

PREPARATION AND PRELIMINARY EVALUATION OF NOVEL β -CYCLODEXTRIN/IUDR PRODRUG FORMULATIONS

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ABSTRACT – PURPOSE. Iododeoxyuridine (IUdR) has a very short *in vivo* half-life and consequently achieves low target-tissue concentrations with concomitant lower efficacy than would be predicted from *in vitro* studies. This work reports the preparation of IUdR: β -cyclodextrin (β -CyD) inclusion complexes designed to reduce *in vivo* inactivation of IUdR. **METHODS.** IUdR was derivatized with either 1-adamantanecarbonyl chloride or 4-(1-adamantylcarbamoyl)butanoic acid, to prepare 5'-O-(1-adamantoyl)-5-iodo-2'-deoxyuridine **1** and 5'-O-(4-(1-adamantylcarbamoyl)butoyl)-5-iodo-2'-deoxyuridine **4**, respectively. β -CyD complexes **5** and **6** were formed by vigorous stirring of 1:1 solutions of β -CyD and **1** or **4**, respectively, in D₂O under argon. Complexation was inferred from DSC, powder x-ray diffractometry and NMR spectrometry. The dissociation of **5** in water and under cholesterol challenge, and the effect of complexation on the stability of **1** was determined by incubation in plasma. **RESULTS.** IUdR coupling with adamantanecarbonyl chloride proceeded smoothly to afford **1** (69 %) and the di-substituted derivative, 3',5'-di-O-(1-adamantoyl)-5-iodo-2'-deoxyuridine **2** (8 %); **4** was obtained in 42 % yield. The formation of 1:1 complexes **5** and **6** was inferred from NMR chemical shift data. In serum, **1** was 90 % hydrolyzed to IUdR in 30 min, compared to 10 % hydrolysis of **1** to IUdR when from complex **5**. **CONCLUSIONS.** Inclusion complexes were formed between β -CyD and adamantamine-IUdR conjugates at 1:1 molar ratios.

The complex **5** was resistant to dissociation by cholesterol challenge, and **5** was more slowly converted to IUdR than non-complexed **1**. *In vivo* studies are required to further exploit the β -CyD inclusion complex approach for improved delivery of nucleoside derivatives.

INTRODUCTION

The application of halogenated pyrimidine nucleosides to the measurement of cell proliferation and to induce radiation sensitization is well established (1,2). Biologically, the halogen-substituted analogues of thymidine (TdR), such as 5-iodo-2'-deoxyuridine (IUdR), compete with TdR for phosphorylation and are subsequently incorporated into DNA. When labeled with radioisotopes of iodine, IUdR is suitable for radiodiagnosis and radiotherapy (3-5). There is compelling evidence from loco-regional administration studies that radiolabelled IUdR is effective for the treatment of tumors characterized by high cell proliferation kinetics, such as liver tumors resulting from the metastasis of colorectal cancer (6), and high grade malignant gliomas (7). In fact, when radiolabeled with an Auger electron emitter such as ¹²³I or ¹²⁵I, IUdR exhibits substantial *in vitro* toxicity when taken up by mammalian cells (8). Although the intranuclear incorporation rate of IUdR by dividing cells *in vitro* is high, IUdR undergoes rapid *in vivo* metabolic degradation, and its short half-life *in vivo* (5-7 minutes)(9) severely hampers its clinical utility.

Like many cell cycle dependent drugs, IUdR is incorporated during S phase to synthesize DNA in preparation for cell duplication. A critical local concentration must be maintained for adequate uptake, usually at the cost of systemic toxicity. Unfortunately, the rapid metabolic degradation of IUdR *in vivo* militates against protracted elevated blood levels even when high doses are administered.

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A formulation which would improve the biological stability of IUdR should therefore facilitate its incorporation into DNA. At the same time, any free compound should be rapidly cleared in order to reduce systemic toxicity by minimizing the dose to normal dividing cells (i.e., bone marrow and intestine) and the thyroid (for radioiodinated IUdR). Cyclodextrins (CyDs) are well suited for oral drug delivery applications (10), but potential toxicity and sheer bulk have precluded their utilization for parenterally-delivered drugs. In the case of radiotherapeutic drugs, however, where the chemical (bulk) dose is very small, CyD toxicity would not be an issue. Due to their intrinsic cyclic nature, CyDs are metabolized at much slower rates than their open chain analogs. In terms of molecular architecture, CyDs can be viewed as hollow truncated conical cylinders with a hydrophilic outer surface inscribing a hydrophobic internal cavity. The cavity sizes for the commonly available α -, β - and γ -CyD are 4.9, 6.2 and 7.9 Å, respectively (11), dimensions which are ideal for the inclusion of low molecular weight lipophilic drugs. This ability to form inclusion complexes has been exploited to alter the chemical and physical properties of guest (drug) molecules, to effect improved water solubility, prolong in vivo stability, reduce toxicity and irritancy, and improve bioavailability (12). Release of the guest molecule is governed by dissociation, and therefore selective chemical modifications can be employed to control equilibrium thermodynamics, and thus release rate.

This paper reports the synthesis of IUdR prodrugs for complexation with β -CyD, with the objective of improving the efficacy of intravenously administered IUdR. Direct complexation of nucleosides and nucleotides with cyclodextrin hosts has been previously reported (13,14), but our attempts to complex IUdR with various CyDs offered no evidence of complexation-induced chemical shifts in the NMR spectra of either the host or guest molecule, thus failing to confirm that inclusion had occurred. The primary task in the current work, therefore, was to address the issue of the restricted host cavity size of β -CyD and dissociation of the inclusion complex. To this end, the strategy for preparing prodrugs equipped with a high affinity ligand for the β -CyD cavity, namely the adamantane skeleton, has been explored.

The high binding affinity of the adamantane skeleton for β -CyD is well documented (15-18), yet the approach of utilizing this ligand for general drug delivery remains relatively unexplored. In the current work, adamantane guest ligands were selected on the basis that they are available with either a carboxyl or amino functionality, and therefore are amenable to standard peptide chemistry. This allows for the convenient assembly of prodrugs from the parent drug via an ester or amide bond, which is bio-hydrolyzable and thereby ensures the release of the free drug in vivo. The preparation of two IUdR-adamantane prodrugs connected via a short and a long bio-cleavable linker arm, and their formulation with β -CyD, are now reported.

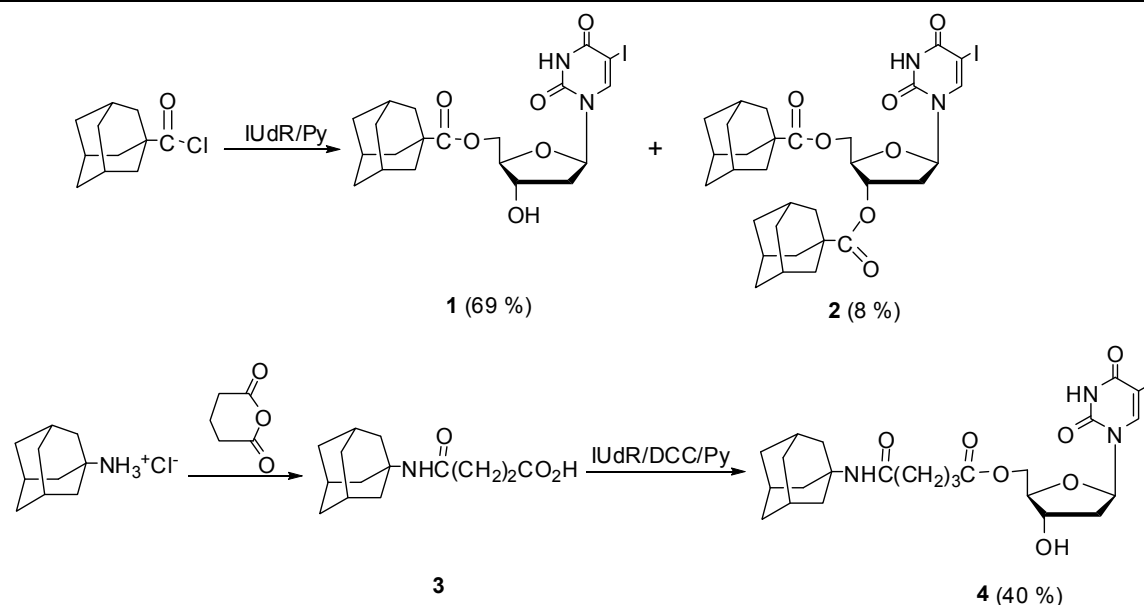
RESULTS

The approach to the synthesis of IUdR-adamantane prodrugs **1** and **4** is shown in Scheme 1. Thus, a solution of IUdR in pyridine was treated with freshly prepared 1-adamantanecarbonyl chloride (1.4 equiv) to give esters **1** and **2** in 69 % and 8 % yield, respectively, after column chromatography over silica gel.

The preparation of an IUdR prodrug with a longer linker arm required modification of 1-adamantanamine hydrochloride. This material was treated with glutaric anhydride (1.5 equiv) in 1:1 pyridine/chloroform at 75 °C. Adamantane derivative **3** was obtained in 81 % yield after aqueous work-up and trituration with chloroform. Dicyclohexylcarbodiimide mediated coupling of **3** to IUdR in pyridine at 70 °C afforded IUdR prodrug **4** (42 %) after purification on silica gel.

Both **1** and **4** were found to be poorly water soluble. The aqueous solubility of **1** was determined to be 26 µg/mL at 25 °C, with an octanol-water partition coefficient (LogP) of 1.91 (calculated 1.85) and an octanol-phosphate buffer partition coefficient of 1.97. The solubility of **1** in the presence of β -CyD determined a water solubility of 8.26 mg/mL (Fig. 1) with an A_L phase solubility diagram.

The formation of 1:1 inclusion complexes between β -CyD and IUdR derivatives **1** and **4** was carried out at 50-60 °C for 30 min with vigorous agitation.



Scheme 1. Synthesis of IUdR-adamantane derivatives.

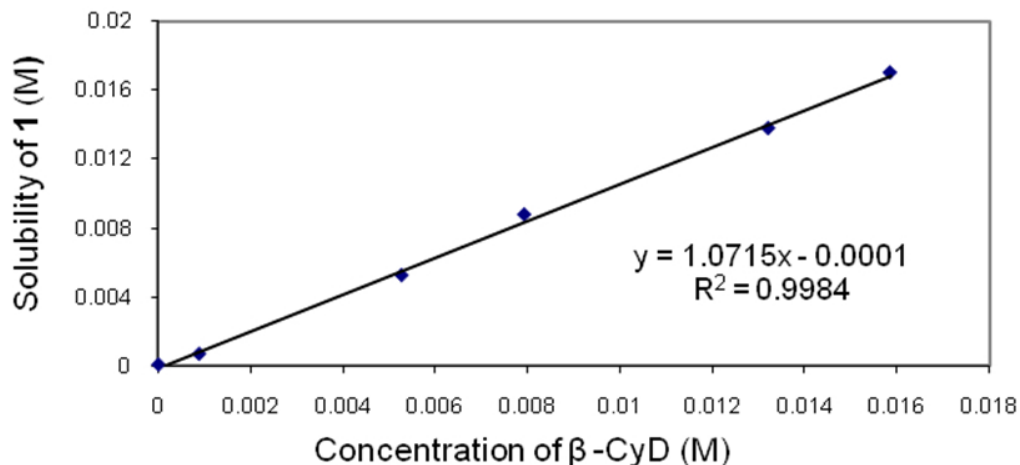


Figure 1. Solubility of **1** as a function of β -CyD concentration. Calculated data were derived using Excel (Microsoft).

The non-complexed prodrug was then removed by filtration prior to analysis of the complex by differential scanning calorimetry (DSC), powder X-ray diffraction and ^1H NMR. DSC scans of **1**, β -CyD, **1** and β -CyD mixed and complex **5** were obtained in the temperature range from 40-250 $^\circ\text{C}$ (data not shown). The thermogram of **1** featured a single exothermic peak at 208 $^\circ\text{C}$ whereas β -CyD had a very broad transition range from 70-180 $^\circ\text{C}$ and a small endothermic peak at 220 $^\circ\text{C}$. The mixture of **1** and β -CyD produced two endothermic

peaks (220 $^\circ\text{C}$ and 227 $^\circ\text{C}$) and an exothermic peak at 240 $^\circ\text{C}$. The complex **5** had an endothermic peak at 235 $^\circ\text{C}$. Prodrug **1** decomposed at its endothermic peak temperature (208 $^\circ\text{C}$).

Further evidence of complex formation was obtained from X-ray power diffraction studies. The X-ray powder diffractogram of the physical mixture was simply the superposition of each component (**1** and β -CyD) with the peaks having lower intensity, whereas the diffractogram of **5** consisted of a single broad peak of moderate intensity (data not shown).

The proton NMR spectra of complexes **5** and **6** provided the strongest evidence of complex formation, inferring the presence of a 1:1 mixture of host β -CyD and guest **1** by proton resonance integration (data not shown). Formation of inclusion complexes **5** and **6** was further confirmed by the presence of upfield shifts for H-3 and H-5 (0.08 and 0.09 ppm, respectively) for the corresponding complexes **5** and **6** (Scheme 2).

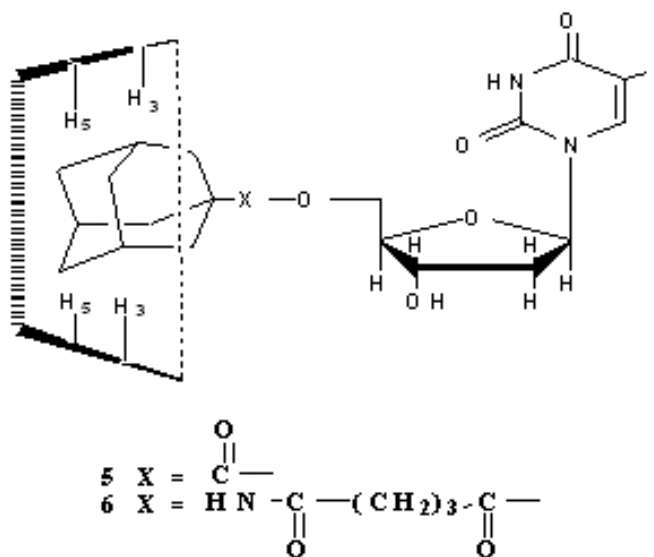
The dissociation of complex **5** in aqueous solution (1.2 and 6.2 mM) was determined as a function of time at 37 °C (Fig. 2). Aliquots taken at prescribed intervals over 3 h were extracted with octanol and the octanol phase was assayed for **1** by HPLC. The regression lines for the semi-log plots of the 0-3 h data are described by the equations $y = -0.6178x + 90.908$, $R^2 = 0.9554$ and $y = -0.6143x + 97.408$, $R^2 = 0.9756$; dissociation half-lives were 40 and 65 min, respectively, based on 3-h data.

The stability of **5** in the presence of cholesterol, a major, high-affinity physiological ligand for β -CyD was determined using this simple experimental approach, using various concentrations of cholesterol in the octanol phase of the two-phase system. The presence of cholesterol had no discernable influence on the release of **1** from **5** (Fig. 3) in that the data for control (no cholesterol) was not measurably different from the experiment containing equimolar or a ten-fold molar excess of cholesterol.

It was also of interest to observe the dissociation of **5** (to **1** and β -CyD) and subsequent hydrolysis of **1** in plasma to form IUdR. Data were obtained at 1.2 mM and 6.2 mM **5** in plasma-octanol. Data for the 6.2 mM experiment are shown in Figure 4. At 1.2 mM, approximately 60 % of **1** remained after 80 min (~40 % is hydrolyzed to IUdR), while at 6.2 mM, even after 5 h, 60 % of the prodrug **1** still remained. Slopes of the regression lines for concentrations of **1** were -0.5338 ($R^2 = 0.9972$) and -0.6143 ($R^2 = 0.9756$) at 1.2 and 6.2 mM, respectively. The apparent transfer of **1** from plasma into octanol is much slower (slope -0.174, $R^2 = 0.8907$; 1.2 mM), reflecting the hydrolysis of **1** as a competing reaction in plasma. In another experiment, when dissolved in plasma:DMSO=19:1 at a lower concentration (0.3 mM) to circumvent difficulties introduced by the low solubility of **1** in water, 90 % of **1** was hydrolyzed in 30 min, compared to 10 % for **5**.

DISCUSSION

The utilization of O'-adamantyl derivitization as a protective device in nucleoside synthesis is a forty-plus year old concept (19).



Scheme 2. CyD/IUdR-Prodrug complexes **5** and **6**, with schematic representation of β -CyD protons H-3 and H-5. Only two of seven sets of H-3 and H-5 protons are depicted.

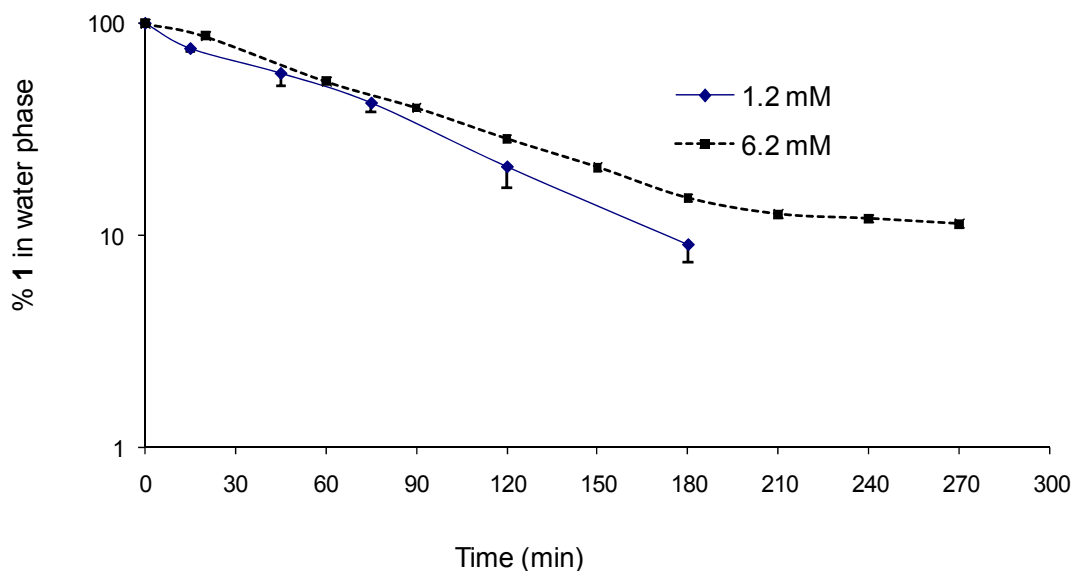


Figure 2. Dissociation of **1** (1.2 and 6.2 mM) in water. Each time interval represents the average of 3 determinations, and lower error bars (1.2 mM only) represent the standard deviation. Lines are smoothed point-to-point fit. Calculated data were derived using Excel (Microsoft).

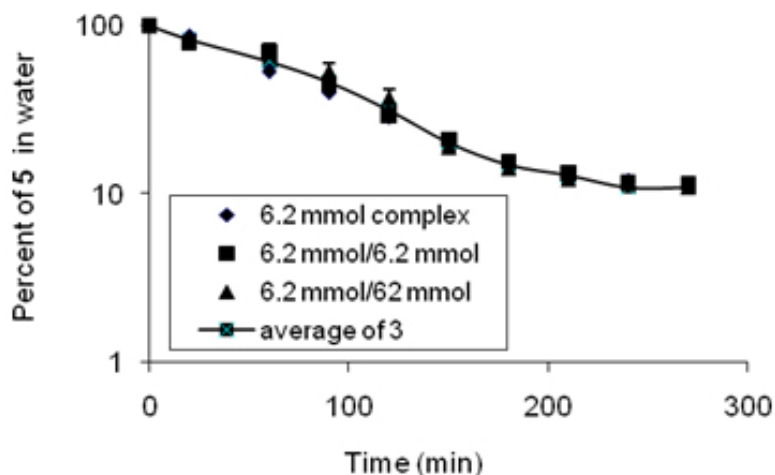


Figure 3. Displacement of **1** from **5** by cholesterol in aqueous solution. The line represents the point-to-point fit to average values for the three concentrations. Error bars (S.D.) are shown only where the scale permits. Calculated data were derived using Excel (Microsoft).

Early biochemical applications of these derivatives included their use as folate metabolism inhibitors (20) and as antiviral agents (21). Technical applications have included the use of microwave-assisted reaction to direct substitution to 5'-O- to

avoid heterocyclic adamantylation (22,23). The current syntheses of adamantanoyl-IUdR ester **1** and analogue **4**, based on 1-adamantanecarbonyl chloride and also an analogue with a 4-carbon spacer between IUdR and adamantamine,

proceeded smoothly. Formation of the 3',5'-di-*O*-adamantantoyl **2** was minimal, and although this compound was characterized, no further studies were undertaken with it. The ^1H NMR spectrum of **1** was in excellent agreement with the proposed structure, and only a single set of adamantane protons was observed, along with a corresponding downfield shift for the H-5' protons of IUdR to 4.48 and 4.25 ppm, respectively. The ^1H NMR data for **2** revealed two sets of adamantane signals, and in addition to the downfield shift for the H-5' protons, the H-3' proton signal was shifted downfield to 5.18 ppm.

Adamantanylation has also been used to increase the lipophilicity of nucleosides and other drugs in attempts to increase their penetration of the blood-brain-barrier (24). Indeed both **1** and **4** were found to be poorly water soluble, whereas the resulting complexes **5** and **6**, respectively, were much more water soluble. This complexation provided a solubility enhancement factor (25) of 300 relative to the solubility of **1**. The A_L phase solubility diagram stoichiometry (Fig. 1) for **1** in aqueous solutions of β -CyD implies a linear increase in solubility with increasing β -CyD concentration, but with unchanged (26). Evidence for inclusion complexation between **1** and β -CyD was provided by DSC, X-ray powder diffractometry and proton NMR. Thermograms for each of the test materials (**1**, β -CyD, their physical mixture and **5**) were markedly different from each

other. Characteristically, the mixture featured two endothermic peaks (220 °C and 227 °C) and an exothermic peak at 240 °C, whereas **5** had an endothermic peak at 235 °C. Prodrug **1** showed one exothermic peak at 208 °C; **1** underwent decomposition at this temperature, as shown by the presence of numerous components upon post-DSC thin-layer chromatographic analysis.

X-ray powder diffractometry has been used for the characterization of β -CyD complexes in solid phase (27). The x-ray powder pattern of every crystalline form of a compound is unique, and indeed the diffractograms for the mixture and inclusion complex **5** differ. However, this technique has limited utility in the identification of non-crystalline (amorphous) materials since their patterns consist of one or more broad diffuse peaks as in the case of **5**.

^1H NMR provided strong evidence of the inclusion of the adamantyl moiety into the β -CyD cavity, and of the host-guest ratio in the complex. The schematic representation of the complexes (Scheme 2) shows that cyclodextrin protons H-3 and H-5 are only accessible from within the cavity, thus, proton NMR provides a means of probing the depth of inclusion of a guest molecule into the CyD cavity (28). Utilization of this approach to determine binding coefficients is beyond the scope of the present work, and will be published elsewhere.

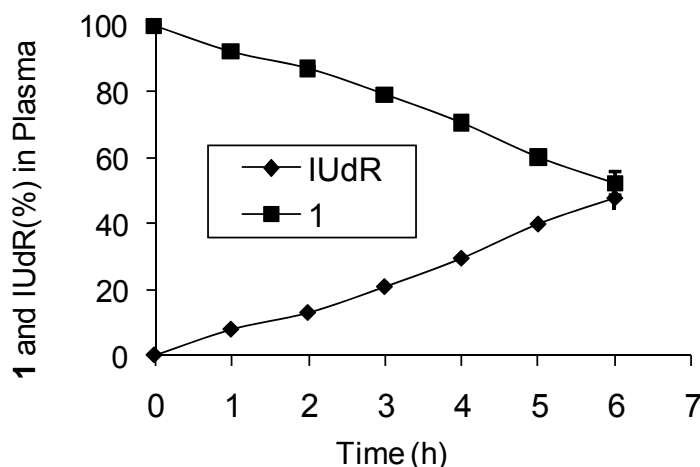


Figure 4. Dissociation of **5** (6.2 mM in plasma) to **1**, and hydrolysis of **1** to IUdR at 37 °C. Calculated data were derived using Excel (Microsoft).

The β -CyD-adamantoyl-IUdR complex formulations **5** and **6** were designed to form high affinity inclusion complexes that would provide longer circulation residence times, as observed for other lipids (29). The transformation of complex **5** to the active form (IUdR) after administration must therefore proceed in two steps: first, **1** must dissociate from the host β -CyD; and second, **1** must be hydrolyzed to the active form (IUdR) by blood and cellular esterases. Data from the simple aqueous solubility and dissociation models support the hypotheses that the β -CyD-adamantoyl-IUdR binding in **5** is sufficient and that dissociation may be low enough to influence (i.e., prolong) circulation times of the adamantoyl-IUdR prodrug. Thus, dissociation of **5** in water was virtually independent of concentration over a 3 h period at these concentrations, but at longer intervals, the system appeared to reach equilibrium (i.e., plateau) at 6.2 mM (Fig. 2). The stability constant for **5**, calculated from the phase solubility diagram (Fig. 1) according to Higuchi and Connors (26) was $1.49 \times 10^5 \text{ M}^{-1}$. It has been reported that a constant of $1 \times 10^5 \text{ M}^{-1}$ would limit dilution-related dissociation at 1:4200 dilution to about 33 % (30), in reasonable agreement with the experimental estimates of about 10 % for **5**, keeping in mind that the current experimental model provides sink conditions, removing the poorly water soluble **1**, thereby shifting equilibrium away from **5**.

To develop a more biologically representative system, dissociation of **5** from plasma was investigated. Fresh plasma contains not only esterases which will hydrolyze **1** to IUdR, and also lipoidal biochemicals such as cholesterol that are effective ligands for inclusion complex formation. The rate of enzymatic hydrolysis of the CyD inclusion complex **5** relative to hydrolysis of **1** to IUdR provided indirect evidence for protection of **1** through CyD inclusion complexation through gradual rather than immediate dissociation of the complex. This process proceeded at nearly identical rates over a five-fold concentration range. Because **1** is sparingly soluble in aqueous media, direct comparison between the hydrolysis of **1** and **5** in plasma was not possible, but in another experiment using a water:DMSO=19:1 solvent mixture similar results were obtained.

These *in vitro* data confirm that release and hydrolysis of the prodrug **1** from the CyD-prodrug complex **5** is slower than hydrolysis of **1** to IUdR, thereby offering the prospect of extended prodrug circulation times (longer plasma clearance $T_{1/2}$) for improved delivery of IUdR to target tissues.

It has been reported that cholesterol forms a complex with β -CyD *in vivo* with an association constant of $1.7 \times 10^4 \text{ M}^{-1}$ (31). In the presence of cholesterol, many drug/ β -CyD complexes quickly dissociate *in vivo* because cholesterol effectively competes with β -CyD for drug binding. This is highly undesirable, as the cholesterol/ β -CyD complex has been found to crystallize in the kidney and cause kidney damage (32). In fact, this is the major drawback of using CyDs for parenteral drug delivery. In this study, we chose to investigate the stability of the complex at two different the cholesterol concentrations: 6.2 mM and 62 mM (in humans the total serum cholesterol concentrations is 5.2–6.2 mM) while the inclusion complex concentration was maintained at 6.2 mM. The experiment data (Fig. 3) show that even at a cholesterol concentration 10 times higher than the complex, dissociation is not changed, that is, cholesterol competed poorly for binding to β -CyD. This effect would be expected based on the apparent ten-fold higher binding constant between β -CyD and **1** than between cholesterol and β -CyD, but the magnitude of the effect is greater than expected. The experimental model used in this challenge study has not been validated. The basic premise is that effective mixing of the two liquid phases will promote sufficient diffusion of cholesterol into the aqueous phase for effective challenge to occur. This is clearly an oversimplification of the partition process (33) and may not adequately counter the impact of the high octanol-water partition coefficient ($\text{Log}_{10}P \sim 8.7$) of cholesterol (34). Clearly additional studies are required to establish the validity of this experimental approach and the stability of these CyD complexes in the presence of cholesterol.

EXPERIMENTAL SECTION

General. NMR spectra were recorded on a Bruker AM 300 spectrometer (^1H : 300 MHz; ^{13}C : 75 MHz) or Varian Unity 500 spectrometer (^1H : 500 MHz) in

CDCl₃ or D₂O solution unless otherwise stated. Chemical shifts in CDCl₃ solutions are reported in parts per million downfield from TMS, or in the case of D₂O solutions, using HOD set at δ 4.82 (25 °C) unless otherwise specified. ¹³C NMR spectral assignments were aided by the J-MOD technique (35). Electrospray (ES) mass spectra were obtained with a Fisons VG Trio-2000 instrument in the negative ion mode. Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F₂₅₄ (Merck) and visualization was accomplished by charring with 5 % methanolic sulfuric acid. Column chromatography was performed using Merck 9385 silica gel (40-63 μ m).

β -Cyclodextrin, 1-adamantanamine hydrochloride, 1-adamantanecarboxylic acid, IUdR and 1-octanol (99.99% spectroscopic grade) were purchased from Sigma Aldrich Canada Ltd. Cyclodextrin was dried *in vacuo* over P₂O₅ at 60 °C prior to use. Chloroform was distilled from P₂O₅ and pyridine was distilled from CaH₂ and stored over molecular sieves 3 Å. For measurement of the rate of hydrolysis in plasma, centrifugation was performed in Eppendorf microcentrifuge tubes using an Eppendorf 5412 microcentrifuge. Samples were filtered through a Millex® low protein binding 0.22 μ m filter (Millipore) was used for filtering plasma samples prior to analysis.

HPLC analysis was performed using a Waters model U6K injector equipped with dual Waters 501 solvent delivery pumps. Chromatographic separation of the IUdR prodrug from IUdR was performed on a reverse-phase column (phenyl radial-pak cartridge; 10 μ m particle size, 8 mm id x 100 mm length) equipped with a guard column (μ Bondapak™ phenyl Guard-pak™) using 4:1 methanol:water as eluant (flow rate of 1 mL/min). Detection was accomplished with a Waters 486 tunable absorbance detector set at 288 nm.

5'-O-(1-Adamantoyl)-5-iodo-2'-deoxyuridine 1 and 3',5'-di-O-(1-adamantoyl)-5-iodo-2'-deoxyuridine 2. A solution of 5-iodo-2'-deoxyuridine (400 mg, 1.13 mmol) in dry pyridine (7 mL) was cooled to 0-5 °C under argon. To this mixture was added drop-wise, a solution of freshly prepared 1-adamantanecarbonyl chloride (300 mg, 1.5 mmol) in dry chloroform (3 mL). The reaction was stirred at this temperature for 30 min, then

allowed to warm to room temperature and stirred overnight. Excess methanol (0.1 mL) was added to the reaction mixture and then mixture was concentrated. The residue was dissolved in chloroform and successively washed with saturated aqueous sodium hydrogen carbonate, water, saturated sodium chloride, and then dried (Na₂SO₄), filtered and concentrated. Column chromatography over silica gel using 12:12:1 hexane:ethyl acetate:ethanol gave first the di-adamantanyl derivative **2** 61 mg (8 %) followed by 498 mg (69 %) of the desired IUdR derivative **1**. Compound **1**: R_f = 0.18 (12:12:1 hexane:ethyl acetate:ethanol); mp 124-125 °C; UV: (1-octanol) λ_{max} 207, 285 nm; ¹H NMR (500 MHz, CDCl₃) δ 9.05 (s, 1H, NH), 7.91 (s, 1H, H-6), 6.23 (t, 1H, $J_{I,2'a} = 6.6$ Hz, H-1'), 4.48 (dd, 1H, $J_{5'a,5'b} = 12.7$ Hz, $J_{5'a,4'} = 1.9$ Hz, H-5'a), 4.37 (m, 1H, H-3'), 4.25 (dd, 1H, $J_{5'b,5'a} = 12.7$ Hz, $J_{5'b,4'} = 1.9$ Hz, H-5'b), 4.23 (s, 1H, H-4'), 2.53 (ddd, 1H, $J_{2'a,2'b} = 13.4$ Hz, $J_{2'a,1'} = 6.6$ Hz, $J_{2'a,3'} = 2.8$ Hz, H-2'a), 2.06 (m, 1H, H-2'b), 2.06 (m, 3H adamantane γ -protons), 1.93 (m, 6H, adamantane α -protons), 1.74 (m, 6H, adamantane β -protons) ¹³C NMR (CDCl₃) δ 175.0, 157.4, 147.3, 141.3, 83.2, 82.5, 68.9, 66.2, 60.9, 38.6, 38.4, 36.5, 33.7, 25.2; Anal. Cal. for C₂₀H₂₅IN₂O₆·1H₂O: C 44.94, H 5.05, N 5.24; Found C 44.53, H 4.65, N 5.42. Data for compound **2**: R_f = 0.36 (12:12:1 hexane/ethyl acetate/ethanol); ¹H NMR (300 MHz, CDCl₃) δ 8.39 (s, 1H, NH), 7.89 (s, 1H, H-6), 6.23 (dd, 1H, $J_{1',2'b} = 8.9$ Hz, $J_{1',2'a} = 5.1$ Hz, H-1'), 5.18 (d, 1H, $J_d = 6.1$ Hz, H-3'), 4.45 (dd, 1H, $J_{5'a,5'b} = 12.5$ Hz, $J_{5'a,4'} = 3.4$ Hz, H-5'a), 4.29 (dd, 1H, $J_{5'b,5'a} = 12.5$ Hz, $J_{5'b,4'} = 2.4$, H-5'b), 4.23 (m, 1H, H-4'), 2.54 (dd, 1H, $J_{2'a,2'b} = 14.0$ Hz, $J_{2'a,1'} = 5.1$ Hz, H-2'a), 2.1 (m, 1H, H-2'b, obscured by adamantane β -protons), 2.04 (m, 6H, adamantane β -protons), 1.92 (m, 6H, adamantane α -protons), 1.87 (m, 6H, adamantane α -protons), 1.72 (m, 12H, adamantane β -protons).

4-(1-Adamantylcarbamoyl)butanoic acid 3. Adamantanamine hydrochloride (188 mg, 1.0 mmol) was dissolved in 1:1 anhydrous pyridine:chloroform, and glutaric anhydride (180 mg, 1.5 mmol) was added to the reaction mixture. The reaction mixture was stirred at 75 °C under argon for 18 h. The solvent was evaporated *in vacuo* and the residue redissolved and evaporated sequentially with chloroform (15 mL x 3), methanol

(15 mL) and, lastly, chloroform (15 mL x 2). The residue was then triturated with chloroform and left overnight at room temperature. The precipitate was collected by filtration, washed with cold chloroform and dried *in vacuo* to afford 215 mg (81%) of **3** as a white solid: mp 225-227 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.24 (s, 1H, NH), 3.34 (br s, 1H, CO₂H), 2.17 (t, 2H, *J*_t = 7.3 Hz, CH₂CO₂H) 2.02 (t, 2H, *J*_t = 7.3 Hz, CH₂CON), 2.0 (br s, 3H, adamantane β-protons), 1.89 (br s, 6H, adamantane α-protons), 1.65 (quintet, 2H, *J* = 7.3 Hz, CH₂CH₂CH₂) 1.60 (br s, 6H, adamantane γ-protons) ¹³C NMR (DMSO-*d*₆) δ 174.0, 170.8, 50.4, 50.0, 36.0, 35.2, 32.9, 29.0, 20.8; ES MS (negative ion) calc. for C₁₅H₂₂NO₃ [M-H]⁻: 264.3; found: 264.6.

5'-O-(4-(1-Adamantylcarbonyl)butoyl)-5-iodo-2'-deoxyuridine 4. A mixture of **3** (121 mg, 0.46 mmol), 5-iodo-2'-deoxyuridine (82 mg, 0.23 mmol) and dicyclo-hexylcarbodiimide (100 mg, 0.48 mmol) in dry pyridine (3 mL) was stirred at 70 °C under argon, overnight. The TLC profile (9:1 chloroform:methanol) of the reaction mixture revealed that one major and two relatively minor components were present as well as a significant amount of starting material. The solvent was removed, the mixture dissolved in chloroform and the organic solution was washed sequentially with saturated aqueous sodium hydrogen carbonate and water, dried (Na₂SO₄), filtered, and concentrated. Purification of the residue by column chromatography over silica using 97:3 chloroform:methanol as eluent gave 59 mg (42%) of **4** as a white solid: R_f = 0.42 (9:1 chloroform/methanol); mp 105 °C; ¹H NMR (500 MHz, CDCl₃) δ 8.22 (s, 1H, NH), 8.04 (s, 1H, H-6), 6.16 (dd, 1H, *J*_{1',2'a} = 6.3 Hz, *J*_{1',2'b} = 4.4 Hz, H-1'), 5.2 (br s, 1H, NH), 4.55 (q, 1H, *J*_t = 6.5 Hz, H-3'), 4.52 (dd, 1H, *J*_{5'a,5'b} = 12.5 Hz, *J*_{5'a,4'} = 2.5 Hz, H-5'a), 4.22 (dd, 1H, *J*_{5'b,5'a} = 12.5 Hz, *J*_{5'b,4'} = 3.0 Hz, H-5'b), 4.09 (dt, 1H, *J*_{4',3'} = 6.0 Hz, *J*_{4',5'a,b} = 3.0 Hz, H-4'), 2.65 (dt, 1H, *J*_d = 15.0 Hz, *J*_t = 6.0 Hz, CHCO₂) 2.49 (dd, 1H, *J*_{2'a,2'b} = 13.5 Hz, *J*_{2'a,1'} = 6.3 Hz, H-2'a), 2.42 (ddd, 1H, *J*_d = 15.0 Hz, *J*_d = 8.0 Hz, *J*_d = 6.0 Hz, CHCO₂) 2.25 (m, 2H, CHCON and H-2'b), (m, 1H, CHCON), 2.06 (br s, 3H, adamantane β-protons), 1.95 (m, 8H, CH₂CH₂CH₂ and adamantane α-protons), 1.66 (br s, 6H, adamantane γ-protons) ¹³C NMR (1:1 CD₃OD/CDCl₃) 172.8, 172.2, 160.5, 149.9, 144.2,

85.6, 84.3, 69.9, 68.2, 63.2, 52.2, 41.2, 40.4, 36.1, 35.7, 33.2, 29.4, 21.0; Anal. Calc. for C₂₄H₃₂IN₃O₇.1/4H₂O: Calc. C 47.56, H 5.36, N 6.94; Found C 47.09, H 5.24, N 7.24 %.

β-cyclodextrin complex 5. A 1:1 solution of 5 μmol each of β-cyclodextrin and IUdR prodrug **1** (log P = 1.91) in D₂O (600 μL) was stirred vigorously under argon at 50 °C for 30 min, then cooled to room temperature and filtered into an NMR tube. The ¹H NMR spectrum (500 MHz, D₂O) revealed (integration) that a 1:1 mixture was present in solution; δ 8.16 (s, 1H, H-6 IUdR), 6.26 (t, 1H, *J*_t = 6.4 Hz, H-1' IUdR), 5.14 (d, 7H, *J*_d = 3.4 Hz, H-1 CyD), 4.58 (m, 1H, H-3' IUdR), 4.47 (m, 2H, H-5'a, H-5'b IUdR), 4.28 (br m, 1H, H-4' IUdR), 3.95 (t, 7H, *J*_t = 9.8 Hz, H-3 CyD), 3.93-3.88 (m, 17H, H-4', H-5'a, H-5'b IUdR, H-6a, H-6b CyD), 3.85 (br d, 7H, *J*_d = 9.8 Hz, H-5 CyD), 3.73 (dd, 7H, *J*_{2,3} = 9.8 Hz, *J*_{2,1} = 3.4 Hz, H-2 CyD), 3.67 (t, 7H, *J*_t = 9.8 Hz, H-4 CyD), 2.64 (br m, 1H, H-2'a IUdR), 2.40 (br m, 1H, H-2'b IUdR), 2.25 (br s, 3H, adamantane β-protons), 2.00 (br s, 6H, adamantane α-protons), 1.91 (d, 3H, *J*_d = 12.0 Hz adamantane γ_a-protons), 1.82 (d, 3H, *J*_d = 12.0 Hz adamantane γ_b-protons).

β-cyclodextrin complex 6. A 1:1 solution of 5 μmol each of β-cyclodextrin and IUdR prodrug **4** in D₂O (600 μL) was stirred under argon at 50 °C for 30 min as described for **5**, then cooled to room temperature and filtered into an NMR tube. The ¹H NMR spectrum (500 MHz, D₂O) revealed that a 1:1 mixture was present in solution; δ 8.22 (s, 1H, H-6 IUdR), 6.31 (t, 1H, *J*_t = 6.4 Hz, H-1' IUdR), 5.18 (br s, 7H, H-1 CyD), 4.58 (m, 1H, H-3' IUdR), 4.50 (dd, 1H, *J*_{5'a,5'b} = 12.2 Hz, *J*_{5'a,4'} < 1.0 Hz, H-5'a), 4.45 (dd, 1H, *J*_{5'b,5'a} = 12.2 Hz, *J*_{5'b,4'} < 1.0 Hz, H-5'b), 4.34 (br s, 1H, H-4'), 3.96 (t, 7H, *J*_t = 9.8 Hz, H-3 CyD), 3.94 (m, 14H, H-6a, H-6b CyD), 3.85 (br d, 7H, *J*_d = 9.3 Hz, H-5 CyD), 3.73 (br dd, 7H, *J*_{2,3} = 9.8 Hz, H-2 CyD), 3.64 (t, 7H, *J*_t = 9.8 Hz, H-4 CyD), 2.63-2.54 (br m, 3H, CH₂ and H-2'a IUdR), 2.40 (br m, 1H, H-2'b IUdR), 2.25 (br s, 3H, adamantane β-protons), 2.00 (br s, 6H, adamantane α-protons), 1.91 (d, 3H, *J*_d = 12.0 Hz adamantane γ_a-protons), 1.82 (d, 3H, *J*_d = 12.0 Hz adamantane γ_b-protons).

Differential scanning calorimetry. Samples (2 mg each) of free prodrug **1**, β-CyD, the physical

mixture of **1** and β -CyD and the inclusion complex **5** were loaded into aluminum pans and measured using a sealed aluminum pan as the reference. The instrument was calibrated with indium (156 °C). Thermograms were obtained at a heating rate of 10 °C/min from 40 °C - 180 °C and 5 °C/min from 180 °C - 260 °C. A nitrogen purge was maintained throughout each run.

X-ray diffractometry. Powder samples of prodrug **1**, β -CyD, the physical mixture of the prodrug **1** and β -CyD, and the inclusion complex **5** were pressed onto a sample slide, and then covered with double-sided cellophane tape. An automated X-ray diffractometer equipped with a 2 θ compensating slit, a nickel filter and Cobalt tube (40 kV, 30 mA). Co ($\lambda\alpha = 1.7962\text{\AA}$) was used to obtain the diffraction patterns of the samples. The patterns were recorded from 2 °C - 90 °C (2 θ) / min.

Partition coefficients (LogP). The LogP of **1** was determined using standard 1-octanol/ water and 1-octanol/phosphate-buffer solution (0.02 M, pH = 7.4) systems. Mechanical shaking was used to achieve equilibration, and UV spectroscopy was used for the quantitation of the analyte in the 1-octanol phase. A standard solution of the prodrug was prepared in water-saturated 1-octanol. The standard sample was analyzed by UV spectrophotometry at 282 nm to prepare a standard curve of UV absorbance vs concentration of the standard solution. Compound **1** (0.5 mg/mL) was partitioned between equal volumes (2 mL) of presaturated 1-octanol-water and 1-octanol:phosphate buffer, respectively. The solutions were gently shaken for 24 h at room temperature. The two phases were then separated and the concentration of compound **1** in the 1-octanol phase was determined by UV spectrophotometry, before and after partitioning. The partition coefficient (P) was calculated as the molar ratio of the concentration in the 1-octanol to the concentration in the water or phosphate buffer phase, respectively, ($P = C_{n\text{-octanol}} / C_{\text{water or buffer}}$).

The LogP for **1** was also calculated by energy optimization of its chemical structure using Alchemy 32; Version 2 (1997), Tripos Inc., Louis, MD (U.S.A), then using subsequent analysis with SciQSAR, version 3.0 (1995-1998 Scivision, Inc.) to provide the calculated LogP values.

Solubility of 1 in water and in aqueous β -CyD solution. Excess prodrug **1** was added to water and an aqueous solution of β -CyD (1.5 mL) in Eppendorf centrifuge tubes, respectively. After 10 min of water bath sonication, the samples were placed in a shaking water bath at 25 °C and mixing was continued overnight. The samples were analyzed by HPLC with uv detection at 282 nm. HPLC analyses were performed using a Waters reverse phase Phenyl Radial-Pak cartridge (10 μ m particle size, 8 mm id x 10 cm length) using 4:1 v/v methanol-water as solvent with a flow rate of 1 mL/min. The retention time of **1** was 4.4 min.

Dissociation of the 1/ β -CyD inclusion complex 5 in water. Two concentrations (1.2 mM and 6.2 mM in water) of the 1:1 molar ratio inclusion complex **5** were investigated. The inclusion complex **5** in water was transferred to a 1.5 mL vial containing 1-octanol (0.5 mL) and placed into shaking water bath kept at 37 °C. Aliquots of the octanol layer were taken at 0, 15, 45, 75, 120, 180, 240 min. The aliquots (50 μ L) were analyzed by HPLC to determine the amount of free **1**.

Stability of the prodrug 1/ β -CyD inclusion complex 5 to cholesterol challenge. The 1:1 molar ratio inclusion complex **5** in water (1 mL; 10.2 mg/mL, 6.2 mM) was added to 1-octanol (1 mL), to 1-octanol (1 mL) containing 2.4 mg (6.2 mmol) cholesterol and to 1-octanol (1 mL) containing 24 mg (62 mmol) cholesterol, respectively. These mixtures were placed into a shaking water bath at 37 °C. Aliquots were taken for HPLC analysis at 0, 20, 60, 90, 120, 150, 180, 210, 240, 270 min.

Hydrolysis of the 1/ β -CyD complex 5 to IUdR in plasma. A solution of **5** in plasma (1.2 mM) was transferred to a 1.5 mL vial containing 1-octanol (0.5 mL) and placed into shaking water bath kept at 37 °C. Aliquots (50 μ L) of the supernatant were taken at 0, 15, 45, 75, 120, 180, 240 min. Each aliquot was quenched with methanol (400 μ L), and the resultant solution was centrifuged at 2500 g (2 min). The supernatant was then carefully removed, filtered through a 0.22 μ m MILLEX GV filter, and analyzed for the **1** and IUdR by HPLC.

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