Syntheses of 5-Chlorouracils/Thymines with 1-[Phosphono(Methyl/Difluoromethyl)]-1,2-Unsaturated-Moiety-Substituted Methyl Groups at N(1) and Human Thymidine Phosphorylase Inhibitory Activity

by **Matthew R. Birck**[b])[1]), **Keith Clinch***[a]), **Graeme J. Gainsford**[a]), **Vern L. Schramm**[b]), and **Peter C. Tyler**[a])

[a]) Carbohydrate Chemistry Team, *Industrial Research Ltd.*, P.O. Box 31-310, Lower Hutt 5040, New Zealand

[b]) Department of Biochemistry, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461, USA (phone: +64-4-931-3261; fax: +64-4-931-3055; e-mail: k.clinch@irl.cri.nz)

Dedicated to Professor Dr. *Andrea Vasella* on the occasion of his 65th birthday

By attaching (methyl)- or (difluoromethyl)-phosphonate groups to the 1-positions of ethene, cyclopentene or benzene, and attaching 1-(methyl)-5-chlorouracil or 1-(methyl)thymine groups to the corresponding 2-positions, compounds **1**–**5** were prepared as potential inhibitors of recombinant human thymidine phosphorylase (TP). The products were designed to mimic the interatomic distance (*ca.* 3.41 Å) between the incoming phosphate and leaving pyrimidine groups at the transition state for the putative S_N2 mechanism of TP. Free rotation around the (unsaturated-CH_2)–pyrimidine bonds in **1**–**5** enabled a span of *ca.* 2.40–4.40 Å between the CH_2 or CF_2 C-atoms in the phosphonates and N(1) of the pyrimidines to be covered. The products were found to be ineffective inhibitors, and some reasons for this are given.

1. Introduction. – Thymidine phosphorylase (TP), synonymous with platelet-derived endothelial growth factor (PD-ECGF), highly expressed in hypoxic regions of many tumors and involved in the pyrimidine nucleotide-salvage pathway, catalyses the reaction shown in *Fig. 1* [1]. The product of the forward reaction, 2-deoxy-α-D-ribose-1-phosphate, after dephosphorylation, stimulates angiogenesis, a process involved in cancer metastases. Inhibition of TP may, therefore, lead to novel anticancer compounds, and this field, including developments in TP inhibitor design and the role played by TP in tumor activity, has been recently reviewed [2]. 5-Chloro-6-[(2-iminopyrrolidin-1-yl)methyl]uracil hydrochloride (TPI) has emerged as a leading TP inhibitor candidate, and a combination of TPI with 2′-deoxy-5-(trifluoromethyl)uridine is currently in phase-II clinical trials as an orally available anticancer treatment under the name of TAS-102 [3].

Recently, several papers have appeared on the syntheses of (phosphonomethoxy)-alkyl-pyrimidines, compounds which have the ability to inhibit TP from rat spontaneous T-cell lymphoma but are somewhat less active against TPs from other sources. Some of the best derivatives found in this series were 5-ethyl-1-[(*R*)-3-hydroxy-2-

[1]) Present address: Homeland Security, 1100 Raymond Blvd., Newark, NJ 07120, USA.

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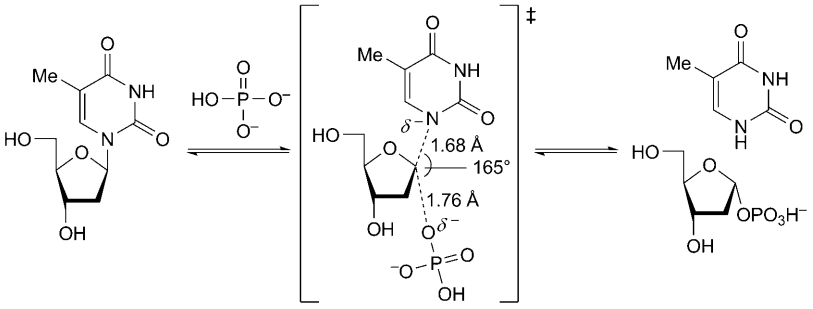


Fig. 1. *Proposed* S_N2 *transition state* (TS) *for thymidine phosphorylase* [1]

(phosphonomethoxy)propyl]uracil [4], 5-ethyl-1-[(*S*)-3-fluoro-2-(phosphonomethoxy)propyl]uracil [4] and 1-[(*R*)-3-fluoro-2-(phosphonomethoxy)propyl]thymine ((*R*)-FPMPT) [5]. In contrast, the structurally simpler 1-[(2-(phosphonomethoxy)-ethyl]thymine (PMET), and consequently a poorer inhibitor than those above, was modified in an attempt to enhance its activity by preparing several 5-alkyl, 5-aryl, or 5-fluoro derivatives or by making *N(1)*-side-chain modifications [6]. Marginal-to-moderate activity was also observed in some 5-aryl analogues of 1-[(*R*)- and (*S*)-3-hydroxy-2-(phosphonomethoxy)propyl]thymines (HMPMTs) [7], and it was found that switching the N(1)-linked side chains found in PMET, (*R*)-HPMPT and (*R*)-FPMPT to the N(3)-position of the nucleobases resulted in significant loss of activity [8].

Replacement of the iminopyrrolidine ring found in TPI with a 3-methylimidazol-3-ium ring led to a series of less active 5-halo-6-[(3-methylimidazol-3-ium-1-yl)methyl]-uracil chlorides [9]. On the other hand, replacement with a 2-aminoimidazole ring, led to a number of 6-[(2-aminoimidazol-1-yl)methyl]-5-chloro-(and 5-bromo)uracil hydrochlorides with comparable inhibitory strength to TPI, but with unfavorable pharmacokinetics [10]. To selectively deliver such compounds to solid tumors, the inactive 6-[(2-nitroimidazol-1-yl)methyl] derivatives were, therefore, prepared and shown to be reduced under anaerobic conditions to the active amines by xanthine oxidase (XO), an enzyme highly expressed in such tumors where oxygen-deprived conditions prevail. Conversely, several known TP inhibitors lacking a C=O function at C(2) and/or C(4) of the uracil ring, and hence inactive, were also prepared in an effort to increase lipophilicity and consequently shown to have their activity restored by XO oxidation [11].

TP Inhibitory activity has also been demonstrated with various 5-chloro-6-[(dialkylamino)methyl]-substituted uracils [12], 6-(alkylamino)-5-halo-uracils or bis(5-halo-substituted uracils) linked at C(6) with a diamine spacer [13], and with 6-chlorouracils substituted at C(5) by a range of halogeno, alkyl, aryl, or carbocyclic side chains [14]. Modification of the 2′,3′-OH groups found in 5-methyluridine into bicyclic nucleosides containing either a 1,3-dioxolane or furan ring bearing *endo*-oriented (methyl)phosphonate, acetic acid, or acetamide appendages have also led to inhibitory activity [15], as have modifications at positions 1, 2, and 6 of the purine ring, or C(5′) of ribose in 5′-*O*-tritylinosine, a known allosteric inhibitor of TP [16].

Computer-modeling simulations have indicated a unimolecular nucleophilic substitution (S_N1)-like mechanism involving oxacarbenium ion formation for the TP reaction [10][17]. However, 5-fluoro-[(6-imidazol-1-yl)methyl]uracil, a TP inhibitor with about half the potency of TPI, was suggested to be a transition state (TS) analogue for the TS involved in a bimolecular nucleophilic substitution (S_N2)-like mechanism [18]; a model developed by two of us (*Fig. 1*) [1]. The proposed S_N2 mechanism for the TP reaction was founded on kinetic isotope effects observed for the analogous arsenolysis of thymidine, and its outcome advocated the incorporation of hydrolytically stable features of 2-deoxy-α-D-ribose, thymine, and phosphate into the design of strong TP inhibitors. Whilst the syntheses of such structures meeting these criteria pose significant challenges [19], some of the examples cited in the references above could be deemed as appropriate. To simplify matters, structures were considered in which the interatomic distance between the thymine, and phosphate groups shown in *Fig. 1* could be imitated. In this model, the distance between the O-atom of the attacking phosphate and the N(1)-atom of the leaving thymine is *ca.* 3.41 Å. Compounds **1**–**5** (*Fig. 2*) in which the 1-(substituted methyl)pyrimidine bases and hydrolytically stable isosteric (methyl/difluoromethyl)phosphonate (as phosphate mimics) appendages are attached 1,2-like to an alkene or aromatic C=C bond were, therefore, regarded as potential inhibitors of TP[2]). In these structures the optimum distance of 3.41 Å between N(1)-atom of the pyrimidines and the methylene or difluoromethylene C-atoms of the phosphonates can be attained because free rotation about the (unsaturated-CH_2)–pyrimidine bond allows for a span of *ca.* 2.40–4.40 Å to be covered. Although it was anticipated that the absence of a 2-deoxy-D-ribose group would result in lower-affinity inhibitors, moderate TP inhibition was still expected to be observed with compounds **1**–**5**. In addition, their structural simplicity made them attractive targets to synthesize.

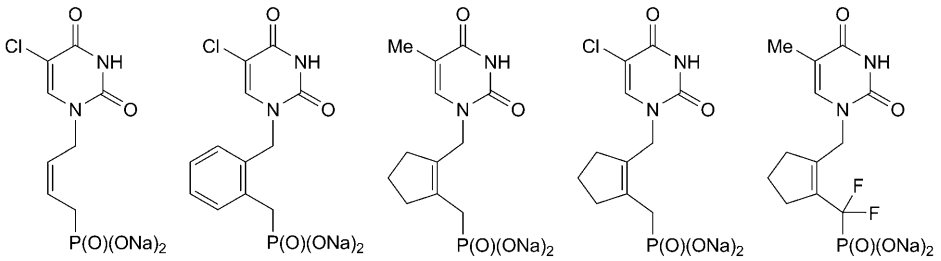


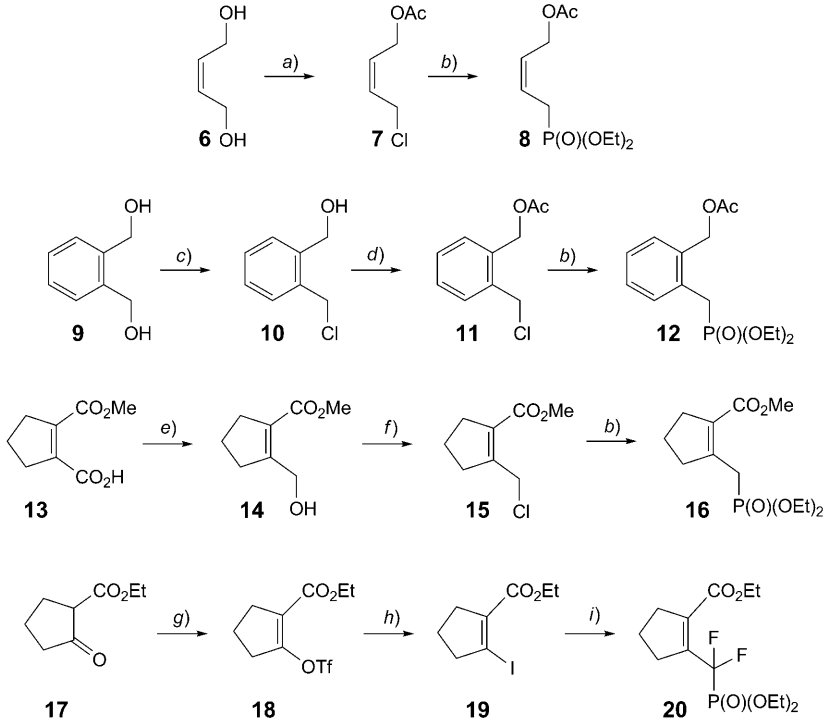
Fig. 2. *Structures of target compounds* **1**–**5**

2. Results and Discussion. – 2.1. *Syntheses of Intermediate Phosphonates* **8**, **12**, **16**, *and* **20**. For synthesis considerations, compounds **1**–**5** were conveniently regarded as derivatives of 1,2-allylic or 1,2-benzylic diols, requiring transformation of the two CH_2OH groups present in these systems into 1-(substituted methyl)-5-chlorouracil/

[2]) Whilst this manuscript was in preparation, the synthesis of the (*E*)-isomer of compound **1** by cross-metathesis reaction between a *N(1)*-crotylated uracil derivative and dimethylallyl phosphonate appeared [20].

thymine or (methyl)/(difluoromethyl)phosphonate functionalities, respectively. We chose to introduce the phosphonate moiety first, and the syntheses of compounds **8**, **12**, **16**, and **20**, which served as precursors to **1**–**5**, are depicted in *Scheme 1*.

Scheme 1. *Introduction of the Methyl- and (Difluoromethyl)phosphonate Moieties*



a) 36% aq. HCl, extract with AcOEt; 7%. *b*) $P(OEt)_3$, reflux; 76% (**8**), 82% (**12**), 86% (**16**). *c*) 36% aq. HCl; 65%. *d*) Ac_2O, I_2; 100%. *e*) 1. $ClCO_2Et$, Et_3N, THF; 2. $NaBH_4$, THF, H_2O; 75%. *f*) Bu_3P, CCl_4; 95%. *g*) NaH, Tf_2O, Et_2O, 99%. *h*) NaI, DMF, reflux; 38%. *i*) $BrCF_2P(O)(OEt)_2$, Zn, CuBr, *N*,*N*-dimethylacetamide (DMA), sonication; 57%.

Treatment of the (*Z*)-diol **6** with 36% aqueous HCl has been reported to give (*Z*)-4-chlorobut-2-en-1-ol [21]. However, we found the main product, albeit in low yield, to be the acetate **7**, which was presumably formed by acid-catalyzed transesterification from the AcOEt used in the initial extractive workup process. Though **7** was only partially purified (*ca.* 80% by ^{1}H-NMR), it nevertheless underwent an efficient *Michaelis–Arbuzov* reaction with $P(OEt)_3$ to give phosphonoacetate **8** which was readily purified and characterized, confirming the presence of acetate. The formation of **7** proved to be fortuitous as the corresponding *Michaelis–Arbuzov* reaction of (*Z*)-4-chlorobut-2-en-1-ol (prepared from **6** with 36% aqueous HCl, omitting AcOEt from the workup and using only CH_2Cl_2 instead) with $P(OEt)_3$ gave the expected product (**21**; *Scheme 2*) only as part of an intractable mixture.

In a similar manner, the benzylic diol **9** underwent mono-chlorination with 36% aqueous HCl [23] to give **10**. The remaining unprotected OH in **10** was efficiently acetylated under solvent free conditions [24] to afford chloro acetate **11**; *Michaelis–Arbuzov* reaction of which cleanly produced **12**. Analogous with the observation above, treatment of chlorido alcohol **10** in refluxing $P(OEt)_3$ did not afford any expected product (**24**; *Scheme 2*) but instead gave an unidentified mixture.

For construction of the (cyclopentenylmethyl)phosphonate **16**, dimethyl cyclopent-1-ene-1,2-dicarboxylate [25] was half-saponified [26] to acid **13**. The carboxylic acid group in **13** was converted into an acyl carbonate derivative [27], then selectively reduced with $NaBH_4$, to give allylic alcohol **14**. Chlorination of **14** with the *Appel* [28] reagent generated from Bu_3P/CCl_4 afforded **15**, which was converted into **16** upon refluxing in $P(OEt)_3$. The known ethyl ester analogue of **15** was also prepared by radical cyclization of an 'α,α-dichloroester alkyne' [29], and whilst this offered an alternative route to the (cyclopentenylmethyl)phosphonate framework, the former method was more useful on a larger scale.

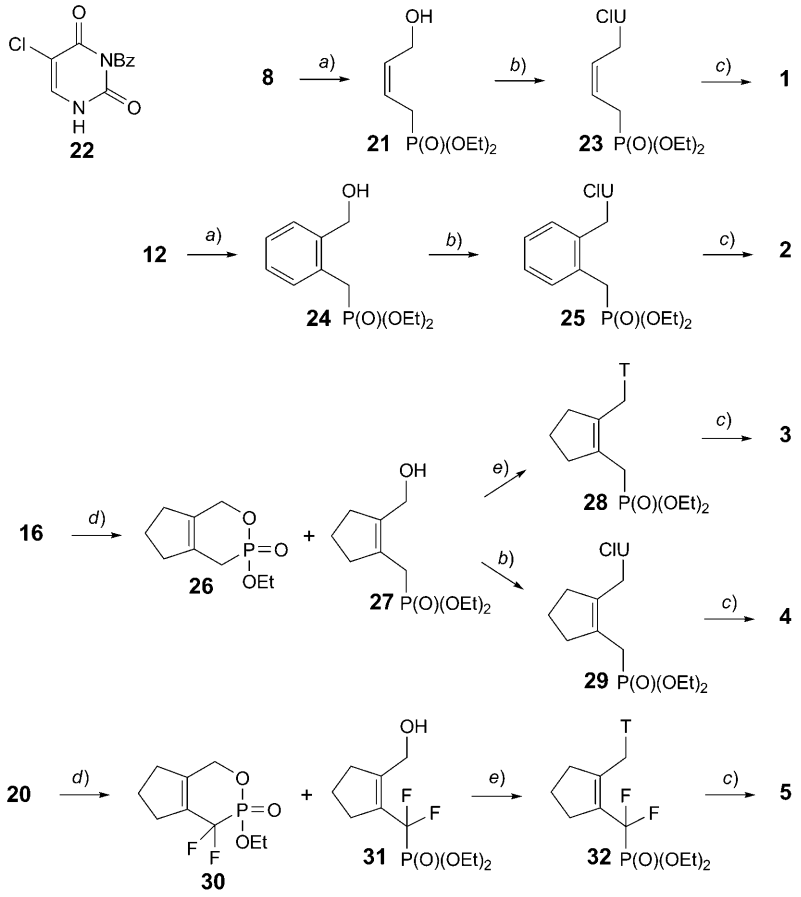
The literature procedure [30] for preparing (α,α-difluoroallyl)phosphonates from vinyl iodides was employed in the synthesis of (difluoromethyl)phosphonate **20**. The keto ester **17** was first converted to the triflate **18**, in the same way as that reported for the corresponding methyl ester [31], which, upon treatment with NaI, in refluxing DMF gave iodo ester **19**. This iodo derivative readily underwent a Cu^I-catalyzed coupling reaction with [(diethoxyphosphoryl)(difluoro)methyl]zinc(II) bromide ($BrZnCF_2P(O)(OEt)_2$) in *N*,*N*-dimethylacetamide (DMA) solution under sonication [30] to give **20**. In an alternative approach to **20**, *Swern* oxidation of **14** cleanly gave the corresponding aldehyde product (^{1}H-NMR) which underwent a KF-catalyzed *Pudovik* reaction [32] with diethyl phosphite producing the corresponding (α-hydroxy)phosphonate (^{1}H-NMR). However, further attempts to oxidize this compound to the (α-keto)phosphonate, which would then be expected to undergo a deoxydifluorination reaction with (diethylamino)sulfur trifluoride to give **20**, failed. This failure may suggest the necessity of using di(*tert*-butyl) phosphite in the initial *Pudovik* reaction as has been observed in the analogous preparation of (α,α-difluorobenzyl)phosphonates where it was found that oxidation of benzyl- or methyl-protected (α-hydroxy)phosphonates did not lead to the corresponding (α-keto)-phosphonates [33]. Recently, however, diethyl phosphite has been used successfully in a similar sequence of reactions to prepare certain (α,α-difluoronaphthyl)phosphonates [34].

Compounds **8**, **12**, and **16** all resonated as *singlet*s in their ^{31}P-NMR spectra with shifts in the range of δ 26.5–27.6. Their ^{1}H-NMR spectra displayed ^2J(H,P) values of between 22.0–24.2 Hz, and their ^{13}C-NMR spectra showed ^1J(C,P) values of between 135.7–140.4 Hz. On the other hand, the (difluoromethyl)phosphonate **20** resonated as a *triplet* (δ 6.6) in its ^{31}P-NMR spectrum with a large ^2J(P,F) value of 111 Hz. In its ^{13}C-NMR spectrum, the CF_2 group was observed as a *doublet* of *triplet*s with ^1J(C,F) and ^1J(C,P) values of 261 and 217 Hz, respectively.

2.2. *Incorporation of the Pyrimidine Rings by* Mitsunobu *Reaction Leading to Compounds* **1**–**5**. Compounds **8**, **12**, **16**, and **20**, after conversion of their AcO or MeOCO/Et functionalities into the corresponding allylic alcohols, served as substrates for the introduction of the pyrimidine moieties using the *Mitsunobu* reaction

(*Scheme 2*). For this purpose, 3-benzoylthymine[3]) [22] and 3-benzoyl-5-chlorouracil[4]) (**22**; prepared by the same method as that used for 3-benzoylthymine [22] and its structure confirmed by X-ray-analysis [37]) were employed. Acid-catalyzed deacetylation of **8** gave allylic alcohol **21** which readily underwent a *Mitsunobu* coupling with **22**, followed by debenzoylation under basic conditions to give **23**. Dealkylation of the diethyl phosphonate groups in **23** with Me_3SiBr (TMSBr) afforded **1**. The same

Scheme 2. *Introduction of the Pyrimidine Bases*



a) $TsOH \cdot H_2O$, EtOH, reflux; 95% (**21**), 88% (**24**). *b*) 1. Diisopropyl azodicarboxylate (DIAD), Ph_3P, **22**, 1,4-dioxane; 2. 7M NH_3/EtOH; 49% (**23**), 69% (**25**), 51% (**29**). *c*) Me_3SiBr (TMSBr), CH_2Cl_2; 63% (**1**), 73% (**2**), 73% (**3**), 49% (**4**), 34% (**5**). *d*) $LiBH_4$, MeOH, Et_2O; 21% (**26**), 63% (**27**), 23% (**30**), 51% (**31**). *e*) 1. DIAD, Ph_3P, 3-benzoylthymine [22], 1,4-dioxane; 2. 7M NH_3/EtOH; 73% (**28**), 20% (**32**).

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[3]) Previously used in the *Mitsunobu* reaction with allylic alcohols [35].

[4]) Previously used in the *Mitsunobu* reaction [36].

sequence of reactions also transformed **12** → **24** → **25** → **2**. The preparation of compounds **3**–**5** followed a similar path as above except that, in these cases, the requisite allylic alcohols were obtained by hydride reduction of the corresponding vinyl esters. The reagent combination of $LiBH_4$/MeOH [38] gave a facile reduction of **16** → **27** and of **20** → **31**, but in both cases cyclic phosphonates **26** (from **16**) and **30** (from **20**) were isolated as by-products. As was the case for alcohols **21** and **24** above, the *Mitsunobu* reaction of **27** with either 3-benzoylthymine or **22**, followed by treatment with 7M NH_3/EtOH solution led to **28** and **29**, respectively. Similarly, alcohol **31** was converted into **32** by *Mitsunobu* coupling with 3-benzoylthymine and subsequent 7M NH_3/EtOH treatment. TMSBr-Promoted deprotection of the diethyl phosphonate groups, **28** → **3**, **29** → **4**, and **32** → **5**, then proceeded uneventfully. Pure compounds **23**, **25**, **28**, and **29** were all obtained as easily crystallizable solids. Compound **32**, however, readily co-crystallized with benzamide, the by-product of debenzoylation with 7M NH_3/EtOH, from which it was also inseparable by chromatography. Surprisingly though, pure crystalline **32** was obtained by seeding a crude solution of **32** with its analogue **28**.

After chromatography to a homogeneous product, but before recrystallization, it was evident from the ^{1}H-NMR spectra of **28** and **29** that they were accompanied by *ca.* 5–10% of isomeric impurities. Isomers can arise during the *Mitsunobu* coupling step because 3-benzoylthymine and **22** are ambident nucleophiles, capable of reacting at N(1) or O(2). The allylic CH_2 groups adjacent to the pyrimidine ring in such structures can be expected to have different chemical shifts in their NMR spectra. A lower-field (deshielded) resonance for the CH_2 unit may be expected when it is attached to the O-atom than when it is attached to the relatively less electronegative N-atom. It was observed in the ^{1}H-NMR spectra of **28** and **29** that such allylic H-atoms resonated at δ 4.43 (4.90 minor isomer) and 4.49 (4.93 minor isomer), respectively. In these two examples, the major isomers and products after recrystallization were the ones that resonated at higher field indicating the structures are as drawn in *Scheme 2*. This analysis was supported by several examples in the literature [4][5a][35b][39] which indicated that H-atoms on the C-atom attached to an O(2)-linked thymine resonated at lower field than the corresponding N(1)-linked derivatives. In the case of **29**, X-ray analysis also confirmed that the 5-chlorouracil moiety in this compound was N(1)-linked (*Fig. 3*). Without observing minor isomers in the ^{1}H-NMR spectra of compounds **23**, **25**, and **32** before crystallization, it was not possible to make the same comparisons as those observed with **28** and **29**. However, the allylic H-atoms of the CH_2 group bonded directly to the 5-chlorouracil structure in **23** resonated as a *multiplet* at δ 4.52–4.45 in its ^{1}H-NMR spectrum which was very close in value to those found in compounds **28** and **29** indicating an N(1)-linkage in **23** also. The chemical shifts observed in the ^{1}H-NMR spectra for the H-atoms on the appropriate CH_2 groups in **25** and **32** were, as a consequence of the benzene ring or electron-withdrawing CF_2 group, too different from those of **28** and **29** to make the same comparisons as for **23**. However, we concluded that **25** and **32** were likely to be N(1)-linked based on the expected similar chemical reactivity of alcohols **24** and **31** involved in the *Mitsunobu* coupling to those of alcohols **21** and **27** (used to prepare **23**, **28** and **29**).

Further confirmation of structure for compounds **1**–**5** was provided by hetero-nuclear multiple-bond coherence (HMBC) experiments. In all cases, the allylic or

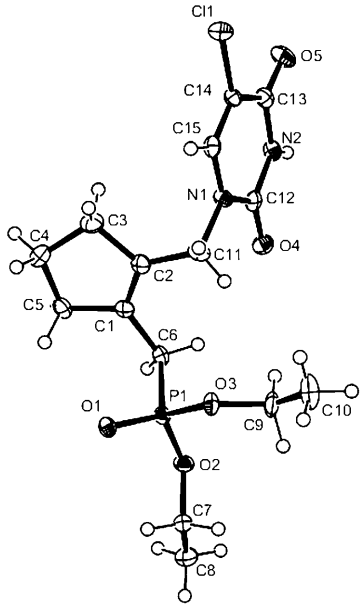

Fig. 3. *ORTEP Plot* [40] *of the independent molecule in* **29** (50% probability ellipsoids for non-H-atoms; arbitrary numbering of the atoms)

benzylic H-atoms attached to the C-atom adjacent to the heterocyclic rings showed strong three-bond interactions with C-atoms C(2) and C(6), and weak four-bond interactions with C(5) in these rings. Additionally, and in all cases, H–C(6) of the pyrimidines showed strong three-bond interactions with the corresponding allylic or benzylic C-atoms. These data supported the N(1)-linked structures drawn for **1**–**5**, because in the alternative O(2)-linked products a similar analysis would require some of the H/C interactions to be over five and six bonds which were less likely to be observed.

Compounds **1**–**5** were tested for their ability to inhibit a recombinant human TP but were found to be inactive with inhibitory constants $K_i > 500$ μM ($K_m = 54$ μM for thymidine). Using the same assay system, control experiments with 5-methyluridine gave a K_i value of 17 ± 2 μM, and uridine gave a K_i value of 13 ± 2 μM, validating the assay method. The lack of useful inhibition was surprising and may reflect the tight geometrical constraints imposed by the S_N2 TS. Alternatively, products **1**–**5** may have too much conformational freedom to enable the pyrimidine and phosphonate groups to attain the optimum spacing required. It may also be essential to have better 2-deoxy-D-ribose mimics present for good inhibitory activity instead of the hydrophobic scaffolds present in **1**–**5** which lack any kind of H-bond contact and probably do not contribute positively to enzyme binding. Whether or not the S_N2 mechanism is the correct pathway for the TP reaction still remains to be validated, and efforts towards establishing this are in progress [19].

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Experimental Part

General. Chromatography solvents were distilled prior to use. Anh. solvents were those commercially available. Org. solns. were dried over anh. $MgSO_4$ and evaporated under reduced pressure. Air-sensitive reactions were performed under Ar. M.p.: uncorrected. Anal. TLC: *Merck* precoated silica gel *60 F254*, detection by UV absorption and/or by heating after dipping in a soln. of $(NH_4)_6Mo_7O_{24}\cdot 4\,H_2O$ (5 wt-%) and $Ce(SO_4)_2\cdot 4\,H_2O$ (0.1 wt-%) in 5% aq. H_2SO_4. Flash chromatography (FC): silica gel *Scharlau* or *Merck 60* (40–60 μm). NMR Spectra: ^{13}C, ^{31}P, and ^{19}F were ^1H-decoupled; chemical shifts in ppm; coupling constants in Hz; ^1H in $CDCl_3$ (internal TMS, δ 0), $(CD_3)_2SO$ (internal TMS, δ 0), or D_2O (internal acetone, δ 2.23); ^{13}C in $CDCl_3$ (center line δ 77.0), $(CD_3)_2SO$ (center line δ 39.7), or D_2O (internal acetone δ 31.5); ^{31}P in $CDCl_3$ or D_2O (external H_3PO_4, δ 0); ^{19}F in $CDCl_3$ or D_2O (external CHF_3, δ 0); assignments of ^1H and ^{13}C resonances were based on 2D (^1H,^1H DQF-COSY, ^1H,^{13}C HSQC, HMBC) and DEPT experiments. MS: Electron impact (EI) at 70 eV and fast atom bombardment (FAB) in a glycerol or nitrobenzyl alcohol matrix, performed by the University of Auckland, New Zealand; electrospray ionization (ESI). Microanalyses were performed by the *Campbell Microanalytical Lab.,* University of Otago, New Zealand.

(Z)-4-(Diethoxyphosphoryl)but-2-en-1-yl Acetate (**8**). *(Z)-But-2-ene-1,4-diol* (**6**; 10.0 g, 113.5 mmol) was dissolved in 36% aq. HCl (24 ml) and left at r.t. for 3 h [21]. The mixture was extracted with AcOEt (2 × 50 ml) and CH_2Cl_2 (2 × 50 ml), then dried, and the solvent was evaporated to give an oil (3.4 g). Bulb-to-bulb distillation gave two fractions. The first fraction (70°/20 Torr) was discarded. The second fraction (130°/20 Torr) consisted mainly of *(Z)-4-chlorobut-2-en-1-yl acetate* (**7**; 1.4 g) of *ca.* 80% purity. Compound **7** (0.51 g, 4.69 mmol) was heated under reflux in $P(OEt)_3$ (5 ml, 28.6 mmol) for 2 h, then excess $P(OEt)_3$ was distilled off and traces removed on a *Kugelrohr* apparatus (150°/0.1 Torr) to leave a colorless residue. FC (AcOEt/hexanes, 8:2 → 9:1) gave **8** (0.50 g, 2.04 mmol, 76%). Colorless oil. ^1H-NMR (300 MHz, $CDCl_3$): 5.81–5.63 (*m*, 2 H); 4.66–4.63 (*m*, 2 H); 4.11 (*quint.*, J = 7.3, 4 H); 2.69 (*dd*, J = 22.5, 7.3, 2 H); 2.06 (*s*, 3 H); 1.32 (*t*, J = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.7 (C); 128.0 (*d*, J = 13.8, CH); 123.2 (*d*, J = 11.3, CH); 62.0 (*d*, J = 6.7, CH_2); 59.9 (CH_2); 26.3 (*d*, J = 140.4, CH_2); 20.8 (Me); 16.4 (*d*, J = 5.9, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 27.6 (*s*). FAB-MS (pos.): 251.1048 ($[M + H]^+$, $C_{10}H_{20}O_5P^+$; calc. 251.1048).

2-[(Diethoxyphosphoryl)methyl]benzyl Acetate (**12**). *Benzene-1,2-dimethanol* (**9**; 2.0 g, 14.48 mmol) was converted to **10** (1.48 g, 65.3%) in the same way as described for the preparation of the corresponding (D_4)phenyl compound [23]. The NMR data were in agreement with those reported for **10** prepared by a different method [41]. Compound **10** (0.62 g, 3.96 mmol) was stirred for 15 min at r.t. with Ac_2O (0.45 ml, 4.75 mmol) and I_2 (0.10 g, 0.40 mmol) [24]. After dilution with Et_2O (50 ml), the mixture was washed successively with 10% aq. $Na_2S_2O_3$, sat. aq. $NaHCO_3$, and brine, then dried, and the solvent was evaporated to give clean acetate **11** (0.79 g, 3.96 mmol, 100%) as a colorless oil with spectroscopic data in agreement with those reported for a sample of **11** obtained by a different method [42]. The *Michaelis–Arbuzov* reaction of **11** (0.79 g, 3.96 mmol) with $P(OEt)_3$ (7 ml, 40.0 mmol) was performed as described for the preparation of **8**. FC (CH_2Cl_2/MeOH 97:3) gave **12** (0.97 g, 3.23 mmol, 82%). Colorless oil. ^1H-NMR (300 MHz, $CDCl_3$): 7.42–7.22 (*m*, 4 H); 5.25 (*s*, 2 H); 4.06–3.95 (*m*, 4 H); 3.28 (*d*, J = 22.0, 2 H); 2.09 (*s*, 3 H); 1.24 (*t*, J = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 170.7 (C); 134.8 (*d*, J = 6.3, C); 131.2 (*d*, J = 5.4, CH); 130.8 (*d*, J = 9.4, C); 130.1 (*d*, J = 2.2, CH); 128.5 (*d*, J = 3.0, CH); 127.3 (*d*, J = 3.2, CH); 64.2 (CH_2); 62.2 (*d*, J = 6.9, CH_2); 30.6 (*d*, J = 138.1, CH_2); 20.9 (Me); 16.3 (*d*, J = 5.8, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 27.3 (*s*). ESI-MS (pos.): 323.1019 ($[M + Na]^+$, $C_{14}H_{21}NaO_5P^+$; calc. 323.1024).

Methyl 2-(Hydroxymethyl)cyclopent-1-ene-1-carboxylate (**14**). Dimethyl cyclopent-1-ene-1,2-dicarboxylate [25] (5.0 g, 27.1 mmol) was dissolved in MeOH (70 ml) and stirred for 16 h with a soln. of KOH (1.52 g, 27.1 mmol) in 80% aq. MeOH (5 ml) [26]. The solvent was evaporated to low volume, H_2O (100 ml) was added and the mixture was washed with CH_2Cl_2 (2 × 50 ml). The aq. phase was acidified with 36% aq. HCl and extracted with CH_2Cl_2 (2 × 100 ml) then dried, and the solvent was evaporated to give **13** as a colorless oil (2.05 g, 12.05 mmol, 44.4%). The ^1H-NMR spectrum was in agreement with that reported in the literature for a sample prepared by a different method [43]. Compound **13** was dissolved in THF (15 ml) containing Et_3N (2.18 ml, 15.66 mmol) and cooled to −5°. $ClCO_2Et$ (1.15 ml,

12.05 mmol) was added dropwise and the mixture was stirred for 30 min [27]. The precipitate was filtered off and washed with THF, and the combined filtrates were added dropwise to a stirred soln. of $NaBH_4$ (1.14 g, 30.1 mmol) in H_2O (15 ml) keeping the temp. within 10–15° with ice cooling. After stirring at r.t. for 2 h, the mixture was cooled in an ice-bath, acidified with 5% aq. HCl and extracted with Et_2O (3 × 50 ml). The combined extracts were washed with 10% aq. NaOH and brine then dried, and the solvent was evaporated to an oil (1.9 g). FC (AcOEt/hexanes 25:75) and then bulb-to-bulb distillation (100°/0.1 Torr) gave **14** (1.41 g, 9.03 mmol, 74.9%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.44 (*d*, *J* = 6.5, *s* after D_2O exchange, 2 H); 3.94 (*t*, *J* = 6.5, exchanged to D_2O, 1 H); 3.76 (*s*, 3 H); 2.71–2.58 (*m*, 4 H); 1.88 (*quint.*, *J* = 7.7, 2 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 167.2 (C); 160.2 (C); 128.6 (C); 60.5 (CH_2); 51.6 (Me); 37.1 (CH_2); 33.7 (CH_2); 21.5 (CH_2). ES-MS (pos.): 156.0786 ($M^{+\bullet}$, $C_8H_{12}O_3^{+\bullet}$; calc. 156.0786).

Methyl 2-(Chloromethyl)cyclopent-1-ene-1-carboxylate (**15**). Bu_3P (4.72 ml, 19.1 mmol) was added to a soln. of **14** (1.57 g, 10.05 mmol) in CCl_4 (50 ml) at 0°. The mixture was warmed to r.t. and stirred for 16 h, then diluted with CH_2Cl_2 (30 ml) and washed with H_2O, dried, and the solvent was evaporated. FC (AcOEt/hexanes 2:98) gave **15** (1.66 g, 9.51 mmol, 95%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.63 (*s*, 2 H); 3.75 (*s*, 3 H); 2.69 (*t*, *J* = 7.7, 4 H); 1.89 (*quint.*, *J* = 7.7, 2 H). ^{1}H-NMR (300 MHz, C_6D_6): 4.50 (*s*, 2 H); 3.31 (*s*, 3 H); 2.46 (br. *t*, *J* = 7.7, 2 H); 2.32 (br. *t*, *J* = 7.7, 2 H); 1.43 (*quint.*, *J* = 7.7, 2 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 165.5 (C); 152.2 (C); 131.2 (C); 51.4 (Me); 40.1 (CH_2); 36.3 (CH_2); 33.9 (CH_2); 21.2 (CH_2). ESI-MS (pos.): 174.0446 ($M^{+\bullet}$, $C_8H_{11}{}^{35}ClO_2^{+\bullet}$; calc. 174.0448); 176.0417 ($M^{+\bullet}$, $C_8H_{11}{}^{37}ClO_2^{+\bullet}$; calc. 176.0418).

Methyl 2-[(Diethoxyphosphoryl)methyl]cyclopent-1-ene-1-carboxylate (**16**). The *Michaelis–Arbuzov* reaction of **15** (1.50 g, 8.59 mmol) with $P(OEt)_3$ (15 ml) was carried out for 1 h as for the preparation of **8**. FC (CH_2Cl_2/MeOH 97:3) gave **16** (2.03 g, 7.35 mmol, 86%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.10 (*quint.*, *J* = 7.1, 4 H); 3.73 (*s*, 3 H); 3.37 (*d*, *J* = 24.2, 2 H); 2.73–2.58 (*m*, 4 H); 1.86 (*quint.*, *J* = 7.6, 2 H); 1.30 (*t*, *J* = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 166.0 (C); 148.8 (*d*, *J* = 11.4, C); 130.3 (*d*, *J* = 12.4, C); 61.9 (*d*, *J* = 6.6, CH_2); 51.0 (Me); 39.0 (CH_2); 33.4 (CH_2); 28.5 (*d*, *J* = 135.7, CH_2); 21.4 (CH_2); 16.4 (*d*, *J* = 6.2, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 26.5 (*s*). ESI-MS (pos.): 276.1124 ($M^{+\bullet}$, $C_{12}H_{21}O_5P^{+\bullet}$; calc. 276.1127).

Ethyl 2-{[(Trifluoromethyl)sulfonyl]oxy}cyclopent-1-ene-1-carboxylate (**18**). *Ethyl 2-oxocyclopentanecarboxylate* (**17**; 3.71 ml, 25.6 mmol) was converted to **18** (7.30 g, 25.3 mmol, 99%) by treatment with 60% NaH (1.28 g, 32.0 mmol) and Tf_2O (5.63 ml, 33.3 mmol) in the same way as described for the corresponding methyl ester [31]. Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.27 (*q*, *J* = 7.2, 2 H); 2.78–2.67 (*m*, 4 H); 2.01 (*quint.*, *J* = 7.7, 2 H); 1.32 (*t*, *J* = 7.2, 3 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 162.3 (C); 153.4 (C); 123.4 (C); 118.4 (*q*, *J* = 320, CF_3); 61.1 (CH_2); 32.7 (CH_2); 29.3 (CH_2); 18.8 (CH_2); 14.0 (Me). ^{19}F-NMR (282.4 MHz, $CDCl_3$): −75.0 (*s*). FAB-MS (pos.): 289.0358 ($[M + H]^+$, $C_9H_{12}F_3O_5S^+$; calc. 289.0358).

Ethyl 2-Iodocyclopent-1-ene-1-carboxylate (**19**). Compound **18** (4.00 g, 13.88 mmol) and NaI (3.12 g, 20.82 mmol) were stirred in DMF (10 ml) and heated under reflux (oil bath 180°) for 2 h. The soln. was cooled to r.t., Et_2O (100 ml) was added and the mixture washed with H_2O (5 × 30 ml), then dried, and the solvent was evaporated. FC (AcOEt/hexanes 2:98) gave crude **19** as a pale purple oil. Bulb-to-bulb distillation (110°/0.1 Torr) afforded pure **19** (1.39 g, 5.22 mmol, 37.6%). Pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.24 (*q*, *J* = 7.1, 2 H); 2.88–2.82 (*m*, 2 H); 2.65–2.58 (*m*, 2 H); 1.97 (*quint.*, *J* = 7.7, 2 H); 1.32 (*t*, *J* = 7.1, 3 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 164.2 (C); 138.4 (C); 106.0 (C); 60.5 (CH_2); 47.9 (CH_2); 33.2 (CH_2); 23.6 (CH_2); 14.2 (Me). FAB-MS (pos.): 266.9880 ($[M + H]^+$, $C_8H_{12}IO_2^+$; calc. 266.9882).

Ethyl 2-[(Diethoxyphosphoryl)(difluoro)methyl]cyclopent-1-ene-1-carboxylate (**20**). A soln. of diethyl [bromo(difluoro)methyl]phosphonate [44] (0.80 g, 3.01 mmol) in anh. *N*,*N*-dimethylacetamide (DMA; 0.2 ml) was added dropwise over 10 min to a suspension of freshly activated Zn dust (0.20 g, 3.01 mmol) in DMA (3 ml; exotherm, 22.6 → 42.3°) [30]. The mixture was stirred at r.t. for 3 h, then CuBr [45] (0.43 g, 3.01 mmol, dried over P_2O_5 under vacuum) was added, followed 30 min later by the dropwise addition of **19** (0.40 g, 1.50 mmol) in DMA (0.2 ml) keeping the temp. below 25°. The mixture was stirred and sonicated (80 W, 50/60 Hz) at r.t. for 20 h (temp. gradually increased to 30°). Et_2O (80 ml) and H_2O (15 ml) were added, and the mixture was filtered through *Celite*. The org. layer was separated and washed with H_2O and brine, then dried, and the solvent was evaporated to leave a colorless

oil which was heated in a *Kugelrohr* apparatus at 70°/0.1 Torr to remove unreacted starting materials. FC (AcOEt/hexanes 1:1) gave **20** (0.28 g, 0.85 mmol, 56.7%). Yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.37–4.19 (*m*, 6 H); 2.82–2.70 (*m*, 4 H); 1.97 (*quint.*, *J* = 7.7, 2 H); 1.38 (*t*, *J* = 7.1, 6 H); 1.30 (*t*, *J* = 7.1, 3 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 166.0 (C); 138.6 (*q*, *J* = 5.8, C); 136.2 (*dt*, *J* = 21.0, 13.5, C); 117.1 (*dt*, *J* = 261, 217, CF_2); 64.7 (*d*, *J* = 6.9, CH_2); 60.8 (CH_2); 36.3 (CH_2); 33.7 (*t*, *J* = 2.7, CH_2); 22.0 (CH_2); 16.3 (*d*, *J* = 5.6, Me); 13.9 (Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 6.6 (*t*, *J* = 111). ^{19}F-NMR (282.4 MHz, $CDCl_3$): −106.6 (*d*, *J* = 111). FAB-MS (pos.): 327.1171 ($[M + H]^+$, $C_{13}H_{22}F_2O_5P^+$; calc. 327.1173).

Diethyl [(Z)-4-Hydroxybut-2-en-1-yl]phosphonate (**21**). A stirred mixture of **8** (0.43 g, 1.70 mmol) and $TsOH \cdot H_2O$ (0.32 g, 1.70 mmol) in EtOH (30 ml) was heated under reflux for 4 h. The EtOH was evaporated, and the residue was dissolved in $CHCl_3$ and washed with sat. aq. $NaHCO_3$ and brine, then dried, and the solvent was evaporated. FC (CH_2Cl_2/MeOH 98:2 → 95:5) afforded **21** (0.34 g, 1.61 mmol, 95%). Light yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.98 (*m*, 1 H); 5.58 (*m*, 1 H); 4.20–4.03 (*m*, 6 H); 3.31 (*t*, *J* = 6.3, exchanged to D_2O, 1 H); 2.72 (*dd*, *J* = 22.9, 8.2, 2 H); 1.33 (*t*, *J* = 7.0, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 134.2 (*d*, *J* = 12.8, CH); 120.3 (*d*, *J* = 12.3, CH); 62.3 (*d*, *J* = 6.5, CH_2); 57.9 (*d*, *J* = 2.1, CH_2); 25.6 (*d*, *J* = 138.0, CH_2); 16.4 (*d*, *J* = 5.8, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 28.7 (*s*). FAB-MS (pos.): 209.0943 ($[M + H]^+$, $C_8H_{18}O_4P^+$; calc, 209.0943).

*3-Benzoyl-5-chloropyrimidine-2,4(1*H*,3*H*)-dione* (**22**). Following the reported method for preparing 3-benzoyl derivatives of uracil and thymine [22], BzCl (6.97 ml, 60.1 mmol) was added in one portion to a stirred suspension of 5-chlorouracil (4.00 g, 27.3 mmol) in a mixture of MeCN (18 ml) and pyridine (7 ml, 90 mmol), and the resulting clear soln. was stirred at r.t. for 16 h. The solvent was evaporated, and the residue was dissolved in AcOEt and washed successively with 5% aq. HCl, sat. aq. $NaHCO_3$, and brine, then dried, and the solvent was evaporated to leave 1,3-dibenzoyl-5-chlorouracil. The latter was dissolved in MeOH (*ca.* 140 ml) and heated under reflux for 15 min with concomitant 1-debenzoylation. After cooling to r.t., the solvent was evaporated leaving an amorphous solid which was washed with Et_2O, then recrystallized (MeOH) to give **22** (2.97 g, 11.85 mmol, 43.4%). Colorless crystals. M.p. 175°, instantly solidifying to an amorphous solid. ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): 12.0 (br. *s*, 1 H); 8.12 (*s*, 1 H); 8.04 (*m*, 2 H); 7.79 (*m*, 1 H); 7.61 (*m*, 2 H). ^{13}C-NMR (75.5 MHz, $(CD_3)_2SO$): 169.0 (C); 159.0 (C); 149.4 (C); 141.0 (CH); 135.8 (CH); 131.1 (C); 130.7 (CH); 129.7 (CH); 106.1 (C). ESI-MS (pos.): 273.0042 ($[M + Na]^+$, $C_{11}H_7{}^{35}ClN_2NaO_3^+$; calc. 273.0043); 275.0024 ($[M + H]^+$, $C_{11}H_7{}^{37}ClN_2NaO_3^+$; calc. 275.0013). Anal. calc. for $C_{11}H_7ClN_2O_3$: C 52.71, H 2.82, N 11.18; found: C 52.70, H 2.70, N 11.26. X-Ray analysis: see [37].

*Diethyl [(Z)-4-(5-Chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)but-2-en-1-yl]phosphonate* (**23**). Diisopropyl azodicarboxylate (DIAD; 0.28 ml, 1.44 mmol) was added dropwise to a soln. of **21** (0.15 g, 0.72 mmol), Ph_3P (0.38 g, 1.44 mmol), and **22** (0.18 g, 0.72 mmol) in anh. 1,4-dioxane (10 ml). The mixture was stirred 16 h at r.t., and then the solvent evaporated. FC (AcOEt/hex 8:2 → AcOEt/MeOH 98:2) gave crude diethyl [(*Z*)-4-(3-benzoyl-5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)but-2-en-1-yl]phosphonate (0.33 g) which was dissolved in 7M NH_3/EtOH soln. (20 ml) and left at r.t. for 3 h. The solvent was evaporated, and FC (AcOEt → AcOEt/MeOH 98:2) gave the crude product as a bright yellow gum (168 mg). Trituration with Et_2O gave a colorless solid (130 mg) which was recrystallized from AcOEt/hexanes to give pure **23** (118 mg, 0.35 mmol, 48.8%, 2 crops). Colorless rhomboids. M.p. 94–95°. ^{1}H-NMR (300 MHz, $CDCl_3$): 9.96 (*s*, exchanged to D_2O, 1 H); 7.76 (*s*, 1 H); 5.85–5.60 (*m*, 2 H); 4.52–4.45 (*m*, 2 H); 4.16 (*quint.*, *J* = 7.2, 4 H); 2.76 (*dd*, *J* = 22.8, 7.9, 2 H); 1.34 (*t*, *J* = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 159.5 (C); 150.2 (C); 141.1 (CH); 127.2 (*d*, *J* = 13.3, CH); 124.4 (*d*, *J* = 11.4, CH); 108.8 (C); 62.4 (*d*, *J* = 6.7, CH_2); 44.8 (CH_2); 25.9 (*d*, *J* = 139.5, CH_2); 16.5 (*d*, *J* = 5.6, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 27.1 (*s*). FAB-MS (pos.): 337.0719 ($[M + H]^+$, $C_{12}H_{19}{}^{35}ClN_2O_5P^+$; calc. 337.0720); 339.0691 ($[M + H]^+$, $C_{12}H_{19}{}^{37}ClN_2O_5P^+$; calc. 339.0691). Anal. calc. for $C_{12}H_{18}ClN_2O_5P$: C 42.81, H 5.39, N 8.32; found: C 43.08, H 5.55, N 8.32.

*Disodium [(Z)-4-(5-Chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)but-2-en-1-yl]phosphonate* (**1**). Compound **23** (0.10 g, 0.297 mmol) and TMSBr (0.39 ml, 2.97 mmol) were dissolved in anh. CH_2Cl_2 (3 ml) and left to stand at r.t. for 16 h. The solvent was evaporated and the residue was dissolved in H_2O and passed through a column of *Dowex 50X8-100* (Na^+) resin. The fractions containing the product were pooled and the H_2O was evaporated. FC (i-PrOH/H_2O 9:1) gave **1** (0.061 g, 0.188 mmol, 63.3%). Colorless solid. ^{1}H-NMR (300 MHz, D_2O): 7.99 (*s*, 1 H); 5.93–5.78 (*m*, 1 H); 5.66–5.53 (*m*, 1 H); 4.53–

4.42 (*m*, 2 H); 2.60 (*dd*, *J* = 21.3, 8.1, 2 H). ^{13}C-NMR (75.5 MHz, D_2O): 163.5 (C); 152.7 (C); 144.8 (CH); 129.2 (*d*, *J* = 10.3, CH); 125.7 (*d*, *J* = 12.9, CH); 109.2 (C); 46.8 (CH_2); 29.6 (*d*, *J* = 129.7, CH_2). ^{31}P-NMR (121.5 MHz, D_2O): 21.7 (*s*). ESI-MS (neg.): 278.9920 ($[M + H - 2Na]^-$, $C_8H_9{}^{35}ClN_2O_5P^-$; calc. 278.9938); 280.9899 ($[M + H - 2Na]^-$, $C_8H_9{}^{37}ClN_2O_5P^-$; calc. 280.9908).

Diethyl [2-(Hydroxymethyl)benzyl]phosphonate (**24**). Compound **12** (0.88 g, 2.92 mmol) was treated with $TsOH \cdot H_2O$ (0.61 g, 3.22 mmol) in EtOH (20 ml) for 2 h as described for the preparation of **21**. FC (CH_2Cl_2/MeOH, 97:3) afforded **24** (0.67 g, 2.58 mmol, 88%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.44 (*m*, 1 H); 7.34–7.19 (*m*, 3 H); 4.73–4.60 (*m*, 3 H, after D_2O exchange became a *s*, 2 H); 4.09–3.89 (*m*, 4 H); 3.30 (*d*, *J* = 21.9, 2 H); 1.24 (*t*, *J* = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 140.4 (*d*, *J* = 5.8, C); 131.4 (*d*, *J* = 6.0, CH); 131.1 (*d*, *J* = 3.1, CH); 129.5 (*d*, *J* = 9.2, C); 128.0 (*d*, *J* = 3.1, CH); 127.8 (*d*, *J* = 3.8, CH); 63.0 (CH_2); 62.7 (*d*, *J* = 6.9, CH_2); 31.1 (*d*, *J* = 137.4, CH_2); 16.3 (*d*, *J* = 5.9, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 28.5 (*s*). ESI-MS (pos.): 281.0916 ($[M + Na]^+$, $C_{12}H_{19}NaO_4P^+$; calc. 281.0919).

*Diethyl {2-[(5-Chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]benzyl}phosphonate* (**25**). The *Mitsunobu* reaction of **24** (0.146 g, 0.58 mmol) with **22** (0.15 g, 0.58 mmol), Ph_3P (0.305 g, 1.16 mmol), and DIAD (0.226 ml, 1.16 mmol) was performed for 48 h in the same way as described for the preparation of **23**. FC (AcOEt/hexanes 7:3) gave crude diethyl {2-[(3-benzoyl-5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]benzyl}phosphonate (0.29 g, 0.58 mmol) which was dissolved in 7M NH_3/EtOH soln. (20 ml) and left at r.t. for 3 h. The solvent was evaporated then FC (AcOEt/hexanes 8:2 → AcOEt) afforded crude **25**. Further FC (CH_2Cl_2/MeOH 98:2) gave a pale yellow gum which was triturated to a colorless solid with Et_2O and recrystallized from AcOEt/hexanes to give pure **25** (0.15 g, 0.40 mmol, 68.5%). Colorless needles. M.p. 132–133°. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.51 (br. *s*, exchanged to D_2O, 1 H); 7.65 (*s*, 1 H); 7.33–7.27 (*m*, 3 H); 7.14–7.08 (*m*, 1 H); 5.15 (*s*, 2 H); 4.09–3.95 (*m*, 4 H); 3.24 (*d*, *J* = 21.8, 2 H); 1.26 (*t*, *J* = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 159.4 (C); 150.6 (C); 141.4 (CH); 134.0 (*d*, *J* = 6.1, C); 131.9 (*d*, *J* = 5.6, CH); 130.2 (*d*, *J* = 9.1, C); 128.5 (CH); 128.0 (CH); 109.1 (C); 62.6 (*d*, *J* = 6.9, CH_2); 48.6 (CH_2); 30.8 (*d*, *J* = 137.9, CH_2); 16.4 (*d*, *J* = 5.8, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 26.8 (*s*). ESI-MS (pos.): 409.0695 ($[M + Na]^+$, $C_{16}H_{20}{}^{35}ClN_2NaO_5P^+$; calc. 409.0696); 411.0686 ($[M + Na]^+$, $C_{16}H_{20}{}^{37}ClN_2NaO_5P^+$; calc. 411.0667). Anal. calc. for $C_{16}H_{20}ClN_2O_5P$: C 49.69, H 5.21, N 7.24; found: C 49.86, H 5.19, N 7.18.

*Disodium {2-[(5-Chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]benzyl}phosphonate* (**2**). Compound **25** (0.128 g, 0.33 mmol) was deprotected with TMSBr (0.43 ml, 3.31 mmol) in the same way as described for **1**. FC (i-PrOH/H_2O, 9:1), followed by freeze drying, gave **2** (0.091 g, 0.24 mmol, 73.4%). Colorless solid. ^{1}H-NMR (300 MHz, D_2O): 7.90 (*s*, 1 H); 7.42–7.26 (*m*, 3 H); 7.16 (*d*, *J* = 7.5, 1 H); 5.12 (*s*, 2 H); 3.12 (*d*, *J* = 20.7, 2 H). ^{13}C-NMR (75.5 MHz, D_2O): 163.4 (C); 152.8 (C); 144.8 (CH); 135.2 (*d*, *J* = 9.1, C); 134.2 (*d*, *J* = 5.7, C); 132.7 (*d*, *J* = 4.6, CH); 129.6 (CH); 128.9 (CH); 128.1 (CH); 109.6 (C); 50.8 (CH_2); 34.1 (*d*, *J* = 127.8, CH_2). ^{31}P-NMR (121.5 MHz, D_2O): 20.9 (*s*). ESI-MS (neg.): 329.0090 ($[M - 2Na + H]^-$, $C_{12}H_{11}{}^{35}ClN_2O_5P^-$; calc. 329.0094); 331.0070 ($[M - 2Na + H]^-$, $C_{12}H_{11}{}^{37}ClN_2O_5P^-$; calc. 331.0065).

*3-Ethoxy-1,3,4,5,6,7-hexahydrocyclopenta[*d*][1,2]oxaphosphinine 3-Oxide* (**26**) *and Diethyl {[2-(Hydroxymethyl)cyclopent-1-en-1-yl]methyl}phosphonate* (**27**). A mixture of $LiBH_4$ (0.163 g, 7.49 mmol), **16** (1.38 g, 5.00 mmol), MeOH (0.30 ml, 7.49 mmol), and Et_2O (45 ml) was stirred at r.t. for 25 min [38]. After cooling in an ice-bath, 5% aq. HCl (5 ml) was added, then Et_2O (50 ml). The org. phase was separated and washed with H_2O and brine, then dried, and the solvent was evaporated. The ^{1}H-NMR indicated the residue to be a *ca.* 1:3 mixture of **26**/**27**. FC (toluene/acetone 85:15) gave first **26**. Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.79–4.60 (*m*, 2 H); 4.24–4.13 (*m*, 2 H); 2.56–2.23 (*m*, 6 H); 1.97 (*quint.*, *J* = 7.5, 2 H); 1.35 (*t*, *J* = 7.1, 3 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 131.8 (*d*, *J* = 12.4, C); 129.7 (*d*, *J* = 8.3, C); 68.6 (*d*, *J* = 6.3, CH_2); 61.5 (*d*, *J* = 6.1, CH_2); 37.5 (*d*, *J* = 12.2, CH_2); 32.3 (CH_2); 23.6 (*d*, *J* = 131.9, CH_2); 22.4 (CH_2); 16.5 (*d*, *J* = 5.0, Me). ^{31}P-NMR (121.5 Hz, $CDCl_3$): 23.1 (*s*). ESI-MS (pos.): 202.0753 ($M^{+\bullet}$, $C_9H_{15}O_3P^{+\bullet}$; calc. 202.0759).

The column was further eluted with toluene/acetone 6:4 to give **27** contaminated with 'diacetone alcohol'. The latter was removed at 80°/0.1 Torr. Further FC (AcOEt → AcOEt/MeOH 97:3) gave pure **27** (0.783 g, 3.15 mmol, 63.1%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.16–4.04 (*m*, 6 H); 3.85 (br. *s*, exchanged to D_2O, 1 H); 2.74 (*d*, *J* = 22.3, 2 H); 2.53–2.41 (*m*, 4 H); 1.84 (*quint.*, *J* = 7.6, 2 H); 1.32 (*t*,

$J = 7.0$, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 140.9 (*d*, $J = 12.0$, C); 127.4 (*d*, $J = 12.8$, C); 62.2 (*d*, $J = 6.9$, CH_2); 59.2 (CH_2); 38.4 (CH_2); 35.2 (*d*, $J = 3.1$, CH_2); 26.4 (*d*, $J = 138.0$, CH_2); 21.8 (CH_2); 16.4 (*d*, $J = 5.9$, Me). ^{31}P-NMR (121.5 Hz, $CDCl_3$): 29.5 (*s*). ESI-MS (pos.): 248.1176 ($M^{+\bullet}$, $C_{11}H_{21}O_4P^{+\bullet}$; calc. 248.1178).

*Diethyl ({2-[(3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}methyl)phosphonate* (**28**). DIAD (0.24 ml, 1.21 mmol) was added dropwise to a stirred soln. of **27** (0.15 g, 0.60 mmol), Ph_3P (0.32 g, 1.21 mmol), and 3-benzoylthymine [22] (0.14 g, 0.60 mmol) in anh. 1,4-dioxane (8 ml). After 3 h at r.t., the solvent was evaporated. FC (AcOEt/hexanes 8:2) gave crude diethyl ({2-[(3-benzoyl-3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}methyl)phosphonate (0.24 g) as a pale yellow gum. The latter (0.23 g) was dissolved in 7M NH_3/EtOH soln. (20 ml) and left to stand at r.t. for 2 h, then the solvent was evaporated. FC (CH_2Cl_2/MeOH, 98:2 → 95:5) afforded a colorless gum (172 mg) which soon crystallized. Recrystallization from AcOEt/hexanes gave pure **28** (0.13 g, 0.37 mmol, 73.4%). Colorless needles. M.p. 130–131°. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.08 (br. *s*, exchanged to D_2O, 1 H); 7.28 (*q*, $J = 1.1$, 1 H); 4.43 (br. *s*, 2 H); 4.12 (*quint.*, $J = 7.5$, 4 H); 2.77 (*d*, $J = 22.6$, 2 H); 2.58–2.47 (br. *m*, 2 H); 2.29 (br. *q*, $J = 7.7$, 2 H); 1.91 (*d*, $J = 1.1$, 3 H); 1.85 (*quint.*, $J = 7.4$, 2 H); 1.34 (*t*, $J = 7.1$, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 164.3 (C); 151.2 (C); 140.2 (CH); 134.0 (*d*, $J = 12.6$, C); 132.3 (*d*, $J = 11.8$, C); 110.6 (C); 62.0 (*d*, $J = 6.7$, CH_2); 45.0 (CH_2); 37.8 (CH_2); 34.0 (CH_2); 26.8 (*d*, $J = 139.6$, CH_2); 21.4 (CH_2); 16.5 (*d*, $J = 5.8$, Me); 12.3 (Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 27.6 (*s*). ESI-MS (pos.): 356.1501 ($M^{+\bullet}$, $C_{16}H_{25}N_2O_5P^{+\bullet}$; calc. 356.1501). Anal. calc. for $C_{16}H_{25}N_2O_5P$: C 53.93, H 7.07, N 7.86; found: C 54.15, H 7.00, N 7.91.

*Disodium ({2-[(3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}methyl)phosphonate* (**3**). Compound **28** (0.11 g, 0.31 mmol) was deprotected with TMSBr (0.40 ml, 3.09 mmol) in the same way as described for **1**. FC (i-PrOH/H_2O 9:1) gave **3** (0.078 g, 0.227 mmol, 73.4%). Colorless solid. ^{1}H-NMR (300 MHz, D_2O): 7.47 (*s*, 1 H); 4.39 (*s*, 2 H); 2.70 (*d*, $J = 21.4$, 2 H); 2.55–2.44 (br. *m*, 2 H); 2.21 (br. *q*, $J = 7.2$, 2 H); 1.86 (*s*, 3 H); 1.79 (*quint.*, $J = 7.5$, 2 H). ^{13}C-NMR (75.5 MHz, D_2O): 168.2 (C); 153.7 (C); 143.9 (CH); 137.2 (*d*, $J = 10.9$, C); 132.2 (*d*, $J = 11.8$, C); 112.0 (C); 47.1 (CH_2); 38.6 (CH_2); 34.4 (CH_2); 30.4 (*d*, $J = 130.1$, CH_2); 22.5 (CH_2); 12.6 (Me). ^{31}P-NMR (121.5 MHz, D_2O): 22.0 (*s*). FAB-MS (pos.): 345.0589 ($[M + H]^+$, $C_{12}H_{16}N_2Na_2O_5P^+$; calc. 345.0592).

*Diethyl ({2-[(5-Chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}methyl)phosphonate* (**29**). The *Mitsunobu* reaction of **27** (0.2 g, 0.81 mmol) with **22** (0.20 g, 0.81 mmol), Ph_3P (0.42 g, 1.61 mmol), and DIAD (0.31 ml, 1.61 mmol) was performed for 2 h in the same way as for the preparation of **23**. FC (AcOEt/hexanes 8:2) gave crude diethyl ({2-[(3-benzoyl-5-chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}methyl)phosphonate which was dissolved in 7M NH_3/EtOH soln. (20 ml) and left at r.t. for 2 h, then the solvent was evaporated. FC (CH_2Cl_2/MeOH 98:2 → 95:5) afforded a colorless gum which was crystallized from AcOEt/hexanes, then recrystallized from the same solvents to give pure **29** (155 mg, 0.41 mmol, 51.1%). Colorless irregular plates. M.p. 132.5–133.5°. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.30 (br. *s*, exchanged to D_2O, 1 H); 7.86 (*s*, 1 H); 4.49 (br. *s*, 2 H); 4.13 (*quint.*, $J = 7.2$, 4 H); 2.75 (*d*, $J = 22.8$, 2 H); 2.59–2.48 (br. *m*, 2 H); 2.30 (br. *q*, $J = 7.4$, 2 H); 1.87 (*quint.*, $J = 7.6$, 2 H); 1.34 (*t*, $J = 7.1$, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 159.4 (C); 150.4 (C); 141.2 (CH); 133.4 (*d*, $J = 11.7$, C); 133.3 (*d*, $J = 12.3$, C); 108.7 (C); 62.2 (*d*, $J = 6.7$, CH_2); 45.5 (CH_2); 37.9 (CH_2); 33.9 (CH_2); 26.8 (*d*, $J = 139.3$, CH_2); 21.4 (CH_2); 16.5 (*d*, $J = 5.7$, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 27.4 (*s*). ESI-MS (pos.): 376.0955 ($M^{+\bullet}$, $C_{15}H_{22}{}^{35}ClN_2O_5P^{+\bullet}$; calc. 376.0955); 378.0924 ($M^{+\bullet}$, $C_{15}H_{22}{}^{37}ClN_2O_5P^{+\bullet}$; calc. 378.0925). Anal. calc. for $C_{15}H_{22}ClN_2O_5P$: C 47.82, H 5.89, N 7.44; found: C 47.88, H 5.90, N 7.38.

X-Ray Structure of **29**[5]). Crystal data: formula $C_{15}H_{22}ClN_2O_5P$; M_r 376.77; crystal size 0.52 × 0.24 × 0.04 mm; crystal system: orthorhombic; crystallization solvent: AcOEt/hexanes; space group: *Pbca*; $Z = 8$; unit cell dimensions; $a = 16.477(3)$, $b = 9.5794(17)$, $c = 22.324(4)$ Å, $V = 3523.6(11)$ Å^3; *Bruker Nonius APEX2 CCD* area detector diffractometer; MoK_α radiation (λ 0.71073 Å); T 93(2) K; $D_x = 1.420$ Mg/m^3, μ(MoK_α) = 0.335 mm^{-1}; measured using φ- and ω-scans; empirical multi-scan absorption correction *Bruker SADABS* [46]; T_{max}, T_{min} 1.0, 0.729. A total of 13176 reflections were measured within the θ range

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[5]) CCDC-693159 contains the supplementary data for this structure, available free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk.

of 1.82–28.11° giving 3996 independent and 2215 observed ($I > 2\sigma(I)$) data. Data reduction and cell refinement (1677 reflections with θ range 2.20–24.86°) was carried out using *Bruker SAINT* [47]. The structure was solved by direct methods and refined on F^2 (284 parameters) with programs SHELXS97 and SHELX97 [48]. For all non-H-atoms, anisotropic displacement parameters were refined. All Me and non-Me H-atoms were refined isotropically with U_{iso} 1.5 and 1.2 times, resp., that of the equivalent U of their parent atom. Final $R(F)$ for $I > 2\sigma(I)$ data = 0.043; $wR(F^2)$ for all data = 0.111; $S = 0.99$. Maximum positive and negative electron density in final difference *Fourier* synthesis 0.40, $-0.32\ e \cdot Å^{-3}$. Calculations and plots were generated using ORTEP-32 [40].

*Disodium ({2-[(5-Chloro-3,4-dihydro-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}methyl)phosphonate* (**4**). Compound **29** (0.14 g, 0.37 mmol) was deprotected with TMSBr (0.48 ml, 3.66 mmol) as for **1**. FC (i-PrOH/H_2O, 9:1) gave **4** (0.065 g, 0.18 mmol, 48.7%). Colorless solid. ^{1}H-NMR (300 MHz, D_2O): 7.94 (*s*, 1 H); 4.48 (*s*, 2 H); 2.66 (*d*, *J* = 21.4, 2 H); 2.56–2.24 (br. *m*, 2 H); 2.24 (br. *q*, *J* = 7.1, 2 H); 1.80 (*quint.*, *J* = 7.5, 2 H). ^{13}C-NMR (75.5 MHz, D_2O): 163.5 (C); 152.9 (C); 144.6 (CH); 138.5 (*d*, *J* = 10.9, C); 131.4 (*d*, *J* = 11.9, C); 109.3 (C); 47.6 (CH_2); 38.5 (CH_2); 34.4 (CH_2); 30.6 (*d*, *J* = 129.9, CH_2); 22.5 (CH_2). ^{31}P-NMR (121.5 MHz, D_2O): 24.1 (*s*). FAB-MS (pos.): 365.0043 ($[M + H]^+$, $C_{11}H_{13}{}^{35}ClN_2Na_2O_5P^+$; calc. 365.0046); 367.0013 ($[M + H]^+$, $C_{11}H_{13}{}^{37}ClN_2Na_2O_5P^+$; calc. 367.0017).

*3-Ethoxy-4,4-difluoro-1,3,4,5,6,7-hexahydrocyclopenta[*d*][1,2]oxaphosphinine 3-Oxide* (**30**) *and Diethyl {Difluoro[2-(hydroxymethyl)cyclopent-1-en-1-yl]methyl}phosphonate* (**31**). Compound **20** (0.50 g, 1.54 mmol) and MeOH (0.09 ml, 2.31 mmol) were dissolved in Et_2O (6 ml) and cooled to 0°. $LiBH_4$ (0.05 g, 2.31 mmol) was added in one portion [38]. After stirring at 0° for 40 min, 5% aq. HCl (2 ml) was added to give a clear soln., which was warmed to r.t., diluted with Et_2O (40 ml) and washed with H_2O and brine, then dried and the solvent was evaporated. FC (AcOEt/hexanes, 4:6) gave first **30** (84 mg, 0.35 mmol, 22.9%). Colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.95–4.69 (*m*, 2 H); 4.45–4.27 (*m*, 2 H); 2.68–2.58 (*m*, 2 H); 2.48–2.34 (*m*, 2 H); 2.08 (*quint.*, *J* = 7.5, 2 H); 1.41 (*t*, *J* = 7.1, 3 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 144.8 (*d*, *J* = 4.7, C); 131.9 (*dt*, *J* = 24.7, 11.9, C); 111.9 (*dt*, *J* = 252, 201, CF_2); 68.1 (*d*, *J* = 4.5, CH_2); 65.2 (*d*, *J* = 5.7, CH_2); 33.0 (CH_2); 28.9 (*d*, *J* = 4.2, CH_2); 22.5 (CH_2); 16.3 (*d*, *J* = 3.9, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 3.42 (*dd*, *J* = 104, 101). ^{19}F-NMR (282.4 MHz, $CDCl_3$): −110.3 (*ABX*, *J* = 315, 104, 101). ES-MS (pos.): 238.0569 ($M^{+\bullet}$, $C_9H_{13}F_2O_3P^{+\bullet}$; calc. 238.0570).

Further elution of the column with AcOEt/hexanes 6:4 gave **31** (0.23 g, 0.79 mmol, 51.3%). Yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.35–4.19 (*m*, 6 H); 3.22 (br. *s*, exchanged to D_2O, 1 H); 2.75–2.55 (*m*, 4 H); 1.90 (*quint.*, *J* = 7.6, 2 H); 1.39 (*t*, *J* = 7.0, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 149.4 (*d*, *J* = 5.4, C); 128.9 (*dt*, *J* = 21.3, 11.6, C); 118.3 (*dt*, *J* = 261, 219, CF_2); 64.9 (*d*, *J* = 6.7, CH_2); 57.8 (CH_2); 36.5 (CH_2); 33.5 (CH_2); 21.6 (CH_2); 16.3 (*d*, *J* = 4.8, Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 8.0 (*t*, *J* = 117). ^{19}F-NMR (282.4 MHz, $CDCl_3$): −105.6 (*d*, *J* = 117). FAB-MS (pos.): 285.1076 ($[M + H]^+$, $C_{11}H_{20}F_2O_4P^+$; calc. 285.1067).

*Diethyl ({2-[(3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}(difluoro)methyl)phosphonate* (**32**). The *Mitsunobu* reaction of **31** (0.20 g, 0.70 mmol) with 3-benzoylthymine [22] (0.162 g, 0.70 mmol), Ph_3P (0.37 g, 1.41 mmol), and DIAD (0.27 ml, 1.41 mmol) was performed for 2 h in the same way as for the preparation of **28**. FC (AcOEt/hexanes 1:1) gave crude diethyl ({2-[(3-benzoyl-3,4-dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}-difluoromethyl)phosphonate (0.34 g, 0.69 mmol) as a colorless gum. The latter was dissolved in 7M NH_3/EtOH soln. (20 ml), left at r.t. for 2 h, then the solvent was evaporated. FC (CH_2Cl_2/MeOH, 98:2 → 95:5) afforded a mixture of **32** and benzamide as a colorless gum. Crystallization from AcOEt/hexanes rapidly gave benzamide (33 mg) which was filtered off. The filtrate was then seeded with a crystal of **28**, and the resulting crystalline mass was filtered off and washed with a little AcOEt/hexane 1:1 and dried to give pure **32** (53 mg, 0.14 mmol, 19.7%). Colorless needles. M.p. 110–111°. ^{1}H-NMR (300 MHz, $CDCl_3$): 8.00 (br. *s*, exchanged to D_2O, 1 H); 7.34 (*d*, *J* = 1.2, 1 H); 4.68 (br. *s*, 2 H); 4.30 (*quint.*, *J* = 7.3, 4 H); 2.69–2.59 (br. *m*, 2 H); 2.47–2.35 (br. *m*, 2 H); 1.96–1.83 (*m*, 5 H); 1.41 (*t*, *J* = 7.1, 6 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 164.5 (C); 151.4 (C); 143.9 (*d*, *J* = 4.7, C); 140.1 (CH); 132.1 (*dt*, *J* = 21.6, 12.5, C); 118.2 (*dt*, *J* = 261, 219, CF_2); 111.1 (C); 64.9 (*d*, *J* = 6.6, CH_2); 44.4 (CH_2); 35.2 (CH_2); 33.4 (CH_2); 21.3 (CH_2); 16.4 (*d*, *J* = 4.5, Me); 12.3 (Me). ^{31}P-NMR (121.5 MHz, $CDCl_3$): 7.50 (*t*, *J* = 115.9). ^{19}F-NMR (282.4 MHz, $CDCl_3$): −105.0, (*d*, *J* = 115.9). ES-MS (pos.): 392.1313 ($M^{+\bullet}$, $C_{16}H_{23}F_2N_2O_5P^{+\bullet}$; calc. 392.1313). Anal. calc. for $C_{16}H_{23}F_2N_2O_5P$: C 48.98, H 5.91, N 7.14; found: C 49.09, H 5.95, N 7.13. The

material left from the above filtrates after crystallization (197 mg) consisted of a mixture **32**/benzamide *ca.* 1.3:1 (estimated by ^{1}H-NMR).

*Disodium ({2-[(3,4-Dihydro-5-methyl-2,4-dioxopyrimidin-1(2*H*)-yl)methyl]cyclopent-1-en-1-yl}(difluoro)methyl)phosphonate* (**5**). Compound **32** (0.046 g, 0.12 mmol) was deprotected over 2 d with TMSBr (0.15 ml, 1.17 mmol) as for **1**. FC (CH_2Cl_2/i-PrOH/H_2O, 5:4.5:0.5 → i-PrOH/H_2O, 9:1) gave, after freeze drying, **5** (0.015 g, 0.04 mmol, 33.7%). Colorless solid. ^{1}H-NMR (300 MHz, D_2O): 7.49 (*s*, 1 H); 4.66 (*s*, 2 H); 2.62 (br. *s*, 2 H); 2.31 (br. *s*, 2 H); 1.88–1.77 (*m*, 5 H). ^{13}C-NMR (75.5 MHz, D_2O): 168.2 (C); 153.8 (C); 143.7 (CH); 141.1 (C); 135.5 (*dt*, J = 21.3, 11.2, C); 121.9 (*dt*, J = 258, 198, CF_2); 112.5 (C); 46.9 (CH_2); 35.9 (CH_2); 35.0 (CH_2); 22.5 (CH_2); 12.6 (Me). ^{31}P-NMR (121.5 MHz, D_2O): 5.54 (*t*, J = 103). ^{19}F-NMR (282.4 MHz, D_2O): −106.1 (*d*, J = 103). FAB-MS (pos.): 381.0408 ($[M + H]^+$, $C_{12}H_{14}F_2Na_2N_2O_5P^+$; calc. 381.0404).

Preparation of TP. Recombinant human thymidine phosphorylase was provided by Dr. *Paula Krosky* (*National Institute of Health*, USA) from *Escherichia coli* BL21(DE3) cells transformed with the pCal-*n*-EK/TP (pCal-*n*-EK from *Invitrogen*) and pGroESL vectors. Purification was performed on a calmodulin column, and the calmodulin binding protein was cleaved from TP using enterokinase, which was removed using soybean trypsin inhibitor-sepharose. TP was concentrated to *ca.* 3 mg/ml and stored at −70°.

Enzyme Kinetics. Binding constants were determined by steady-state kinetic assays using 2′-deoxy-5-nitrouridine (5NdU) as the substrate (K_m = 50 μM at pH 6.01). Under these conditions, the K_m value for thymidine was 54 μM. The reaction mixture consisted of **1**–**5** (*ca.* 1/10 × K_m to 10 × K_m), 50 mM MES (pH 6.01), 150 mM NaCl, 1 mM EDTA, 5 mM inorg. phosphate, and 250 μM 5NdU in a total of 1 ml. Thymidine phosphorylase was added to *ca.* 10 nM, and the reaction was monitored at λ = 347 nm for 30 min. The extinction coefficient for this conversion is *ca.* 10^4 M^{-1} cm^{-1} [1a]. At least five concentrations of **1**–**5** were used for each compound, and experiments were repeated in triplicate. Initial rates were determined by a linear least-squares fit to the linear portion of the data using KaleidaGraph (v 3.5). The K_i values for compounds **1**–**5** were found to be > 500 μM. 5-Methyluridine and uridine gave K_i values of 17 ± 2 and 13 ± 2 μM, resp., in positive control assays.

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