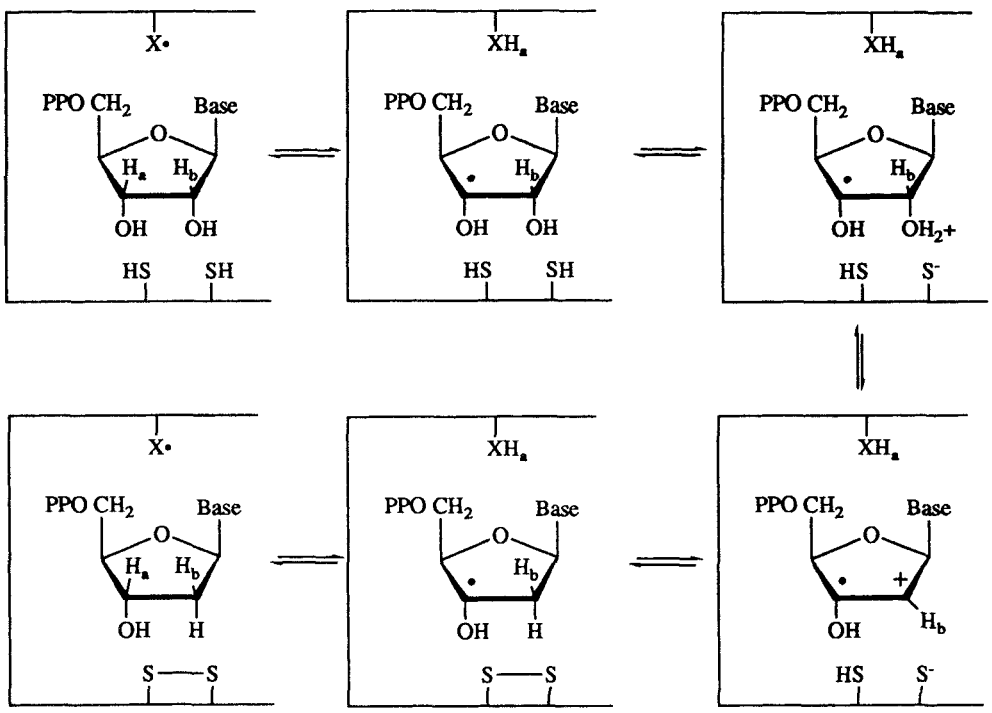
Department of Chemistry, Heriot-Watt University, Edinburgh EH14 4AS, UK.

ABSTRACT: 5-(*N*-Hydroxy)carboxamidouridine (**5**) and 5-(*N*-hydroxy)carboxamidomethyluridine (**6**) have been synthesized; these hydroxamic acids incorporate a radical trap into a nucleoside structure, and are designed as potential inhibitors of ribonucleotide diphosphate reductase.

Introduction

The enzyme ribonucleoside diphosphate reductase (RDPR) catalyses the reductive conversion of ribonucleotides into their deoxy analogues. Since pool sizes of deoxyribonucleotides in mammalian cells are very small and inadequate for cell replication, this enzyme occupies a key role in cellular proliferation and a number of studies have shown correlation between RDPR activity and rates of cell division.[1] Thus the inhibition of RDPR becomes a valid objective in cancer chemotherapy.

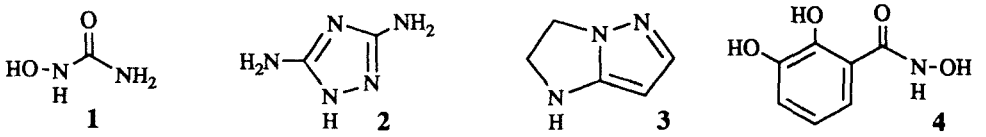
RDPR catalyses the reduction of a nucleoside diphosphate with concomitant oxidation of a dithiol unit; the small protein thioredoxin can function as the immediate hydrogen donor, and must be reduced again by the enzyme thioredoxin reductase, which contains a flavin coenzyme, to complete the catalytic cycle.[2,3] It appears to be generally accepted that RDPR from both bacterial and mammalian sources consists of two subunits (termed B1 and B2 for the bacterial enzyme, M1 and M2 for the mammalian one); of these the B1/M1 subunit binds the substrate and contains the redox-active thiols, whilst the B2/M2 unit contains both two Fe^{III} atoms linked by a μ-oxo bridge and, very unusually, a tyrosyl free radical which is essential for activity.[2,3] As a result of studies with various 2'-deoxy-2'-halo- and 2'-azido-2'-deoxynucleoside diphosphates which act



Scheme 1

as irreversible inactivators of RDPR, and work with specifically-labelled substrates, the mechanism shown in Scheme 1 has been proposed.[3,4]

A number of antitumour agents are known to act by inhibition of RDPR; amongst them are hydroxyurea (**1**), guanazole (**2**) and IMPY (**3**), of which hydroxyurea is in clinical use.[5] More recently, hydroxybenzohydroxamic acid derivatives such as **4** have

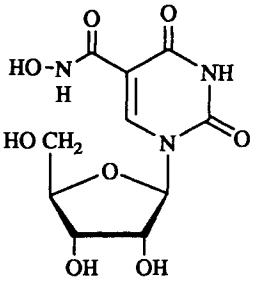


attracted attention as inhibitors of RDPR.[6] However, hydroxyurea is a relatively weak inhibitor, and its *in vitro* effectiveness may suffer because of the difficulty of delivering useful dosages at the tumour site.[5]

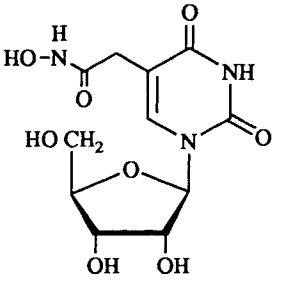
The weight of evidence strongly supports the idea that hydroxyurea (**1**) and related compounds,[5] and the benzohydroxamates (**4**)[6] act as inhibitors of RDPR by destroying the free radical at the active site.

The considerations above suggest that it might be possible to design more potent inhibitors of RDPR by attaching a hydroxamate to a natural nucleotide substrate such that the hydroxamate is positioned above the β-face of the ribose unit in the region of space where the free radical (X• in Scheme 1) lies. It has become clear from the X-ray crystal structure of B2 protein[7] that catalysis requires long-range electron transfer between the tyrosyl radical and the substrate, involving at least one thiol as a relay,[8] so that the positioning of the hydroxamate may not be critical. Additionally we were encouraged by the observation that base modification does not have a major effect on the interaction of nucleotides to the substrate site of RDPR,[9] and by the fact that acyclonucleoside hydroxamic acids have been shown to be potent inhibitors of RDPR.[10]

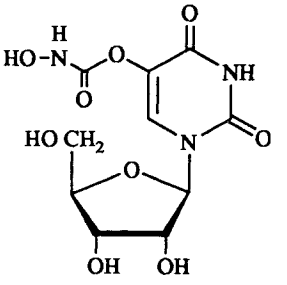
In this paper we report the synthesis of the uridine-based hydroxamic acids **5** and **6** and studies related to the *N*-hydroxycarbamate **7**. Other workers have recently described uridine derivatives with potential radical traps attached to the sugar unit.[11]



5



6

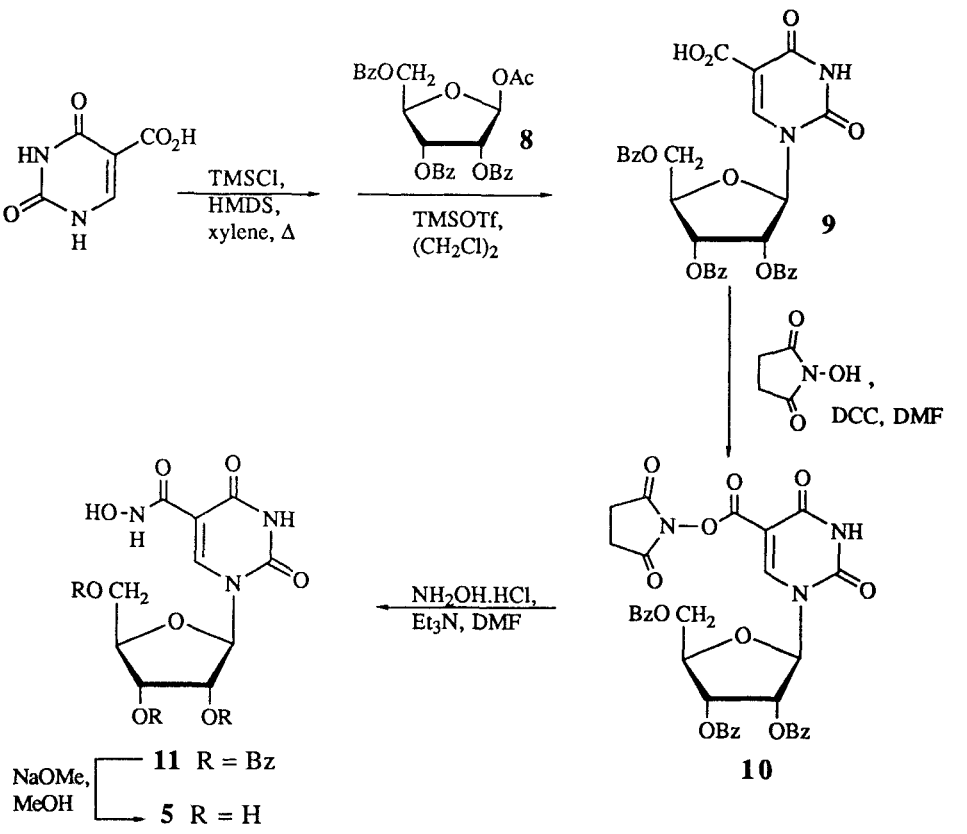


7

Results and Discussion

The synthesis of the hydroxamic acid **5** is outlined in Scheme 2. Silylation of uracil-5-carboxylic acid, followed by condensation with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**8**) under Vorbrüggen-type conditions gave the protected nucleoside **9** in 68% yield. For formation of the hydroxamate, the acid **9** was firstly converted to its N-hydroxysuccinimidyl ester **10** (71%) by DCC-mediated coupling, followed by reaction with hydroxylamine in the presence of triethylamine. Routine debenzoylation then led to the nucleoside **5**.

For the synthesis of the higher homologue **6**, we were initially attracted by the report by Senda and coworkers that a suitable side-chain could be introduced at C-5 of uracil derivatives by the interaction between 5-hydroxyuracils and stabilized phosphoranes.[12] In order to give a side-chain which should be capable of selective deprotection, the reaction of 5-hydroxyuridine (**12**)[13] with *t*-butoxycarbonylmethylene triphenylphosphorane (**13**) was investigated. Reaction in dioxan at reflux[12] gave the triol **14** which was directly acetylated to give the tri-*O*-acetyl compound **15** (Scheme 2)

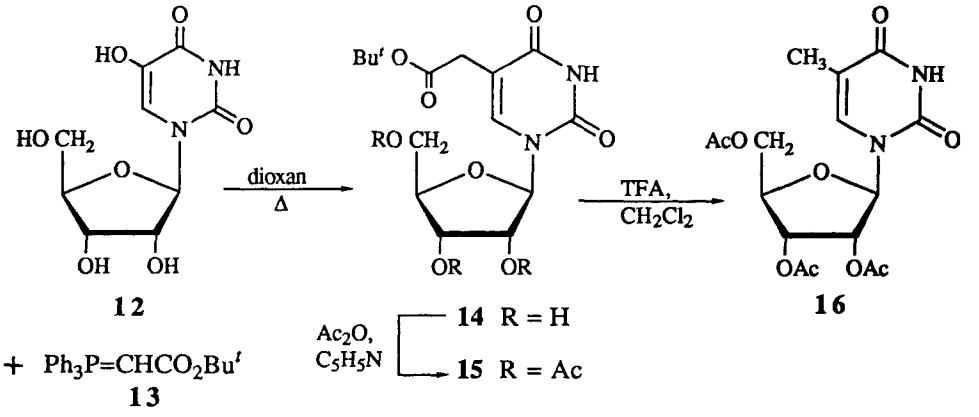


Scheme 2

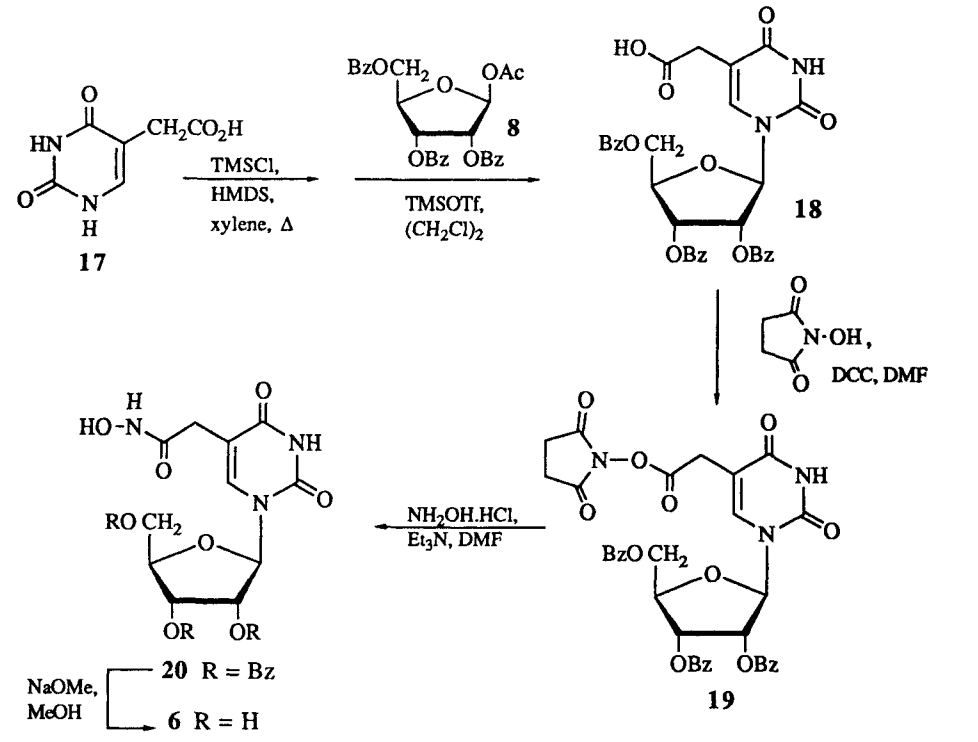
in 68% overall yield. Interestingly, however, when **15** was treated with trifluoroacetic acid (TFA) in dichloromethane, deprotection of the *t*-butyl ester was accompanied by decarboxylation to give tri-*O*-acetyl-5-methyluridine **16**[14] in good yield.

An approach involving base-sugar condensation (Scheme 4) was more successful.[15] Silylation of 5-carboxymethyluracil (**17**)[16] followed by condensation with ribofuranose derivative **8** gave the nucleoside **18**. This was converted into the hydroxamic acid **6** using the same procedures as for the lower homologue. Thus condensation of **18** with *N*-hydroxysuccinimide gave the active ester **19** (73%), which was treated with hydroxylamine to give the triester **20**. This could be debenzoylated with methoxide in methanol to give the triol **6**.

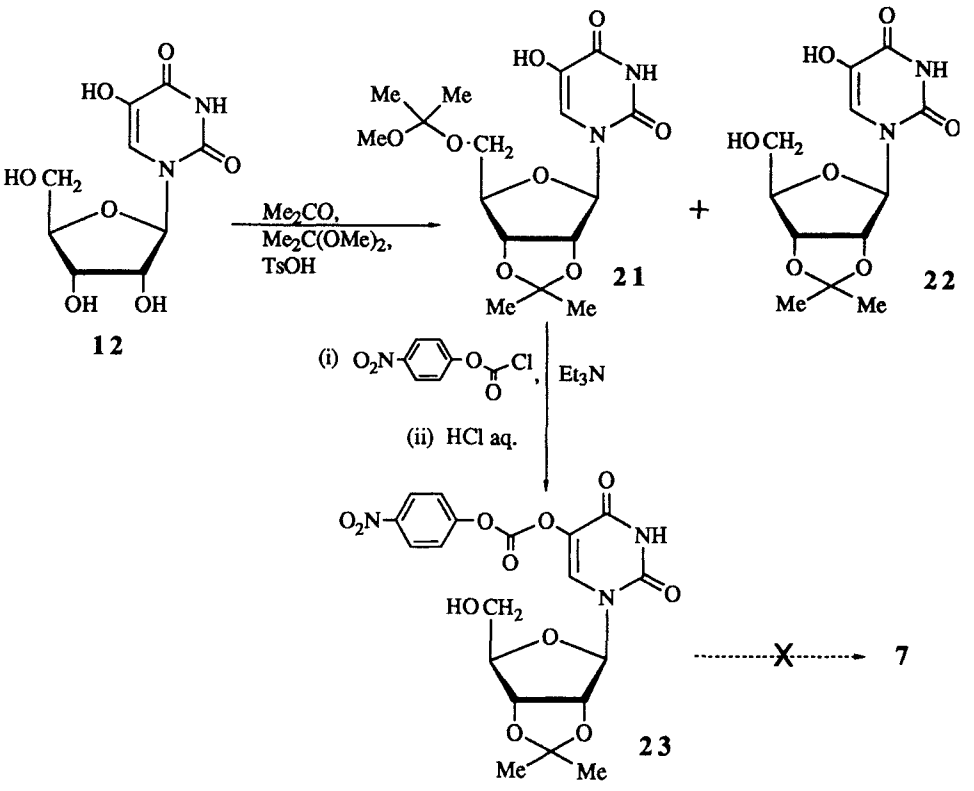
We also considered the *N*-hydroxycarbamate **7** as a target. In an approach to this (Scheme 5), treatment of 5-hydroxyuridine with acetone, 2,2-dimethoxypropane and *p*-



Scheme 3



Scheme 4



Scheme 5

toluenesulfonic acid gave a mixture of about equal amounts of the derivative **21** in which all the sugar hydroxy groups were protected, and the 2',3'-*O*-isopropylidene derivative **22**.[17] When **21** was treated with *p*-nitrophenyl chloroformate, followed by brief treatment with dilute acid, the mixed carbonate **23** was isolated in good yield.

The positioning of the *p*-nitrophenyloxycarbonyl group in **23** on O-5 rather than on N-3 was supported in the ^{1}H-NMR spectrum by the downfield shift (0.9 ppm) of H-6 in **23** as compared to either **21** or **22**, and chemically by a negative ferric chloride test.

Consideration of pK_a values[17,18] led us to believe that the nitrophenolate anion should be the better leaving group in this mixed carbonate, but despite considerable experimentation it was not possible to obtain an N-hydroxycarbamate by interaction of **23** with hydroxylamine.

Conversion of **5** and **6** to their diphosphates and biological evaluation will be reported elsewhere.

Experimental

NMR spectra were recorded on a Bruker WP 200 SY spectrometer. ^{1}H-Spectra were obtained at 200 MHz, and ^{13}C-spectra at 50 MHz, with $CDCl_3$ as solvent unless otherwise stated. Coupling constants are measured in Hz. Mass spectrometry was performed using V.G. updated MS9 and V.G. ZABE high resolution EI/FAB instruments. Specific rotations were measured at room temperature using a Bendix-NPL 143D automatic polarimeter (path length 1 cm); units for $[\alpha]_D$ values are 10^{-1} deg cm^2 g^{-1}. Melting points were determined using an Electrothermal MK II melting point apparatus and are uncorrected.

Column chromatography was carried out using Kieselgel H type 60 (Merck) an external pressure being applied to the top of columns. Organic extracts were dried over anhydrous magnesium sulfate.

2',3',5'-*Tri*-O-*benzoyluridine*-5-*carboxylic acid* (**9**) - Uracil-5-carboxylic acid monohydrate (1.0g, 5.75mmol), hexamethyldisilazane (10 ml), xylene (10 ml) and chlorotrimethylsilane (0.73 ml, 5.75mmol) were stirred and heated under reflux with careful exclusion of moisture. Ammonia was vigorously evolved and NH_4Cl sublimed into the condenser. After two hours the solid had dissolved to give a clear, colourless solution. Evaporation of the excess hexamethyldisilazane and xylene under reduced pressure gave a colourless syrup to which was added sequentially 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**8**) (2.89g, 5.75mmol) in dry 1,2-dichloroethane (20 ml) and trimethylsilyl trifluoromethanesulfonate (1.7 ml, 8.62mmol). The resulting mixture was stirred for two hours at room temperature, then diluted with dichloromethane (30 ml) and shaken with saturated $NaHCO_3$ solution (20 ml). The resulting emulsion was filtered through a bed of Celite-sand and the aqueous layer separated and extracted with further dichloromethane (2 x 20 ml). The combined organic layers were dried and evaporated. The residue was dissolved in dichloromethane containing silica and evaporated to dryness under reduced pressure. The preadsorbed powder was applied to the top of a silica column which was eluted with ethyl acetate-methanol (20:1). Fractions containing the product were evaporated to a small volume (approximately 20 ml). Dropwise addition of hexane resulted in precipitation of the *carboxylic acid* (**9**) (2.63 g, 68%) as a white crystalline solid, m.p. 213-215°C, $[\alpha]_D$ -74.3 (*c* 1.13 in DMSO); δ_H [$(CD_3)_2$ SO] 4.67 (2H, m, H-5'), 4.79 (1H, m, H-4'), 5.90-6.10 (2H, m, H-2', H-3'), 6.30 (1H, d, *J* 2.2, H-1'), 7.30-7.73 (9H, m, Ph), 7.78-8.08 (6H, m, Ph), 8.74 (1H, s, H-6), 12.25 (1H, bs, NH), 12.74 (1H, bs, CO_2H); δ_C [$(CD_3)_2SO$], 63.4 (C-5'), 70.3 (C-3'), 73.6 (C-2'), 79.4 (C-4'), 90.0 (C-1'), 103.6 (C-5), 128.6, 129.1, 129.3, 133.4, 133.74, 133.8 (Ph), 149.2 (C-2) 149.7 (C-6), 162.9 (C-4), 163.0, 164.6 (CO, aromatic esters),

165.5 (CO_2H); m/z(FAB) 623 (MNa), 601 $(MH)^+$ (Found: MH^+ 601.1460; $C_{31}H_{25}N_2O_{11}$ requires 601.1458. Found: C, 61.9%; H, 4.0%; N, 4.6%; calc. for $C_{31}H_{24}N_2O_{11}$: C, 62.0%; H, 4.0%; N, 4.7%).

N-*Succinimidyl* 2',3',5'-*tri*-O-*benzoyluridine*-5-*carboxylate* (**10**) - DCC (309mg, 1.5 mmol) was added to a mixture of the acid **9** (600mg, 1.0 mmol) and *N*-hydroxysuccinimide (115 mg, 1.0 mmol) in dry DMF (10 ml). The reaction mixture was stirred for 72 h at room temperature. Acetic acid (200 mg) was added and the mixture stirred for an additional 1 h to destroy the excess of DCC. The crystalline dicyclohexylurea was filtered and washed with ethyl acetate (15 ml). The residue after evaporation was dissolved in hot ethyl acetate and the solution was cooled to room temperature. Dropwise addition of hexane resulted in precipitation of the *active ester* **10** (493 mg, 71%) as a white crystalline solid, m.p. 238-240°C, $[\alpha]_D$ -139.2 (*c*0.57 in $CHCl_3$), R_f 0.62 (ethyl acetate: chloroform: acetone, 5:3:2); δ_H [$(CD_3)_2SO$], 2.82 (4H, s, $-CH_2-CH_2$), 4.67 (2H, m, H-5'), 4.83 (1H, m, H-4'), 5.96-6.17 (2H, m, H-2', H-3'), 6.38 (1H, d, *J* 4.3, H-1'), 7.32-7.74 (9H, m, Ph), 7.80-8.11 (6H, m, Ph), 8.94 (1H, s, H-6), 12.10 (1H, s, NH); δ_c[$(CD_3)_2SO$]. 25.4 (CH_2 -CH_2), 63.6 (C-5'), 70.4 (C-3'), 73.9 (C-2'), 79.5 (C-4'), 91.1 (C-1'), 99.9 (C-5), 128.6, 129.1, 129.3, 133.4, 133.8 (Ph), 149.1 (C-2), 158.3, 151.9 (C-6), 158.4, 164.5, 164.6, 165.4, and 170.0 (6x*C*O); m/z (FAB) 698 $(MH)^+$ (Found: MH^+ 698.1620; $C_{35}H_{28}N_3O_{13}$ requires 698.1622. Found: C, 60.0%; H, 3.9%; N, 5.8%; calc. for $C_{35}H_{27}N_3O_{13}$: C, 60.3%; H, 3.9%; N, 6.0%).

2',3',5'-*Tri*-O-*benzoyl*-5-*[(*N-*hydroxy)carboxamido]uridine* (**11**) - A solution of hydroxylamine hydrochloride (28mg, 0.41 mmol), the active ester **10** (286mg, 0.41 mmol) and triethylamine (86µl, 0.61 mmol) in dry DMF (10ml) was stirred for 24 h at room temperature. Triethylamine hydrochloride was filtered and washed with ethyl acetate (15 ml). The combined filtrates were diluted with more ethyl acetate (25ml), washed with water (25ml), dried and evaporated to give a light yellow syrup. Trituration of this material in ether gave the *hydroxamate* **11** (178mg, 71 %) as a white powder, mp 234-237 °C, $[\alpha]_D$ -64.2 (*c* 1.06 in DMSO), and giving a deep blue colour when treated with ferric chloride solution; δ_H [$CDCl_3$+$(CD_3)_2SO$] 4.61 (3H, m, H-4', H-5'), 5.84 (2H, m, H-2', H-3'), 5.94 (1H, d, *J* 3.2, H-1'), 7.16-7.52 (9H, m, Ph), 7.7-8.0 (6H, m, Ph), 8.43 (1H, s, H-6), 8.87 (1H, bs, NH), 10.64 (1H, s, OH), 11.72 (1H, bs, NH); δ_c[$CDCl_3$+$(CD_3)_2SO$] 63.2 (C-5'), 70.5 (C-3'), 73.3 (C-2'), 79.6 (C-4'), 91.7 (C-1'), 105.5 (C-5), 127.8, 128.0, 128.7, 129.0, 129.1, 132.6, 133.0, 133.1 (Ph), 146.4 (C-6), 148.7 (C-2), 159.1 (C-4), 162.0, 164.4, 164.5 and 165.4 (4 x CO); m/z(FAB) 638 $(MNa)^+$, 616 $(MH)^+$ (Found: MH^+ 616.1510; $C_{31}H_{26}N_3O_{11}$ requires 616.1567. Found: C, 60.8%; H, 4.5%, N, 7.2%; calc. for $C_{31}H_{25}N_3O_{11}$: C, 60.5%; H, 4.1%; N, 6.8%).

5-(N-*Hydroxy*)-*carboxamidouridine* (**5**) - To a solution of sodium methoxide [from sodium (11 mg) in dry methanol (5 ml)] was added the triester **11** (170mg, 0.28 mmol). The mixture was stirred for 15 min. at room temperature, after which the solution was evaporated to dryness. The resulting white solid was dissolved in water (25 ml) and extracted with ether (2 x 25 ml). Amberlite IR 120 (H^+) resin was added to the aqueous phase and the suspension was stirred for 30 min. The resin was filtered and washed with water (2 x 10ml). Evaporation of the combined filtrate and washings afforded the *hydroxamate* **5** (51 mg, 61%) as a straw-coloured solid, m.p. 170-172 °C, and giving a deep blue colour when treated with ferric chloride solution; δ_H [$(CD_3)_2SO$] 3.69 (2H, m, H-5'), 3.94 (2H, m, H-2', H-3'), 4.08 (1H, q, *J*~4.8, H-4'), 5.13 (2H, m, 2 x OH), 5.47 (1H, d, *J* 5.3, OH), 5.78 (1H, d, *J* 4.7, H-1'), 8.70 (1H, bs, OH), 10.57 (1H, s, NH), 11.92 (1H, bs, NH); δ_c[$(CD_3)_2SO$] 60.8 (C-5'), 69.8 (C-3'), 74.0 (C-2'), 85.1 (C-4'), 88.8 (C-1), 105.6 (C-5), 145.4 (C-6), 149.7 (C-2), 159.7 (C-4), 162.4 (CONHOH); m/z(FAB) 304 $(MH)^+$ (Found: MH^+ 304.0781; $C_{10}H_{14}N_3O_8$ requires 304.0783).

2',3',5'-*Tri*-O-*acetyl*-5-t-*butoxycarbonylmethyluridine* (**15**) - A solution of 5-hydroxyuridine (**12**) (2.01 g, 7.73 mmol) and t-butoxycarbonylmethylene triphenylphosphorane (**13**) (3.50 g, 9.28 mmol) in dry dioxan (100 ml) was heated under reflux for 9 h. Evaporation of the solvent gave a light brown syrup. Water (100 ml) was added which resulted in the precipitation of triphenylphosphine oxide. After filtration the aqueous solution was extracted with ether (2 x 50 ml). Evaporation of the aqueous solution under reduced pressure afforded the triol **14** (2.59 g) as a dark brown syrup. A solution of this material in dry pyridine (40 ml) containing acetic anhydride (2.58 g, 25.3 mmol) was stirred at room temerature for 16 h. Evaporation of the solution gave a dark brown syrup, which was preadsorbed onto silica and applied to the top of a silica column. Chromatography with ethyl acetate-methanol (95:5) as eluent gave the *triacetate* **15** (2.55 g, 68%) as a white amorphous foam which softened at 41°C, $[\alpha]_D$ -1.9 (*c* 1.07 in $CHCl_3$; δ_H 1.47 (9H, s, C*Me*$_3$), 2.12, 2.16 and 2.18 (each 3H, s, OAc), 3.28 (2H, s, α-CH_2), 4.60-4.83 (3H, H-4', H-5'), 5.27-5.42 (2H, m, H-2', H-3'), 6.12 (1H, d, *J*, 4.7, H-1'), 7.49 (1H, s, H-6), 9.54(1H, s, NH); δ_c 20.3, 20.4 and 20.6 (*Me*CO), 28.0 (Me$_3$*C*), 32.4 (α-CH_2), 63.2 (C-5'), 70.4 (C-3'), 72.7 (C-2'), 80.1 (C-4'), 81.5 (O*C*Me$_3$), 87.3 (C-1'), 109.6 (C-5), 137.4 (C-6), 150.1 (C-2), 162.5 (C-4), 169.4 and 169.5 (x2) (Me*C*O), 170.2 (*C*OtBu); m/z (FAB) 485 $(MH)^+$ [Found: $(MH)^+$ 485.1771. $C_{21}H_{29}N_2O_{11}$ requires 485.1771].

2',3',5'-*Tri*-O-*acetyl*-5-*methyluridine* (**16**) - To a solution of 2',3',5'-tri-*O*-acetyl-5-*t*-butoxycarbonylmethyluridine (**15**) (100 mg, 0.21mmol) in dichloromethane (10 ml) was

added TFA/CH_2Cl_2 (1:1, 10 ml). The mixture was stirred at room temperature for 1 h, and then evaporated to give a light yellow syrup. This was taken up in dichloromethane (20 ml) and the solution was washed with saturated $NaHCO_3$ (20 ml) and dried. Evaporation and chromatography of the residue on silica, with toluene : ethyl acetate (1:1) as eluent afforded the 5-methyluridine derivative **16**[14] (52 mg, 68%) as a clear oil, R_f 0.38 (toluene : ethyl acetate, 1:2); δ_H 2.13, 2.15 and 2.17 (each 3H, s, OAc), 2.30 (3H, s, CH_3), 4.29-4.43 (3H, m, H-4', H-5'), 5.29-5.40 (2H, m, H-2', H-3'), 6.09 (1H, d, *J* 4.6, H-1'), 7.53 (1H, s, H-6), 9.51 (1H, bs, NH); δ_C 20.1, 20.3, 20.4 and 20.5 (4 x CH_3), 62.9 (C-5'), 70.2 (C-3'), 72.8 (C-2'), 79.9 (C-4'), 87.2 (C-1'), 127.8 (C-5), 130.3 (C-6), 149.4 (C-2), 158.0 (C-4), 168.2, 169.5 and 170.1 (*C*OMe).

2',3',5'-*Tri*-O-*Benzoyl*-5-*carboxymethyluridine* (**18**) - 5-Carboxymethyluracil (**17**)[16] (0.5 g, 2.94 mmol), hexamethyldisilazane (5 ml), xylene (5 ml), and chlorotrimethylsilane (0.37 ml, 2.94 mmol) were stirred and heated under reflux with careful exclusion of moisture. Ammonia was vigorously evolved and NH_4Cl sublimed into the condenser. After 1 h the solid had dissolved to give a clear, colourless solution. Evaporation gave a colourless syrup, to which was added a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**8**) (1.48 g, 2.94 mmol) in dry 1,2-dichloroethane (15 ml) followed by trimethylsilyl trifluoromethanesulfonate (0.85 ml, 4.41 mmol). The resultant solution was stirred for 2 h at room temperature, then diluted with dichloromethane (20 ml) and shaken with saturated $NaHCO_3$ solution (10 ml). The resulting emulsion was filtered through a bed of Celite sand and the aqueous layer extracted with further dichloromethane (2 x 20 ml). The combined organic extracts were washed with water (2 x 25 ml), dried and evaporated. Chromatography on silica, eluting with ethyl acetate, afforded a white foam. This was dissolved in boiling ethyl acetate and the solution was cooled to room temperature. Dropwise addition of hexane resulted in precipitation of the *carboxylic acid* **18** (900 mg, 50%) as a white solid, mp 161-163°C, $[\alpha]_D$ -74.3 (*c* 1.05 in $DMSO_1$); δ_H [$CDCl_3$+$(CD_3)_2$ SO], 3.0 (2H, AB dd, *J* 17.5, CH_2CO_2H), 4.70 (2H, m, H-5'), 4.84 (1H, m, H-4'), 5.87 (1H, t, *J* 5.7, H-3'), 5.97 (1H, m, H-2'), 6.38 (1H, d, *J* 5.4, H-1'), 7.28-7.69 (10H, m, Ph + H-6), 7.89-8.14 (6H, m, Ph), 10.20 (1H, bs, NH); δ_C [$CDCl_3$+$(CD_3)_2SO$], 31.5 (α-CH_2), 63.8 (C-5'), 71.2 (C-3), 73.7 (C-2'), 80.3 (C-4'), 88.3 (C-1'), 109.4 (C-5), 128.3, 128.6, 129.1, 129.4, 129.6, 129.7, 133.5 (Ph), 138.0 (C-6), 150.0 (C-2), 163.1 (C-4), 165.1 and 165.9 (aromatic esters), 172.4 (CO_2H); m/z (FAB) 615 $(MH)^+$ [Found: MH^+ 615.1615; $C_{32}H_{27}N_2O_{13}$ requires 615.1615. Found: C, 62.2%; H, 4.0%; N, 4.5%; calc. for $C_{32}H_{26}N_2O_{11}$: C, 62.5%; H, 4.2%; N, 4.6%.]

N-Succinimidyl -2',3',5'-*Tri*-O-*benzoyl*-5-*carboxymethyluridine* (**19**) - DCC (185 mg, 0.89mmol) was added to a mixture of the acid **18** (0.5 g, 0.81mmol) and *N*-

hydroxysuccinimide (94 mg, 0.81mmol) in dry DMF (10 ml) and the reaction mixture was stirred for 72 h at room temperature. Acetic acid (100 mg) was added and the reaction mixture stirred for an additional 1 h to destroy the excess of DCC. The crystalline dicyclohexylurea was filtered and washed with ethyl acetate (15 ml). The light yellow filtrate was diluted with more ethyl acetate (25 ml), washed with water (2x30 ml) and dried. Evaporation afforded an oily solid, which was dissolved in dichloromethane containing silica and evaporated to dryness. The resultant silica was applied to the top of a silica column and chromatographed with ethyl acetate-methanol (20:1) as eluent, to give the *active ester* **19** (426 mg, 73%) as a white solid, mp 83-85 °C, R_f 0.60 (ethyl acetate-chloroform-acetone, 5:3:2), $[\alpha]_D$ -32.6 (*c* 1.08 in $CHCl_3$); δ_H 2.76 (4H, s, CH_2CH_2), 3.39 (2H, AB dd, *J* 17.4, α-CH_2), 4.63-4.88 (3H, m, H-4', H-5'), 5.83 (1H, t, *J* 5.8, H-2'), 5.87 (1H, dd, $J_{3',2'}$ 6.0, $J_{3',4'}$ 4.2, H-3'), 6.39 (1H, d, *J* 5.5, H-1'), 7.33-7.64 (9H, m, Ph), 7.67 (1H, s, H-6), 7.92-8.13 (6H, m, Ph), 8.69 (1H, bs, NH); δ_C 25.49 (-CH_2CH_2 succinimidyl), 33.7 (α-CH_2), 63.8 (C-5'), 71.1 (C-3'), 73.6 (C-2'), 80.3 (C-4'), 88.1 (C-1'), 107.2 (C-5), 128.4, 128.6, 129.3, 129.6, 129.7, 129.8, 133.5, 133.6 (Ph), 138.5 (C-6), 149.9 (C-2), 162.1 (C-4), 165.2, 165.6, 166.0 and 166.0 (ester), 169.1 (*C*O, succinimidyl); m/z (FAB) 712 $(MH)^+$ (Found: MH^+ 712.1779; $C_{36}H_{30}N_3O_{13}$ requires 712.1778).

2',3',5'-*Tri*-O-*benzoyl*-5-*[(*N-*hydroxy)-carboxamidomethyl]uridine* (**20**). - A solution of hydroxylamine hydrochloride (30 mg, 0.43 mmol), the active ester **19** (300 mg, 0.43 mmol) and dry triethylamine (90μl, 0.65 mmol) in dry DMF (10 ml), was stirred for 24 h at room temperature. Triethylamine hydrochloride was filtered and washed with ethyl acetate (15 ml). The light yellow filtrate was diluted with more ethyl acetate (30 ml), washed with water (2 x 30 ml), dried, and evaporated to give a syrup. This was dissolved in boiling acetone and the solution was cooled to room temperature. Dropwise addition of ether resulted in precipitation of the *hydroxamate* **20** (143 mg, 54%), as a white solid, mp 137-138°C, $[\alpha]_D$ -54.1 (*c* 1.09 in DMSO), giving a deep red colour when treated with ferric chloride solution; ^1H and ^{13}C NMR data unresolved; m/z (FAB) 630 $(MH)^+$ (Found MH^+, 630.1724. $C_{32}H_{28}N_3O_{11}$ requires 630.1724. Found: C, 60.7%; H, 4.2%; N, 6.8%; calc. for $C_{32}H_{27}N_3O_{11}$: C, 61.0%; H, 4.3%; N, 6.7%.)

5-(N-*Hydroxy)carboxamidomethyluridine* (**6**). - To a solution of sodium methoxide [from sodium (12 mg) in dry methanol (5 ml)] was added the triester **20** (140 mg, 0.22 mmol) and the mixture was stirred for 15 min at room temperature. The solid residue after evaporation was dissolved in water (25 ml) and extracted with ether (2 x 25 ml). Amberlite IR 120 (H^+) resin was added to the aqueous phase and the mixture was stirred

for 30 min. The resin was filtered and washed with water (2 x 10 ml). Evaporation of the combined filtrate and washings afforded the *triol* (**6**) (45 mg, 64%) as a white amorphous solid, mp 200-202°C, which gave a deep red colour when treated with ferric chloride solution; δ_H [$(CD_3)_2SO$], 2.97 (2H, s, α-CH_2), 3.60 (2H, m, H-5'), 3.81 (1H, m, H-4'), 4.99 (2H, m, H-2', H-3'), 5.11 (2H, m, OH), 5.39 (1H, d, *J* 5.3, OH), 5.78 (1H, d, *J* 4.6, H-1'), 7.78 (1H, s, H-6), 8.74 (1H, s, NH hydroxamate), 10.40 (1H, s, OH) 11.34 (1H, s, NH); δ_C [$(CD_3)_2$ SO], 30.5 (α-CH_2), 61.0 (C-5'), 69.8 (C-3'), 73.4 (C-2'), 84.8 (C-4') 87.9 (C-1'), 108.0 (C-5), 138.8 (C-6) 150.7 (C-2), 163.1 (C-4), 166.3 (*C*O, hydroxamate); m/z (FAB 318 $(MH)^+$ (Found: MH^+ 318.0937. $C_{11}H_{16}N_3O_8$ requires 318.0937).

Acetonation of 5-hydroxyuridine (**12**). - A suspension of 5-hydroxyuridine (**12**)[13] (3 g, 1.5 mmol) and toluene *p*-sulfonic acid (100 mg) in anhydrous acetone (160 ml) and 2,2-dimethoxypropane (16 ml) was stirred for 24 h at room temperature. T.l.c. (chloroform-methanol, 10:1) indicated the formation of two major products. After neutralisation with anhydrous sodium carbonate, the clear filtrate was evaporated to give a white foam. Chromatography on silica gel eluting with chloroform-methanol (97:3) afforded the faster moving adduct, R_f 0.47, as a white solid. A solution of this material in boiling acetone was cooled to room temperature. Dropwise addition of hexane resulted in precipitation of 2',3'-O-*isopropylidene*-5'-O-(2-*methoxy*-2-*propyl*)-5-*hydroxyuridine* (**21**) (1.29 g, 30 %) as a white crystalline solid, mp 165-167°C, $[\alpha]_D$ -29.3 (*c* 1.03 in DMSO), which gave a blue colour when treated with ferric chloride solution; δ_H [$(CD_3)_2SO$], 1.28 (9H, s, 3xMe), 1.48 (3H, s, Me), 3.09 (3H, s, OMe), 3.51 (2H, m, H-5'), 4.14 (1H, q, *J*~3.0, H-4'), 4.74 (1H, dd, $J_{3',4'}$ 3.6, H-3'), 4.86 (1H, dd, $J_{2',3'}$ 6.3, H-2'), 5.87 (1H, d, $J_{1',2'}$ 2.5, H-1'), 7.30 (1H, s, H-6), 8.73 (1H, bs, 5-OH), 11.58 (1H, bs, NH); δ_C [$(CD_3)_2SO$], 23.8, 23.9, 25.1 and 26.9 (4 x Me), 47.8 (OMe), 60.7 (C-5'), 80.4 (C-3'), 83.4 (C-2'), 84.3 (C-4'), 90.3 (C-1'), 99.8 (*C*Me_2), 113.0 (*C*Me_2), 120.5 (C-5), 138.5 (C-6), 148.8 (C-2), 160.4 (C-4). (Found: C, 51.6%; H, 6.5%; N, 7.5%; calc. for $C_{16}H_{24}N_2O_8$: C, 51.8%; H, 6.6%; N, 7.2%).

Further elution of the column with chloroform-methanol (95:5) yielded 2',3'-*O*-isopropylidene-5-hydroxyuridine (**22**) (1.27 g, 37%) as a white amorphous solid, mp 215-218°C (Lit.[17] 215-217°C, R_f 0.20 (chloroform-methanol, 10:1), $[\alpha]_D$ -37.7 (*c* 1.05 in DMSO), giving a blue colour when treated with ferric chloride solution; δ_H [$(CD_3)_2SO$], 1.28 and 1.47 (each 3H, s, Me) 3.57 (2H, m, H-5'), 4.01 (1H, m, H-4'), 4.74 (1H, dd, $J_{3',2'}$ 6.3, $J_{3',4'}$ 3.5, H-3'), 4.85 (1H, dd, $J_{2',3'}$ 6.3, $J_{2',1'}$ 2.9, H-2'), 5.13 (1H, bs, 5'-OH), 5.86 (1H, d, *J* 2.9, H-1'), 7.30 (1H, s, H-6), 8.75 (1H, bs, 5-OH), 11.58 (1H, bs,

NH); δ_C [$(CD_3)_2SO$] 25.7 and 27.0 (CMe_2), 61.3 (C-5'), 80.3 (C-3'), 83.2 (C-2'), 85.7 (C-4'), 90.2 (C-1'), 113.1 (CMe_2), 120.3 (C-5), 133.1 (C-6), 148.9 (C-2), 160.8 (C-4).

2',3'-O-*Isopropylidene-5-(*p-*Nitrophenoxycarbonyloxy)uridine* (**23**). - To a solution of 2',3'-*O*-isopropylidene-5'-*O*-(2-methoxy-2-propyl)-5-hydroxyuridine (**21**) (400 mg, 1.09 mmol) and triethylamine (0.23 ml, 1.63 mmol) in dry dichlomethane (10 ml), under a nitrogen atmosphere, was added *p*-nitrophenyl chloroformate (220 mg, 1.09 mmol), and the mixture was stirred at room temperature for 1 h. The yellow solution was diluted with dichloromethane (10 ml) and shaken with aqueous HCl (10%, 10 ml). The organic layer was separated and washed with aqueous $NaHCO_3$ (10% 10 ml), water (2 x 20 ml), dried, and evaporated to give an off-white foam. Trituration of this material in chloroform (5 ml) afforded the p-*nitrophenyl carbonate* **23** (379 mg, 75%) as a white crystalline solid, mp 172-174°C, $[\alpha]_D$ -22.9 (*c* 1.05 in DMSO); δ_H [$CDCl_3$+$(CD_3)_2SO$], 1.36 (3H, s, CH_3), 1.57 (3H, s, CH_3), 3.04 (1H, bs, 5-OH), 3.83 (2H, m, H-5'), 4.33 (1H, m, H-4'), 4.80 (H, dd, $J_{2',3'}$ 6.2, $J_{2',1'}$ 2.8, H-2'), 4.88 (H, dd, $J_{3',2'}$ 6.2, $J_{3',4'}$ 2.6, H-3'), 5.97 (1H, d, *J* 2.8, H-1'), 7.50 (2H, d, *J* 8.0, PNP), 8.20 (1H, s, H-6), 8.3 (2H, d, *J* 8.0, PNP), 11.58 (1H, bs, NH); δ_C [$CDCl_3$+ $(CD_3)_2SO$], 24.9 and 26.8 (CH_3), 61.5 (C-5'), 80.1 (C-3'), 84.5 (C-2'), 86.5 (C-4'), 90.1 (C-1'), 113.5 (CMe_2), 121.4 and 124.9 (CH, PNP), 127.1 (C-5), 132.5 (C-6), 145.3 (quaternary, PNP), 149.7 (C-2), 154.8 (OCOO), 157.9 (C-4); m/z (FAB) 466 (Found: MH^+ 466.1100; $C_{19}H_{20}N_3O_{11}$ requires 466.1098. Found: C, 48.8%; H, 4.1%; N, 9.0%; calc. for $C_{19}H_{19}N_3O_{11}$: C, 49.0%; H, 4.1%; N, 9.0%).

Acknowledgements

We thank Cancer Research Campaign for financial support, and EPSRC for access to facilities at the Mass Spectrometry Service Centre, University of Wales, Swansea.

References

1. e.g., Weber, G. *New Engl. J. Med.*, **1977**, *296*, 486 and refs. therein.
2. Reichard, P.; Threnberg, A. *Science*, **1983**, *221*, 514, and refs. therein.
3. Stubbe, J.; Ashley, G.W. *Pharmac. Ther.*, **1985**, *30*, 301.
4. (a) Mao, S.-S.; Johnson, M.I.; Bollinger, J.M.; Baker, C.H.; Stubbe, J. in *Molecular Mechanisms in Bioorganic Processes*; Bleasdale, C; Golding, B.T. Eds.; Royal Society of Chemistry : London, 1990; p. 305. (b) Stubbe, J. *J. Biol. Chem.*, **1990**, *265*, 5329.
5. Moore, E.C.; Hurlbert, R.B. *Pharmac. Ther.*, **1985**, *27*, 167.
6. Elford, H.L.; van't Riet, B. *Pharmac. Ther.*, **1985**, *27*, 239.

7. Nordlund, P.; Sjöberg, B.-M.; Eklund, H. *Nature*, **1990**, *345*, 593.
8. (a) Climent, I.; Sjöberg, B.-M.; Huang, Y. *Biochemistry*, **1992**, *31*, 4801. (b) Mao, S.-S.; Yu, G.X.; Chalfoun, D.; Stubbe, J. *Biochemistry*, **1992**, *31*, 9752.
9. Roy, B.; Lepoivre, M.; Decout, J.L.; Lhomme, J.; Fontecave, M. *Biochem. Biophys. Res. Commun.*, **1992**, *187*, 432.
10. Farr, R.A.; Bey, P.; Sunkara, P.S.; Lippert, B.J. *J. Med. Chem.*, **1989**, *32*, 1879.
11. Auguste, S.P.; Young, D.W. *J. Chem. Soc., Perkin Trans. 1*, **1995**, 395.
12. Hirota, K.: Suematsu, M.; Kuwabara, Y.; Asao, T.; Senda, S. *J. Chem. Soc., Chem. Commun.*, **1981**, 623.
13. Visser, D.W. in *Synthetic Procedures in Nucleic Acid Chemistry*; Zorbach, W.N.; Tipson, R.S. Eds., Vol. 1; Interscience: New York, 1965, p.428.
14. Beranek, J.; Hrebabecky, H. *Nucleic Acids Res.*, **1976**, *3*, 1387.
15. For an approach to 5-carboxymethyluridine by chain-extension of a 5-hydroxymethyluridine derivative, see: Ikeda, K.; Tanaka, S.; Mizuno, Y.; *Chem. Pharm. Bull.*, **1975**, *23*, 2958.
16. Fissekis, J.D.; Sweet, F. *Biochemistry*, **1970**, *9*, 3136.
17. Otter, B.A.; Falco, E.A.; Fox, J.J. *J. Org. Chem.*, **1969**, *34*, 2636.
18. e.g. Sykes, P. *A Guidebook to Mechanism in Organic Chemistry*; Longman: London, 1986, p. 61.

Received September 5, 1996
Accepted November 19, 1996