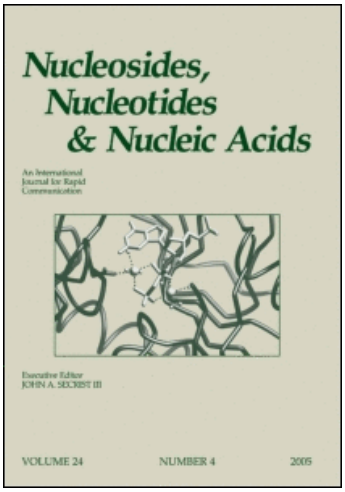

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SYNTHESIS OF CERTAIN 2'-DEOXYURIDINE DERIVATIVES CONTAINING SUBSTITUTED PHENOXY GROUPS ATTACHED TO C-5'; EVALUATION AS POTENTIAL dUTP ANALOGUES

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SYNTHESIS OF CERTAIN 2′-DEOXYURIDINE DERIVATIVES CONTAINING SUBSTITUTED PHENOXY GROUPS ATTACHED TO C-5′; EVALUATION AS POTENTIAL dUTP ANALOGUES

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ABSTRACT

Derivatives of 2′-deoxyuridine in which the 5′-OH group is replaced by a 2,3,6-trifluoro-5-hydroxy-4-nitrophenoxy or a 4-carboxy-2,3,6-trifluoro-5-hydroxyphenoxy group have been prepared for evaluation as possible dUTP analogues. They showed a weak ability to displace radiolabelled dUTP from a dUTP-binding antiserum. The corresponding compounds lacking the three fluorine substituents were prepared for comparison.

INTRODUCTION

We have speculated that the 2,3,6-trifluoro-5-hydroxy-4-nitrophenoxy and 4-carboxy-2,3,6-trifluoro-5-hydroxyphenoxy groups (**1** and **2**) may have some di- and/or tri-phosphate-like properties, such that compounds corresponding to natural substrates of di- or tri-phosphate-utilising enzymes,

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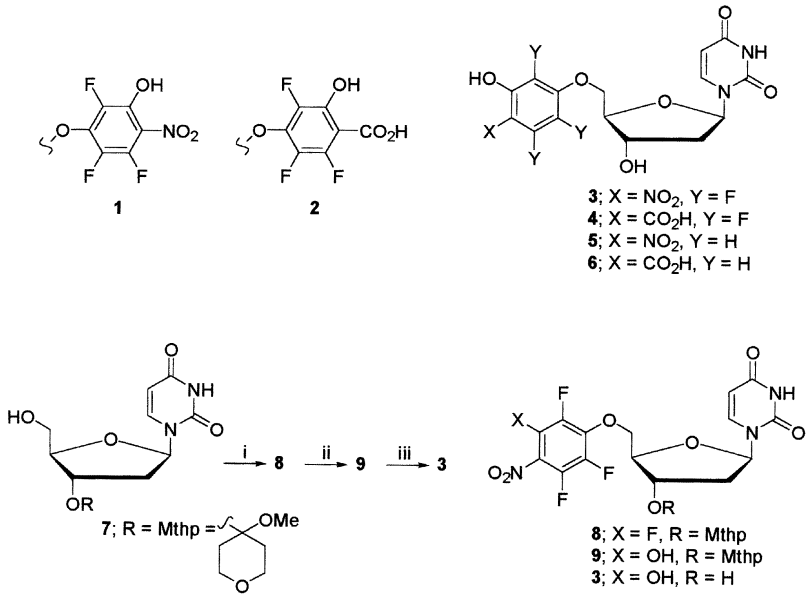
with the di/tri-phosphate group replaced by the group **1** or **2**, might in some instances prove to be inhibitors of the relevant enzymes. Following preliminary reports involving compounds containing the group **1** or **2** attached to isoprenoid groups[1], this paper describes the synthesis and biochemical evaluation of compounds **3** and **4**, in which these groups are attached to C-5′ of 2′-deoxyuridine, in place of the 5′-OH group[2a]. We required these compounds for testing 1) for ability to displace radiolabelled dUTP from a dUTP-binding antiserum, and 2) for ability to inhibit the activity of the enzyme 2′-deoxyuridine 5′-triphosphate nucleotidohydrolase (dUTPase), which catalyses the hydrolysis of dUTP to dUMP and inorganic pyrophosphate. We regarded these assays as a means of evaluating possible triphosphate mimicking ability associated with the groups **1** and **2**; the dUTPase inhibition assay was carried out for the additional reason that dUTPase has been suggested as a possible target for design of therapeutic agents against cancer[3]. In order to determine the effect of the fluorination within the groups **1** and **2** on the properties observed in the assays, we also prepared and evaluated the unfluorinated analogues of **3** and **4**, *i.e.* compounds **5** and **6**.

SYNTHESIS

The four desired compounds **3**–**6** were each prepared from 3′-*O*-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (**7**)[4]. The synthesis of compound **3**, which contains a 2,3,6-trifluoro-5-hydroxy-4-nitrophenoxy group replacing the 5′-OH of 2′-deoxyuridine, is shown in Scheme 1. Compound **7** reacted with pentafluoronitrobenzene under liquid-liquid phase transfer catalysis conditions[5] to give the 5′-*O*-aryl derivative **8**. Aqueous sodium hydroxide (0.1 M at room temperature) effected substitution of one of the fluorines *ortho* to the nitro group to give **9**, which was deprotected with aqueous acetic acid, producing **3**.

Compound **5**, the unfluorinated analogue of **3**, was produced according to Scheme 2. A Mitsunobu reaction between compound **7** and 3-fluoro-4-nitrophenol gave compound **10**, and this was converted to the phenol **11** with aqueous sodium hydroxide; a longer reaction time and more concentrated sodium hydroxide (1.5 M) were used in this case than for the analogous transformation of **8** into **9**. Deprotection of **11** gave the desired compound **5**.

The synthesis of compound **4**, which contains a 4-carboxy-2,3,6-trifluoro-5-hydroxyphenoxy group replacing the 5′-OH of 2′-deoxyuridine, employed compound **13**, which may be synthesised from 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid (**12**)[2b]. Mitsunobu etherification between compounds **13** and **7** (Scheme 3), followed by removal of the Mthp group, gave the crystallisable intermediate **14**. Aqueous sodium hydroxide (0.1 M) rapidly



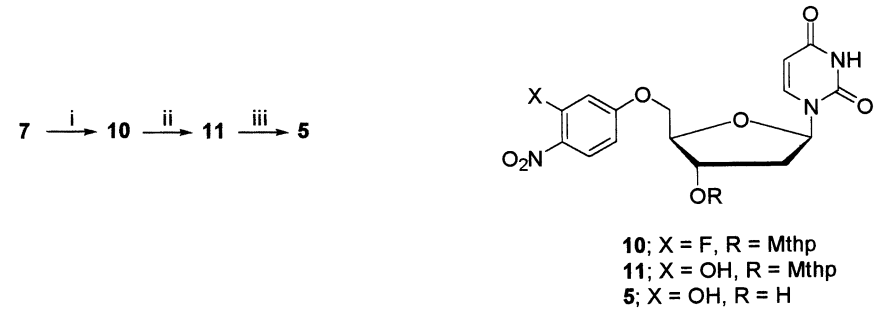
Scheme 1. i) pentafluoronitrobenzene, $Bu^n_4N^+HSO_4^-$, 1 M aq. NaOH, CH_2Cl_2, 37.5%; ii) 0.1 M aq. NaOH, 90.5%; iii) AcOH-H_2O, 85%.

removed the methylene protecting group from **14**, and compound **4** was isolated after neutralisation.

For the synthesis of compound **6**, the unfluorinated analogue of **4**, the intermediate **17** was required; this was prepared by a reaction[6] between phenyl 2,4-dihydroxybenzoate (**16**)[7] and paraformaldehyde using Dabco (Scheme 4). A Mitsunobu reaction between **17** and **7** gave the protected intermediate **18**. To obtain the desired compound **6** from **18**, we chose to remove the methylene protecting group first, then the Mthp group (Scheme 4).

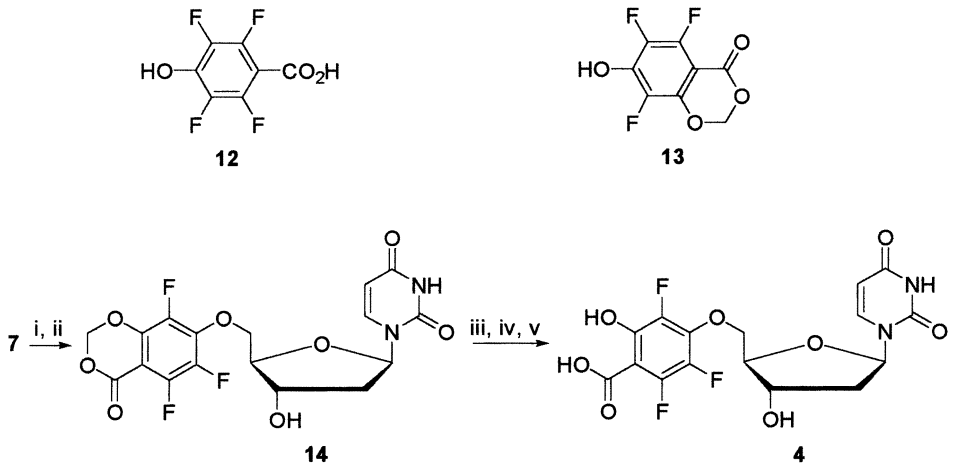
BIOCHEMICAL ASSAYS

dUTP Radioimmunoassay. This assay[8] may be used to compare a compound with dUTP, with respect to ability to displace radiolabelled dUTP from an antiserum that binds dUTP (the antiserum was raised to a dUTP-protein conjugate). Radiolabelled dUTP was mixed with a limited amount of dUTP antiserum and various amounts of unlabelled dUTP, and after incubation, antiserum-bound and unbound ligand were separated by addition of dextran-coated charcoal and centrifugation. The radioactivity in the supernatant (bound fraction) was measured by liquid scintillation

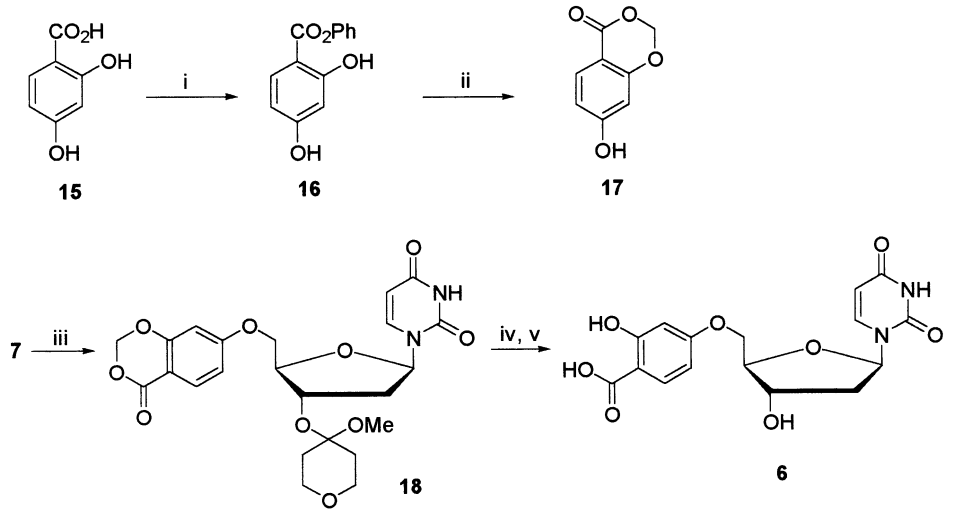


Scheme 2. i) 3-fluoro-4-nitropohenol, DIAD, PPh_3, THF, 38%; ii) 1.5 M aq. NaOH, 85%; iii) AcOH-H_2O, 83%.

counting. In the Figure, the black squares (■) plot shows the effect of the amount of unlabelled dUTP added on B/B_0, where B is the amount of bound radiolabel, and B_0 is the amount of bound radiolabel when no unlabelled dUTP was added. The other plots in the Figure show corresponding results obtained when compounds **3**–**6** were added in place of unlabelled dUTP, and, for comparison, results for addition of UTP in place of unlabelled dUTP. Percent cross-reactivity for each compound was calculated as the ratio of the concentration of compound and unlabelled dUTP, required to reduce the amount of bound radiolabel (in the absence of unlabelled dUTP) by 50% or by 30%. The Table shows calculated percentage cross-reactivity figures for compounds **3**–**6** and UTP at 50% and 30% displacement, and the percentage B/B_0 value in the presence of 20000 pmol of each compound. The antiserum did not have a



Scheme 3. i) 13, DIAD, PPh_3, THF; ii) AcOH-H_2O, 61.5% overall from **7**; iii) 0.1 M aq. NaOH; iv) AcOH; v) Bio-Rad AG50W X-4 (H^+ form), 81% overall from **14**.



Scheme 4. i) PhOH, $POCl_3$, PhMe, 45%; ii) paraformaldehyde, Dabco, $CHCl_3$, 31.5%; iii) **17**, DEAD, PPh_3, THF, 33.8%; iv) 0.05 M aq. NaOH; v) AcOH-H_2O, 80% overall from **18**.

high cross-reactivity with any of the compounds **3**–**6**. Cross-reactivity with the non-fluorinated compounds was slightly less than with their fluorinated counterparts. Cross-reactivity with the carboxylic acids was slightly greater than with the nitro compounds, in each pair of compounds. Cross-reactivity was greatest with compound **4**, but was still 65–100-fold less than with UTP. The 5′-substituents in **4** and **6** may be compared with the salicyl

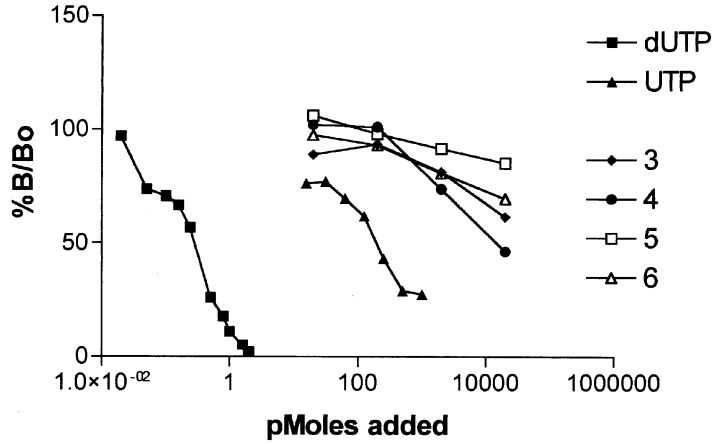


Figure. Cross-reaction of dUTP-binding antiserum with compounds **3**–**6** and UTP; B = amount of bound radiolabel and B_0 = amount of bound radiolabel when no unlabelled dUTP added.

Table. Percentage Cross-Reactivity of Compounds **3**–**6** and UTP with dUTP-Binding Antiserum; B = Amount of Bound Radiolabel and B_0 = Amount of Bound Radiolabel When No Unlabelled dUTP Added; ne = Not Evaluable

Compound	Percentage Cross-reactivity Compared to dUTP		% B/B_0 in Presence of 20000 pmol Added Compound
	@ 50% binding	@ 70% binding	
dUTP	100	100	0
UTP	0.13	0.4	0
3	ne	0.001	61.4
4	0.002	0.004	46.3
5	ne	ne	85.1
6	ne	0.0006	69.5

group present in certain EGF receptor-associated protein tyrosine kinase inhibitors[9]. The carboxyl together with the hydroxyl group have been proposed in this context as superimposable on oxygen atoms of the γ and β phosphoryl groups of ATP. It may be speculated that the analogous substituents in **4** and **6** perform a similar role in relation to dUTP and in the present context it is noteworthy that the inclusion of the fluorine atoms appears beneficial.

dUTPase Activity Assay[10]. None of the compounds **3**–**6** was found to inhibit the activity of dUTPase.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 250 MHz using the residual undeuterated solvent signal as internal standard, on a Bruker spectrometer. ^{19}F spectra were obtained at 235 MHz on the same instrument, and chemical shifts are relative to $FCCl_3$ ($\delta_F = 0$). ESI mass spectra were obtained on a Finnigan MAT TSQ700 triple quadrupole mass spectrometer or a Finnigan LCQ ion trap mass spectrometer. FAB mass spectra were obtained at the School of Pharmacy, London University. The IR spectrum of **17** was obtained with a Perkin–Elmer 1720X spectrometer. Melting points were determined with a Reichert micro-hot-stage apparatus, and are uncorrected. Elemental analyses were carried out by C.H.N. Analysis Ltd, Leicester, England. Preparative reactions were monitored by TLC using silica-coated glass plates (Merck), visualised under UV light.

3′-*O*-(4-Methoxytetrahydropyran-4-yl)-5′-*O*-(2,3,5,6-tetrafluoro-4-nitro phenyl)-2′-deoxyuridine (8). Aqueous NaOH solution (1 M; 5 ml) was added to a rapidly stirred, cooled (water bath at 10 °C) mixture of 3′-***O***-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (**7**)[4] (0.344 g, 1 mmol), pentafluoronitrobenzene (0.220 g, 1 mmol), tetra-n-butylammonium hydrogensulfate (0.342 g, 1 mmol) and CH_2Cl_2 (5 ml). After 40 min, further pentafluoronitrobenzene (0.110 g, 0.52 mmol) was added, and after a further 20 min, CH_2Cl_2 (45 ml) was added. The CH_2Cl_2 layer was separated and the aqueous layer diluted with water (15 ml) and extracted with CH_2Cl_2 (4×5 ml). The combined CH_2Cl_2 solution was washed with water (3×10 ml) and dried ($MgSO_4$), then evaporated after addition of silica (Merck 7734; 2.7 g). The impregnated silica was applied to a column of silica (Merck 9385). Elution with CH_2Cl_2-MeOH (95:5) followed by evaporation of appropriate fractions, crystallisation (MeCN) of the residue, and recrystallisation gave **8** (0.202 g, 37.5%), mp 163–165 °C; $\delta_H[(CD_3)_2SO]$ 1.75 (m, 4H, Mthp 3,5-H), 2.33 (m, 2H, 2′-H), 3.15 (s, 3H, Me), 3.48, 3.62 ($2 \times$ m, each 2H, Mthp 2,6-H), 4.24 (m, 1H) and 4.58 (m, 3H) (3′,4′,5′-H), 5.63 (d, J = 8.2 Hz, 1H, 5-H), 6.18 (t, J = 6.8 Hz, 1H, 1′-H), 7.63 (d, J = 8.1 Hz, 1H, 6-H), 11.36 (s, 1H, N-H); $\delta_F[(CD_3)_2SO]$ –155.13 (m, 2F), –147.01 (m, 2F); HRMS (FAB) 536.1270; calcd for $C_{21}H_{22}F_4N_3O_9$ $[(M+H)^+]$, 536.1292; Found: C, 47.05; H, 3.90; N, 7.95; F, 14.06; $C_{21}H_{21}F_4N_3O_9$ requires C, 47.11; H, 3.95; N, 7.85; F, 14.19%.

3′-*O*-(4-Methoxytetrahydropyran-4-yl)-5′-*O*-(2,3,6-trifluoro-5-hydroxy-4-nitro-phenyl)-2′-deoxyuridine (9). Compound **8** (0.122 g, 0.23 mmol) was stirred with aqueous NaOH solution (0.1 M; 11.5 ml) at room temperature. The reaction could be monitored by TLC with CH_2Cl_2-MeOH (4:1) or Pr^iOH-aq. NH_3 (d 0.88)-H_2O (7:1:2). After 44 h the solution was cooled in ice and adjusted to pH 5 with acetic acid whilst being stirred. The resulting suspension was concentrated to *ca.* 10 ml then cooled, and the solid which had separated was collected, washed with cold water, and dried to give **9** (0.110 g, 90.5%), mp 156–158 °C; $\delta_H[(CD_3)_2SO]$ 1.74 (m, 4H, Mthp 3,5-H), 2.31 (m, 2H, 2′-H), 3.15 (s, 3H, Me), 3.48 (m, overlaps HOD peak) and 3.62 (m, 2H) (Mthp 2,6-H), 4.22 (m, 1H) and 4.51 (m, 3H) (3′,4′,5′-H), 5.62 (dd, J = 2.1, 8.1 Hz, 1H, 5-H), 6.19 (t, J = 6.8 Hz, 1H, 1′-H), 7.63 (d, J = 8.1 Hz, 1H, 6-H), 11.35 (s, 1H, N-H); $\delta_F[(CD_3)_2SO]$ – 163.48 (m, 1F), – 151.85 (m, 2F); ESI MS (– ve ion mode) *m/z* 532.1 $[(M-H)^-]$; HRMS (FAB) 534.1337; calcd for $C_{21}H_{23}F_3N_3O_{10}$ $[(M+H)^+]$, 534.1336; Found: C, 47.05; H, 4.04; N, 7.85; $C_{21}H_{22}F_3N_3O_{10}$ requires C, 47.29; H, 4.16; N, 7.88%.

5′-*O*-(2,3,6-Trifluoro-5-hydroxy-4-nitrophenyl)-2′-deoxyuridine (3). Compound **9** (0.090 g, 0.169 mmol), acetic acid (2 ml) and water (0.5 ml) were stirred together at 23 °C. After 15 h the mixture was evaporated and the solid residue triturated with Et_2O to give a solid (0.071 g). Part (0.045 g) of this material was

crystallised from MeOH-H_2O to give **3** (0.039 g, 85%), mp 178–179 °C; δ_H[$(CD_3)_2SO$] 2.19 (m, 2H, 2′-H), 4.05 and 4.33 (2 × m, each 1H, 3′-H, 4′-H), 4.47 (m, 2H, 5′-H), 5.48 (br, 1H, OH), 5.61 (dd, J = 2.1, 8.1 Hz, 1H, 5-H), 6.20 (t, J = 6.9 Hz, 1H, 1′-H), 7.63 (d, J = 8.1 Hz, 1H, 6-H), 11.34 (s, 1H, N-H); δ_F[$(CD_3)_2SO$] − 163.22 (m, 1F), − 151.76 (m, 2F); ESI MS (− ve ion mode) *m/z* 418.1 [$(M-H)^-$]; HRMS (FAB) 420.0640; calcd for $C_{15}H_{13}F_3N_3O_8$ [$(M+H)^+$], 420.0655; Found: C, 41.85; H, 2.92; N, 9.74; F, 13.33; $C_{15}H_{12}F_3N_3O_8 \cdot 0.5H_2O$ requires C, 42.07; H, 3.06; N, 9.81; F, 13.31%.

5′-*O*-(3-Fluoro-4-nitrophenyl)-3′-*O*-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (10). Diisopropyl azodicarboxylate (0.392 g, 1.9 mmol) was added dropwise during 5 min to a stirred, cooled (ice-water bath) solution of 3′-O-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (**7**)[4] (0.516 g, 1.5 mmol), 3-fluoro-4-nitrophenol (0.262 g, 1.67 mmol) and triphenylphosphine (0.47 g, 1.8 mmol) in dry THF (9 ml) under N_2. The mixture was then allowed to warm to room temperature. After 19 h the mixture was concentrated and the residue chromatographed [Merck 7729 silica; CH_2Cl_2-EtOH (100:0 to 95:5)]; further purification by a second chromatography column [Merck 7729 silica; CH_2Cl_2-EtOH (100:0 to 97:3)] gave a white solid which was crystallised from MeCN to give **10** (0.276 g, 38%), mp 206–208 °C (remelting of resolidified crystals); δ_H[$(CD_3)_2SO$] 1.74 (m, 4H, Mthp 3,5-H), 2.32 (m, 2H, 2′-H), 3.14 (s, 3H, Me), 3.47, 3.62 (2 × m, each 2H, Mthp 2,6-H), 4.24 (m, 1H, 3′-H or 4′-H), 4.34 (m, 2H, 5′-H), 4.56 (m, 1H, 4′-H or 3′-H), 5.65 (d, J = 8.1 Hz, 1H, 5-H), 6.19 (t, J = 6.8 Hz, 1H, 1′-H), 7.02 (dt, J = 1.2, 9.3 Hz, 1H, aryl H), 7.25 (dd, J = 2.6, 13.6 Hz, 1H, aryl H), 7.64 (d, J = 8.0 Hz, 1H, 6-H), 8.16 (t, J = 9.2 Hz, 1H, aryl H), 11.31 (s, 1H, N-H); δ_F[$(CD_3)_2SO$] − 113.86 (t, J = 11.7 Hz); HRMS (FAB) 482.1562; calcd for $C_{21}H_{25}FN_3O_9$ [$(M+H)^+$], 482.1575; Found: C, 52.21; H, 4.97; N, 8.71; F, 3.93; $C_{21}H_{24}FN_3O_9$ requires C, 52.39; H, 5.02; N, 8.73; F, 3.95%.

5′-*O*-(3-Hydroxy-4-nitrophenyl)-3′-*O*-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (11). Compound **10** (0.130 g, 0.27 mmol) was stirred with aqueous NaOH solution (1.5 M; 3.6 ml) at room temperature for 14 days [this reaction may be followed by TLC with Pr^iOH − aq. NH_3 (d 0.88) − H_2O (7:1:2)]. The resulting solution was cooled in ice and acetic acid (0.33 ml) was added with stirring. The solid which separated was collected, washed with water, dried, and crystallised from EtOH to give **11** (0.110 g, 85%) as a yellow solid, mp 172–174 °C; δ_H[$(CD_3)_2SO$-D_2O] 1.75 (m, 4H, Mthp 3,5-H), 2.34 (m, 2H, 2′-H), 3.13 (s, 3H, Me), 3.48, 3.61 (2 × m, each 2H, Mthp 2,6-H), 4.25 (m, 3H, 5′-H and 3′-H or 4′-H), 4.55 (m, 1H, 4′-H or 3′-H), 5.65 (d, J = 8.1 Hz, 1H, uracil 5-H), 6.18 (t, J = 6.7 Hz, 1H, 1′-H), 6.66 (m, 2H, aryl 2,6-H), 7.66 (d, J = 8.1 Hz, 1H, uracil 6-H), 7.98 (d, J = 9.1 Hz, 1H, aryl 5-H); before addition of D_2O, additional signals [10.94 (br s, 1H) and 11.32 (s, 1H)

(OH and N-H)] were apparent; ESI MS (−ve ion mode) *m/z* 478.1 [(M − H)$^-$]; HRMS (FAB) 502.1454; calcd for $C_{21}H_{25}N_3NaO_{10}$ [(M+Na)$^+$], 502.1438; Found: C, 52.40; H, 5.26; N, 8.68; $C_{21}H_{25}N_3O_{10}$ requires C, 52.61; H, 5.26; N, 8.76%.

5′-*O*-(3-Hydroxy-4-nitrophenyl)-2′-deoxyuridine (5). Compound **11** (0.072 g, 0.15 mmol), acetic acid (4 ml) and water (1 ml) were stirred together at room temperature. After 19 h the solution was evaporated to dryness and the residue triturated with Et_2O. The resulting solid was collected after cooling, washed with further Et_2O, and crystallised from water to give **5** (0.047 g, 83%), mp 120−122 °C; δ_H[$(CD_3)_2SO$] 2.21 (m, 2H, 2′-H), 4.07 (m, 1H) and 4.26 (m, 3H) (3′,4′,5′-H), 5.44 (d, J = 4.3 Hz, 1H, 3′-OH), 5.63 (m, 1H, reduced to d, J = 8.1 Hz, 1H, on addition of D_2O, uracil 5-H), 6.20 (t, J = 6.7 Hz, 1H, 1′-H), 6.66 (m, 2H, aryl 2,6-H), 7.66 (dd, J = 1.2, 8.2 Hz, 1H, reduced to d, J = 8.1 Hz, 1H, on addition of D_2O, uracil 6-H), 7.97 (m, 1H, reduced to d, J = 9.4 Hz, 1H, on addition of D_2O, aryl 5-H), 10.93 (s, 1H) and 11.28 (s, 1H) (aryl OH and N-H); ESI MS (−ve ion mode) *m/z* 363.9 [(M − H)$^-$]; HRMS (FAB) 366.0947; calcd for $C_{15}H_{16}N_3O_8$ [(M+H)$^+$], 366.0937; Found: C, 47.53; H, 4.16; N, 11.08; $C_{15}H_{15}N_3O_8 \cdot 0.75H_2O$ requires C, 47.56; H, 4.39; N, 11.09%.

5′-*O*-(4-Oxo-5,6,8-trifluoro-4*H*-1,3-benzodioxin-7-yl)-2′-deoxyuridine (14). Diisopropyl azodicarboxylate (DIAD) (0.066 g, 0.33 mmol) was added dropwise during 5 min to a stirred, cooled (ice-water bath) solution of 3′-*O*-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (**7**)[4] (0.092 g, 0.27 mmol), 5,6,8-trifluoro-7-hydroxy-4H-1,3-benzodioxin-4-one (**13**)[2b] (0.059 g, 0.27 mmol) and triphenylphosphine (0.087 g, 0.33 mmol) in dry THF (2 ml) under argon. The mixture was then allowed to warm to room temperature. After 2 h, further triphenylphosphine (0.028 g, 0.11 mmol) and DIAD (0.022 g, 0.11 mmol) were added, and after 20 h (total) the mixture was concentrated. A portion of CH_2Cl_2 was added and evaporated and the residue chromatographed [Merck 7729 silica; CH_2Cl_2-EtOH (100:0, 98:2 and 97:3 in succession)] to give a colourless glass (0.115 g); δ_H[$(CD_3)_2SO$] 1.75 (m, 4H, Mthp 3,5-H), 2.33 (m, 2H, 2′-H), 3.15 (s, 3H, Me), 3.48, 3.62 (2 × m, each 2H, Mthp 2,6-H), 4.23 (m, 1H) and 4.59 (m, 3H) (3′,4′,5′-H), 5.61 (d, J = 8.2 Hz, 1H, 5-H), 5.89 (s, 2H, O-CH_2-O), 6.18 (t, J = 6.8 Hz, 1H, 1′-H), 7.63 (d, J = 8.2 Hz, 1H, 6-H), 11.31 (s, 1H, N-H); δ_F[$(CD_3)_2SO$] −159.17 (d, J = 22.9 Hz, 1F), −155.07 (d, J = 10.8 Hz, 1F), −139.38 (dd, J = 11.8, 22.0 Hz, 1F); HRMS (FAB) 567.1230; calcd for $C_{23}H_{23}F_3N_2NaO_{10}$ [(M+Na)$^+$], 567.1202. A solution of this material (0.105 g) in acetic acid (2 ml) and water (0.5 ml) was stirred at room temperature. After 23 h the solution was evaporated and portions of PhMe-EtOH (1:1; 4 × 10 ml) were added to the residue and evaporated successively. The resulting white solid was triturated with Et_2O, then crystallised from EtOH to give **14** (0.065 g,

61.5% overall from **7**), mp 195–197 °C; δ_H[$(CD_3)_2SO$] 2.22 (m, 2H, 2′-H), 4.07 and 4.35 (2 × m, each 1H, 3′-H, 4′-H), 4.62 (m, 2H, 5′-H), 5.51 (br s, OH), 5.61 (d, J = 8.1 Hz, 1H, 5-H), 5.90 (s, 2H, O-CH_2-O), 6.20 (t, J = 6.8 Hz, 1H, 1′-H), 7.63 (d, J = 8.1 Hz, 1H, 6-H), 11.35 (s, 1H, N-H); δ_F[$(CD_3)_2SO$] − 159.00 (d, J = 21.2 Hz, 1F), − 154.90 (d, J = 11.8 Hz, 1F), − 139.31 (dd, J = 11.8, 21.2 Hz, 1F); HRMS (FAB) 431.0718; calcd for $C_{17}H_{14}F_3N_2O_8$ [$(M+H)^+$], 431.0702; Found: C, 47.29; H, 3.03; N, 6.42; F, 13.11; $C_{17}H_{13}F_3N_2O_8$ requires C, 47.45; H, 3.05; N, 6.51; F, 13.25%.

5′-*O*-(4-Carboxy-2,3,6-trifluoro-5-hydroxyphenyl)-2′-deoxyuridine (4). Compound **14** (0.048 g, 0.11 mmol) was stirred with aqueous NaOH solution (0.1 M; 3.3 ml) at room temperature. After 40 min the solution was diluted with MeOH (5 ml) and water (5 ml), and acetic acid (0.069 g) was added. The resulting slightly turbid solution was applied to a column (bed volume 9 ml) of Bio-Rad AG 50W-X4 100–200 mesh cation exchange resin (H^+ form) that had been packed in MeOH-H_2O (1:1). MeOH-H_2O (1:1) was passed through the column until the emerging eluate was no longer UV-absorbing. The product-containing fractions were combined and concentrated to *ca.* 4 ml and the solid which separated was collected after cooling the suspension, washed with cold water, and dried to give **4** (0.038 g, 81%), mp 240 °C (decomp.); δ_H[$(CD_3)_2SO$-D_2O] 2.19 (m, 2H, 2′-H), 4.03 and 4.34 (2 × m, each 1H, 3′-H, 4′-H), 4.46 (m, 2H, 5′-H), 5.60 (d, J = 8.1 Hz, 1H, 5-H), 6.19 (t, J = 6.8 Hz, 1H, 1′-H), 7.63 (d, J = 8.1 Hz, 1H, 6-H) [before addition of D_2O, additional signal at 11.33 (s, 1H, N-H) was apparent]; δ_F[$(CD_3)_2SO$] − 165.70 (m, 1F), − 156.84 (m, 1F), − 140.66 (m, 1F); ESI MS (− ve ion mode) *m/z* 416.9 [$(M-H)^-$]; HRMS (FAB) 419.0685; calcd for $C_{16}H_{14}F_3N_2O_8$ [$(M+H)^+$], 419.0702; Found: C, 45.82; H, 3.05; N, 6.64; F, 13.41; $C_{16}H_{13}F_3N_2O_8$ requires C, 45.94; H, 3.13; N, 6.70; F, 13.63%.

Phenyl 2,4-Dihydroxybenzoate (16)[11]. Phosphorus oxychloride (3.4 ml, 36.5 mmol) was added to a mixture of 2,4-dihydroxybenzoic acid (10.0 g, 64.9 mmol), phenol (68.0 g, 72.3 mmol), and dry PhMe (50 ml). The mixture was stirred under Ar at 100 °C (bath temperature) for 2 h, then boiled under reflux (bath temperature = 150 °C) for 30 min. It was then allowed to cool to room temperature and partitioned between $CHCl_3$ (200 ml) and water (200 ml). The aqueous layer was extracted with $CHCl_3$ (4 × 25 ml) and the combined $CHCl_3$ solution washed successively with water (100 ml) and half-saturated brine (3 × 100 ml), dried ($MgSO_4$) and concentrated thoroughly (water pump followed by oil pump). The solid residue was crystallised from hexane-EtOAc to give **16** (6.769 g, 45%) as pale pink crystals, mp 148–150 °C (lit[7a]., 147–149 °C); δ_H[$(CD_3)_2SO$] 6.37 (d, J = 2.2 Hz, 1H, dihydroxyphenyl 3-H), 6.46 (dd, J = 2.0, 8.8 Hz, 1H, dihydroxyphenyl 5-H), 7.28 (m, 3H) and 7.47 (m, 2H) (Ph), 7.88 (d, J = 8.8 Hz, 1H, dihydroxyphenyl 6-H), 10.39 (s, OH), 10.57 (s, OH);

HRMS (FAB) 231.0646; calcd for $C_{13}H_{11}O_4$ [$(M+H)^+$], 231.0657. A sample for analysis was recrystallised with a little silica (Merck 7734) being added to the hot solution before filtration; Found (for recrystallised material): C, 67.54; H, 4.29; N, 0.00; $C_{13}H_{10}O_4$ requires C, 67.82; H, 4.38; N, 0.00%.

7-Hydroxy-4*H*-1,3-benzodioxin-4-one (17). $CHCl_3$ (2.75 ml) was added to a stirred mixture of **16** (3.657 g, 15.9 mmol), paraformaldehyde (2.38 g) and 1,4-diazabicyclo[2.2.2]octane (Dabco) (1.78 g, 15.9 mmol) at room temperature. After 30 h, further Dabco (1.78 g) was added. The reaction was followed by TLC with CH_2Cl_2-EtOAc (19:1). After 97 h (total time) the mixture was partitioned between EtOAc (100 ml) and 10% w/w aqueous citric acid solution (200 ml). The aqueous layer was filtered to remove insoluble solid material, and extracted with EtOAc (5×70 ml). The combined EtOAc solution was washed successively with 10% w/w aqueous citric acid solution (70 ml followed by 25 ml) and saturated brine (35 ml), then dried ($MgSO_4$) and evaporated. The residue was redissolved in EtOAc (50 ml) and the solution evaporated after addition of silica (Merck 7734; 10 g). Hexane was added and evaporated and the impregnated silica applied to a column of silica (Merck 9385). Elution with hexane-EtOAc (3:2 and 1:1 in succession), evaporation of appropriate fractions, and crystallisation (hexane-EtOAc) of the residue gave **17** (0.832 g, 31.5%), mp 128–130 °C; $\delta_H[(CD_3)_2SO]$ 5.73 (s, 2H, 2-H), 6.46 (d, J = 2.2 Hz, 1H, 8-H), 6.66 (dd, J = 2.2, 8.7 Hz, 1H, 6-H), 7.72 (d, J = 8.7 Hz, 1H, 5-H), 10.90 (s, OH); ν_{max}(KBr disc) 1614, 1699 cm^{-1}; ESI MS (−ve ion mode) *m/z* 165.1 [$(M-H)^-$]; HRMS (FAB) 167.0342; calcd for $C_8H_7O_4$ [$(M+H)^+$], 167.0344; Found: C, 57.63; H, 3.57; N, 0.01; $C_8H_6O_4$ requires C, 57.84; H, 3.64; N, 0.00%.

3′-*O*-(4-Methoxytetrahydropyran-4-yl)-5′-*O*-(4-oxo-4*H*-1,3-benzodioxin-7-yl)-2′-deoxyuridine (18). Diethyl azodicarboxylate (0.249 g, 1.4 mmol)[12] was added dropwise during 20 min to a stirred, cooled (water bath at 10 °C) solution of 3′-*O*-(4-methoxytetrahydropyran-4-yl)-2′-deoxyuridine (**7**)[4] (0.500 g, 1.46 mmol), **17** (0.244 g, 1.47 mmol) and triphenylphosphine (0.383 g, 1.46 mmol) in dry THF (8 ml) under argon. After a further 5 min, the solution was allowed to warm to room temperature. After 27 h the solution was evaporated and the residue chromatographed on silica (Merck 7729); higher R_F materials were eluted with CH_2Cl_2 followed by CH_2Cl_2-EtOH (98.5:1.5 then 98:2), and the desired product with CH_2Cl_2-EtOH (97.5:2.5). Appropriate fractions were evaporated and the residual glass was triturated with hexane to give **18** (0.242 g, 33.8%), which was used in the next step; $\delta_H[(CD_3)_2SO]$ 1.76 (m, 4H, Mthp 3,5-H), 2.32 (m, 2H, 2′-H), 3.14 (s, 3H, Me), 3.48, 3.62 ($2 \times$ m, each 2H, Mthp 2,6-H), 4.28 (m, 3H) and 4.55 (m, 1H) (3′,4′,5′-H), 5.66 (d, J = 8.1 Hz, 1H, uracil 5-H), 5.79 (s, 2H, O-CH_2-O), 6.20 (t, J = 6.7 Hz, 1H, 1′-H), 6.82 (m, 2H, benzodioxin 6,8-H),

7.67 (d, J = 8.1 Hz, 1H, uracil 6-H), 7.82 (d, J = 8.7 Hz, 1H, benzodioxin 5-H), 11.35 (s, 1H, N-H); HRMS (FAB) 491.1651; calcd for $C_{23}H_{27}N_2O_{10}$ $[(M+H)^+]$, 491.1666. Attempted crystallisation (MeOH) of a sample of this material from another run gave an amorphous white solid, mp 142–144 °C, which was used for elemental analysis; Found: C, 56.21; H, 5.34; N, 5.69; $C_{23}H_{26}N_2O_{10}$ requires C, 56.32; H, 5.34; N, 5.71%.

5′-*O*-(4-Carboxy-3-hydroxyphenyl)-2′-deoxyuridine (6). Compound **18** (0.168 g, 0.34 mmol) was stirred with aqueous NaOH solution (0.05 M; 34 ml) at room temperature. TLC [$CHCl_3$-MeOH-AcOH (95:5:3)] after 10 min indicated complete conversion to a product of lower R_F than **18**. After 25 min (total time), the solution obtained was cooled in ice and acetic acid (0.1 ml) added; the pH was then found to be *ca*. 5. The mixture was concentrated to *ca*. 3.9 ml , and further acetic acid (7.8 ml) added. The resulting mixture was stirred at room temperature for 34 h, then evaporated, and water (2 ml) was added to the residue and evaporated. The residue was triturated with Et_2O and dried. Water (2 ml) was added and the resulting mixture heated briefly, then cooled in ice. The solid which separated was collected, washed with cold water, and dried to give **6** (0.100 g, 80%), mp 205 °C (decomp.); $\delta_H[(CD_3)_2SO]$ 2.20 (m, 2H, 2′-H), 4.06 (m, 1H, 3′- or 4′-H), 4.21 (m, 2H, 5′-H), 4.31 (m, 1H, 4′- or 3′-H), 5.45 (br s, 1H, 3′-OH), 5.63 (dd, J = 2.1, 8.1 Hz, 1H, uracil 5-H), 6.21 (t, J = 6.8 Hz, 1H, 1′-H), 6.55 (m, 2H, aryl 2,6-H), 7.69 (m, 2H, aryl 5-H, uracil 6-H), 11.33 (s, 1H, N-H), 11.51 (br s, 1H, aryl OH), 13.60 (br s, 1H, CO_2H); ESI MS (−ve ion mode) *m/z* 363.1 $[(M-H)^-]$; HRMS (FAB) 365.0992; calcd for $C_{16}H_{17}N_2O_8$ $[(M+H)^+]$, 365.0985; Found: C, 52.50; H, 4.39; N, 7.65; $C_{16}H_{16}N_2O_8$ requires C, 52.75; H, 4.43; N, 7.69%.

dUTP Radioimmunoassay. Compounds **3**–**6** were dissolved in PBS at an initial concentration of 1 mg/ml. Various dilutions of these solutions were included in a radioimmunoassay for dUTP[8]. The assay was carried out as previously reported except that whole antiserum was used (R9 1/1000) and dilutions and dispensing of reagents were carried out using a Multiprobe (Canberra Packard).

dUTPase Activity Assay. Compounds **3**–**6** were tested for ability to inhibit the activity of dUTPase. The dUTPase activity assay was based on a reported method[10]. A cellular extract from the human lung carcinoma cell line MOR, which has been shown to have high dUTPase activity, was employed[13]. Tritiated dUTP was incubated with the cellular extract and the product, tritiated dUMP, separated from substrate by TLC and quantitated using a plate scanner. Compounds were assayed at 20, 50, 100, and 200 μM.

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