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# Synthesis and $P2Y_2$ receptor agonist activities of uridine 5′-phosphonate analogues

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ABSTRACT

We explored the influence of modifications of uridine 5′-methylenephosphonate on biological activity at the human $P2Y_2$ receptor. Key steps in the synthesis of a series of 5-substituted uridine 5′-methylenephosphonates were the reaction of a suitably protected uridine 5′-aldehyde with [(diethoxyphosphinyl)methylidene]triphenylphosphorane, C-5 bromination and a Suzuki–Miyaura coupling. These analogues behaved as selective agonists at the $P2Y_2$ receptor, with three analogues exhibiting potencies in the submicromolar range. Although maximal activities observed with the phosphonate analogues were much less than observed with UTP, high concentrations of the phosphonates had no effect on the stimulatory effect of UTP. These results suggest that these phosphonates bind to an allosteric site of the $P2Y_2$ receptor.



## 1. Introduction

Extracellular purine and pyrimidine nucleosides and nucleotides act as messengers via specific receptors on the plasma membrane. These receptors exist in two families: P1 receptors (also termed adenosine receptors) activated by adenosine and P2 receptors activated by adenosine 5′-tri- or diphosphate (ATP or ADP) and/or uridine 5′-tri- or diphosphate (UTP or UDP). P2 receptors are further divided as P2X and P2Y receptors, which are ligand-gated ion channels and G protein-coupled receptors (GPCRs), respectively.[1–4] At least eight different subtypes of P2Y receptors are known, that is $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$. The missing numbers belong to non-mammalian receptors, which may be orthologs of mammalian subtypes, or receptors that do not appear to be bona-fide P2Y receptor family members.[5]

The various *human* P2Y receptor subtypes are activated by different physiological nucleotides. The human $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors are activated preferentially by ADP, while the human $P2Y_{11}$ receptor is activated preferentially by ATP. The human $P2Y_4$, $P2Y_6$, and $P2Y_{14}$ subtypes respond exclusively to various uracil nucleotides, and the human $P2Y_2$ receptor is activated by UTP and ATP with similar potency. The P2Y receptors are preferentially coupled to heterotrimeric G proteins of the $G_q$ ($P2Y_1$–$P2Y_{11}$) or $G_i$ ($P2Y_{12}$–$P2Y_{14}$) families, to stimulate phospholipase C (PLC) or to inhibit adenylyl cyclase (AC), respectively. The $P2Y_{11}$ receptor is also coupled to $G_s$ proteins.

The $P2Y_2$ receptor is the most widely studied uracil nucleotide receptor.[5] It is broadly distributed throughout the body and is most prominently expressed in the lung, heart, skeletal muscle, spleen, kidney, and liver.[6,7] The $P2Y_2$ receptor is known to play important physiological roles in epithelial cells of the lung, gastrointestinal tract and the eye, and therefore, it is under investigation as a therapeutic target. Agonists are promising for treatment of cystic fibrosis, cancer and dry eye syndrome,[8,9] while $P2Y_2$ antagonists might have anti-inflammatory[10] and neuroprotective effects.[11]

The major limitations associated with known agonists for the $P2Y_2$ receptor are (i) the lack of selectivity versus closely related P2Y receptor subtypes and (ii) their fast degradation by nucleotide-hydrolyzing ecto-enzymes, which results in a relative short duration of action.[5] In that context, we recently explored to what extent replacement of the α-phosphate group of UTP by an isosteric phosphonate affected $P2Y_2$ receptor activity.[9] Since the carbon–phosphorus bond cannot be hydrolyzed, this analogue was expected to exhibit prolonged metabolic stability. While we initially

*Abbreviations:* NBS, *N*-bromosuccinimide; TMSBr, trimethylsilyl bromide; TFA, trifluoroacetic acid; NMO, *N*-methylmorpholine-*N*-oxide.

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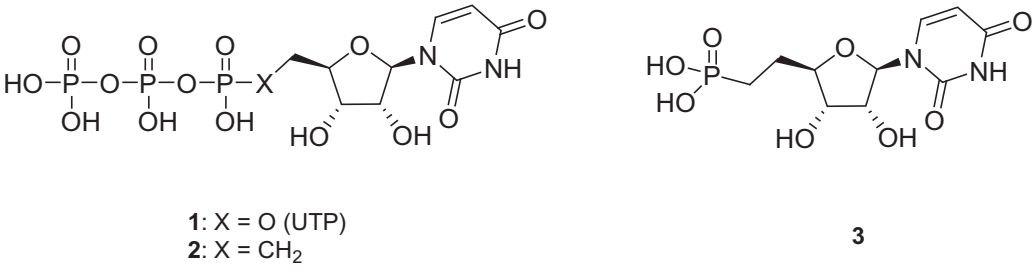


**1**: X = O (UTP)
**2**: X = $CH_2$

**3**

**Figure 1.** Structure of UTP (**1**), diphosphophosphonate **2**, and 5′-methylenephosphonate **3**.

focused on a diphosphophosphonate mimic of UTP (**2**), it was fortuitously discovered that its synthetic precursor **3** was also capable of activating the $P2Y_2$ receptor but was inactive at the $P2Y_4$ receptor (Fig. 1).[9]

In this study, we explore the influence of further modifications of the 5′-methylenephosphonate **3** on activity at the $P2Y_2$ receptor. A patent application from Astra-Zeneca indicated that incorporation of a large semiplanar, hydrophobic aromatic ring at position 5 of thiouridine triphosphate may be accommodated by the $P2Y_2$ receptor but tends to preclude the conformational change required for receptor activation.[12] Therefore, we introduced several smaller (hetero)aromatic substituents at the 5-position of analogue **3** to enhance binding but still allow for receptor activation. To sort out the influence of replacement of the 2′-OH group of **3**, we envisaged the synthesis of a 2′-chloro and a 2′-amino analogue. Besides establishing possible interactions with the target receptor, a 2′-chloro substituent may impact on the furanose ring conformation. In the case of UTP, a 2′-amino modification was associated with increased $P2Y_2$ selectivity while maintaining excellent potency.[13] To assess the influence of rigidifying the ribofuranose conformation of **3**, an *N*-methanocarba analogue was synthesized. Finally, another phosphonate bioisoster of uridine 5′-monophosphate (UMP), obtained by the inversion of the 4′-$CH_2$–*O* group of UMP, was also explored. Transforming a phosphate moiety ($(RO)_2P(O)$–O–C–) to its isomeric catabolically stable phosphonomethyl ether ($(RO)_2P(O)$–C–O–) has proven to be a successful strategy in the development of antiviral drugs.[14]

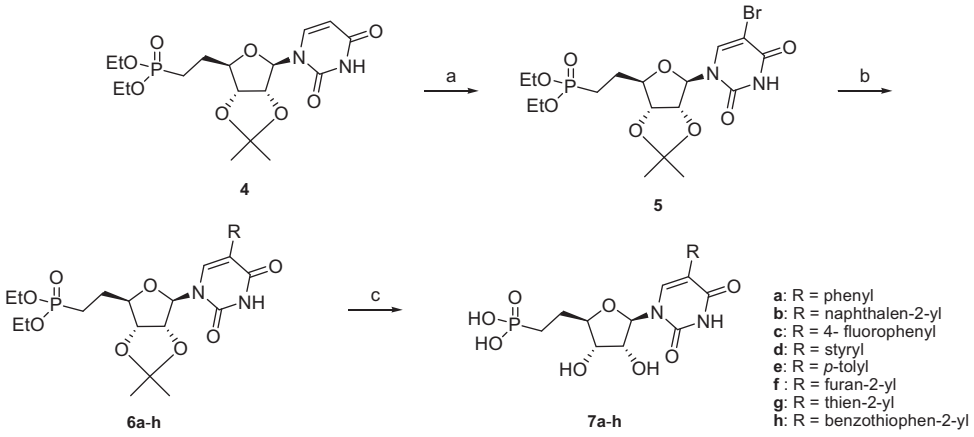
## 2. Results and discussion

### 2.1. Chemistry

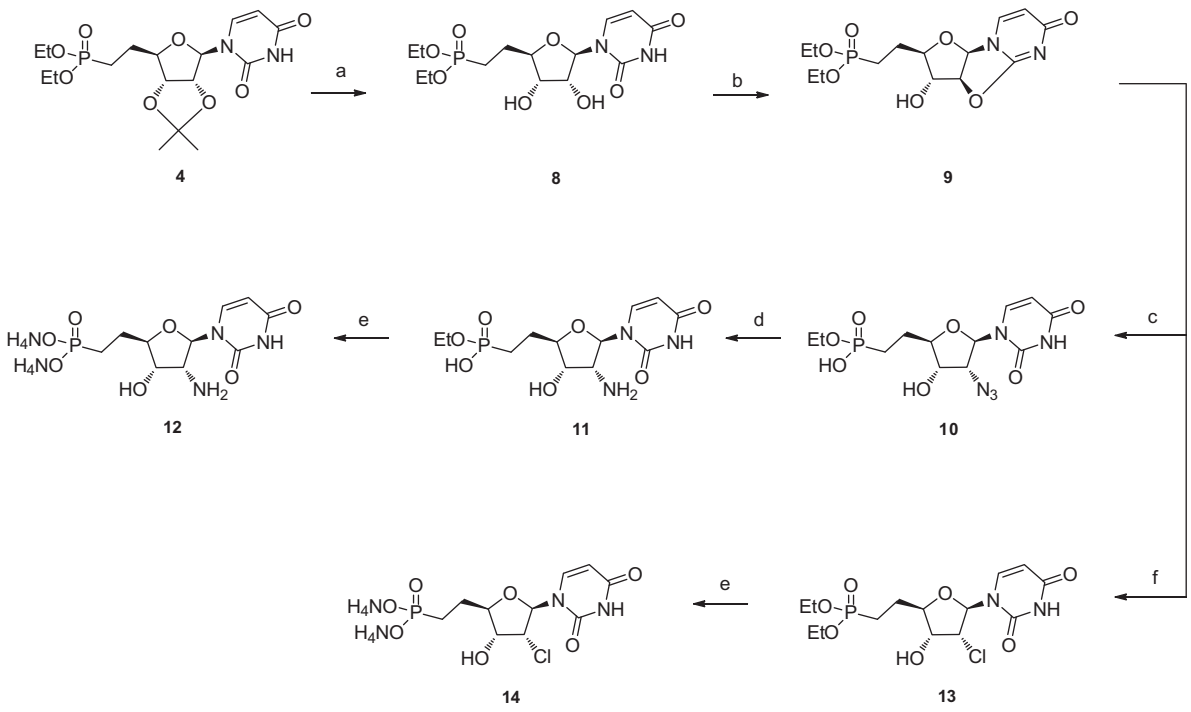
Different methods for the preparation of isosteric phosphonate analogues of nucleoside phosphates have been reported. Most often these involve a Wittig-type[15] or an Arbuzov[16] reaction. In addition, Barton et al.[17] published a radical approach for the introduction of the carbon–phosphorous bond. We decided to follow the method described by Xu et al.[18] in which the isosteric analogue was prepared by treatment of a suitably protected uridine 5′-aldehyde with a stabilized [$Ph_3P$=CHPO$(OEt)_2$] ylide. Hydrogenation of the obtained olefin in MeOH using a 10% palladium on carbon catalyst gave access to the known compound **4**.[9] C-5 selective NBS-mediated bromination of this intermediate followed by a Suzuki–Miyaura coupling with a number of commercial aryl and heteroaryl boronic acids gave access to a series of C-5 substituted analogues. The latter transformation took place in a DMF–$H_2O$ solution and was catalyzed by $Pd(PPh_3)_4$. Sodium carbonate was used for the activation of the boronic acids.[19] One-pot deprotection of the phosphonate diester and the 2′,3′-*O*-isopropylidene group by consecutive treatment with TMSBr and TFA afforded the desired phosphonates **7a–h** in variable yields (Scheme 1).

The synthesis of 2′-substituted 5′-methylenephosphonates **12** and **14** started from intermediate **4** (Scheme 2). Following deprotection of the 2′- and 3′-hydroxyl groups of **4**, 2′,2-*O*-anhydro analogue **9** was formed using thionyl chloride in $CH_3CN$ followed by the addition of NaOAc.[20] Introduction of an amino group at the 2′-position was accomplished via opening of the anhydro-derivative **9** with $NaN_3$ in DMF. Interestingly, these conditions caused concomitant incomplete hydrolysis of the phosphonate ester to afford **10** as reported previously by Holy.[21] Staudinger reduction of the azide followed by deprotection of mono ethylphosphonate **11** resulted in the desired 2′-amino-2′-deoxyuridine-5′-phosphonate analogue **12**. Similarly, treatment of **9** with a 2 N HCl in diethyl ether solution followed by the hydrolysis of the phosphonate diester resulted in 2′-chloro-2′-deoxy analogue **14**.

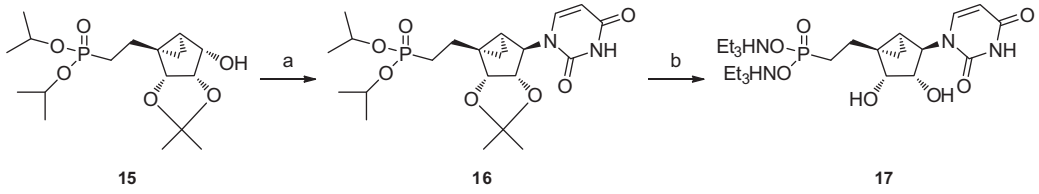
The synthetic procedure for *N*-methanocarba analogue **17** is depicted in Scheme 3. An initial Mitsunobu base coupling reaction of previously reported alcohol **15**[22] using triphenylphosphine,




**Scheme 1.** Reagents and conditions: (a) NBS, DMF, rt, overnight, 79%; (b) R-$B(OH)_2$, $Na_2CO_3$, $Pd(PPh_3)_4$, DMF, $H_2O$, reflux, 4 h, 39–76%; (c) (i) TMSBr, $CH_2Cl_2$, rt, overnight; (ii) TFA, $H_2O$, rt, 4 h, 11–78%.



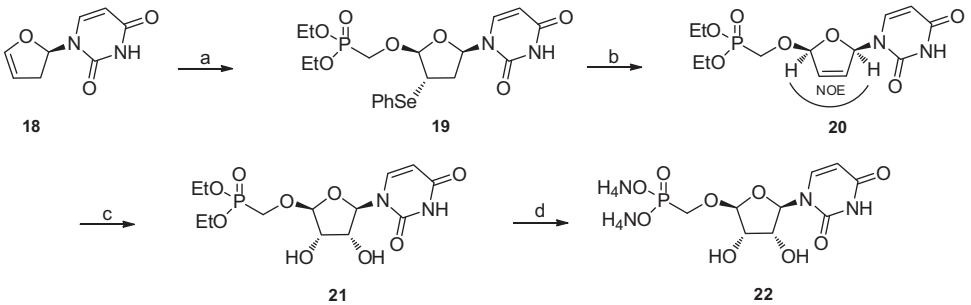

**Scheme 2.** Reagents and conditions: (a) 50% aq HCOOH, rt, 4.5 h, 57%; (b) (i) thionyl chloride, $CH_3CN$; (ii) $CH_3COONa$, DMF, 85 °C, 92%; (c) $NaN_3$, DMF, 150 °C, overnight, 88%; (d) $PPh_3$, THF, $H_2O$, rt, overnight, quant.; (e) TMSBr, $CH_2Cl_2$, rt, overnight, 78% for 2′-amino and 58% for 2′-chloro; (f) 2 N HCl in $Et_2O$–dioxane (3:1), rt, overnight, 54%.




**Scheme 3.** Reagents and conditions: (a) (i) Triphenylphosphine, 3-*N*-benzoyluracil, diisopropyl azodicarboxylate, anhyd THF; (ii) 6 N $NH_3$/MeOH, 50 °C, 24% (over two steps); (b) iodotrimethylsilane, anhyd $CH_2Cl_2$, 19%.

diisopropyl azodicarboxylate and 3-*N*-benzoyluracil[23] followed by a debenzoylation using 6 N $NH_3$/MeOH gave phosphonate diester **16** in 24% overall yield. Simultaneous deprotection of the phosphonate diester and the 2′,3′-*O*-isopropylidene group using freshly opened iodotrimethylsilane[24] afforded target phosphonate **17**.

The synthesis of 4′-*O*–$CH_2$ phosphonate analogue **22** started from 2′,3′-dideoxy-3′,4′-didehydro-β-D-erythrofuranosyl)uracil **18** (Scheme 4).[25] Treatment of this glycal with phenylselenyl chloride at −70 °C followed by the addition of silver perchlorate in the presence of diethyl (hydroxymethyl)phosphonate afforded phosphonate **19** in 35% overall yield. Assignment of the stereoarrangement in **19** was based on mechanistic considerations.[26] Phosphonate **19** was further transformed into olefin **20** via sodium periodate oxidation of selenium and subsequent elimination. $OsO_4$-promoted dihydroxylation in the presence of *N*-methylmorpholine-*N*-oxide followed by TMSBr hydrolysis of the phosphonate ester groups provided compound **22** in moderate yield. A positive NOE was observed between the H-1′ and H-4′ of compound **20**, confirming the *cis* stereoarrangement of the uracil base and the phosphonethoxy side chain.




**Scheme 4.** Reagents and conditions: (a) (i) PhSeCl, $CH_2Cl_2$, −70 °C, 1 h; (ii) $HOCH_2PO(OEt)_2$, $AgClO_4$, $CH_2Cl_2$, $CH_3CN$, −70 to 0 °C, 15 min, 35%; (b) $NaIO_4$, $NaHCO_3$, MeOH, rt, 1 h then 80 °C, 75 min, 68%; (c) $OsO_4$, NMO, acetone–$H_2O$ (5:1), rt, 48 h, 60%; (d) TMSBr, 2,6-lutidine, DMF, 0 °C to rt, overnight, 50%.

### 2.2. Pharmacological evaluation

Activities of analogues at the $P2Y_2$ receptor were determined measuring PLC-dependent phosphoinositide hydrolysis in 1321N1 human astrocytoma cells stably expressing the human $P2Y_2$ receptor ($P2Y_2$-1321N1 cells).[27,28] As previously shown for UTP and UDP,[27] none of the phosphonate analogues reproducibly promoted inositol phosphate accumulation in wild-type 1321N1 cells (Fig. 2A).

In contrast, quantification of inositol phosphate accumulation in $P2Y_2$ receptor-expressing 1321N1 cells revealed that the newly synthesized 5′-methylenephosphonate analogues are $P2Y_2$ receptor agonists (Fig. 2B), although none of the analogues produced maximal effects at 100 μM concentration as great as UTP. Given the novel activity observed with these phosphonate analogues at the $P2Y_2$ receptor, we also determined whether they interacted with the UTP-activated $P2Y_4$ receptor or the UDP-activated $P2Y_6$ receptor. Little or no reproducible stimulation was observed over basal activity for any of these analogues in 1321N1 human astrocytoma cells stably expressing the human $P2Y_4$ (Fig. 2C) or human $P2Y_6$ (Fig. 2D) receptors.

Full concentration effect curves were generated in $P2Y_2$-1321N1 cells with each of the analogues to more clearly assess their maximal effects relative to UTP and to establish their relative potencies as $P2Y_2$ receptor agonists. To enhance clarity of comparison of the relative activities of these analogues, the data are arbitrarily presented in four separate panels (Fig. 3).

The 5-modified uridine 5′-phosphonate analogues activated the $P2Y_2$ receptor with a range of apparent potencies, and the maximal effect observed with each of these molecules was only 40–55% of that observed with UTP (Fig. 3 and Table 1). The most potent agonists were the 5-(4-fluorophenyl) (**7c**), thien-2-yl (**7g**), and 5-(2-furanyl) (**7f**) analogues, which exhibited potencies in the submicromolar range (Table 1).

Both 2′-modified analogues (**12** and **14**) were essentially inactive at the $P2Y_2$ receptor. The negative impact of the replacement of a 2′-OH by a 2′-$NH_2$ group may indicate that these phosphonates have a different mode of binding to the $P2Y_2$ receptor, since a similar modification on UTP was well tolerated.

Conformationally constrained compound **17** behaved as a low efficacy $P2Y_2$ agonist compared to UTP. This indicated that a (*N*) conformation of a ribose-like moiety in this series of nucleoside monophosphonates is compatible with the observed biological effect. Likewise, compound **22** was significantly less potent than the lead phosphonate indicating that replacement of the 5′-$CH_2$ group by an oxygen tends to reduce agonist activity.

The activities of two of the most potent new compounds **7c** and **7f** were directly compared in the same experiments to the 5-unsubstituted parent compound **3** (Fig. 4). Both analogues produced maximal effects at the $P2Y_2$ receptor that were similar to that of **3**, but were approximately 10-fold more potent than this previously studied analogue.

The idea that these modified uridine 5′-phosphonate analogues are orthosteric ligands that exhibit less intrinsic efficacy than UTP was examined by assessing their capacity at high concentrations to inhibit the effect of a near-maximal concentration of UTP (Fig. 5A). No inhibitory effect on the action of UTP was observed with any of these phosphonate analogues. Concentration effect curves for UTP

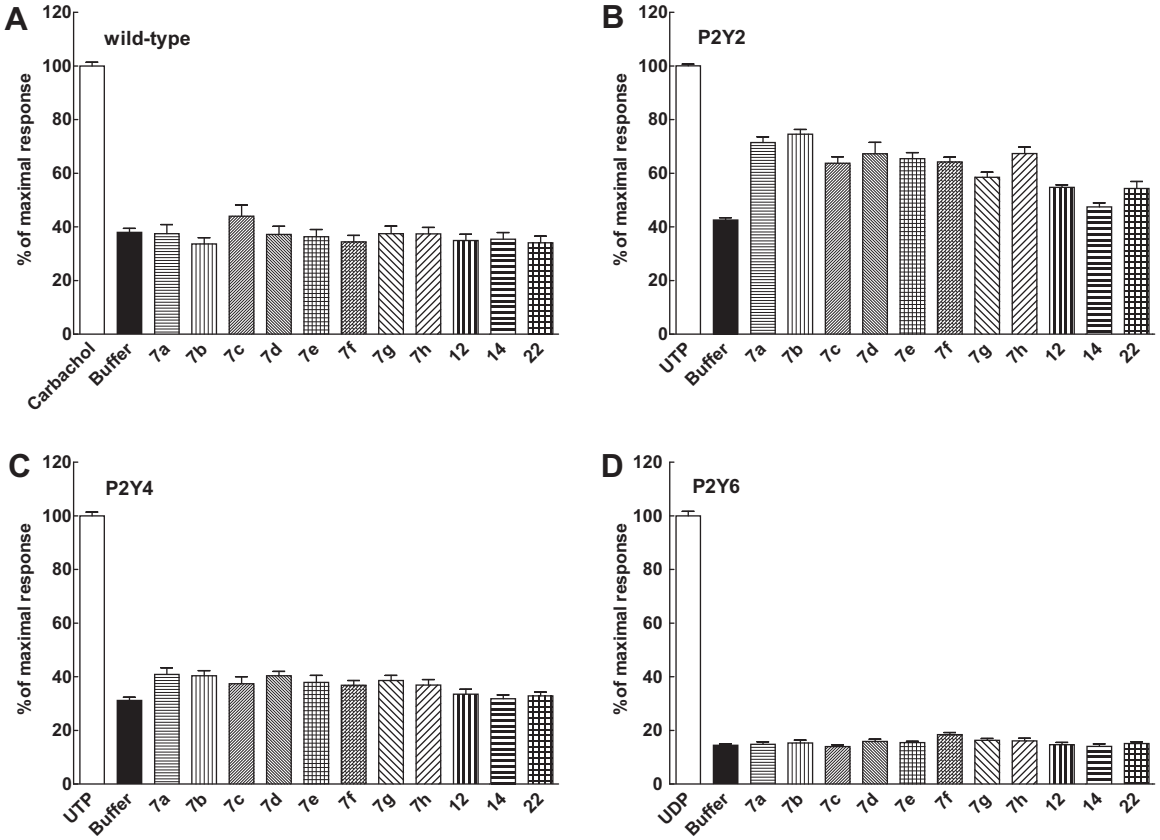



**Figure 2.** Inositol phosphate accumulation in (A) wild-type 1321N1 human astrocytoma cells or in 1321N1 cells stably expressing the human (B) $P2Y_2$, (C) $P2Y_4$, or (D) $P2Y_6$ receptor. The concentration of all phosphonate analogues was 100 μM, of carbachol was 100 μM, of UTP was 100 nM, and UDP was 1 μM. [$^3$H]inositol phosphate accumulation was quantified as described in Methods. The results are presented as the percent of response observed with carbachol (A), UTP (B and C), or UDP (D). Values are presented as the mean ± SEM and are the average of results obtained in at least three separate experiments.

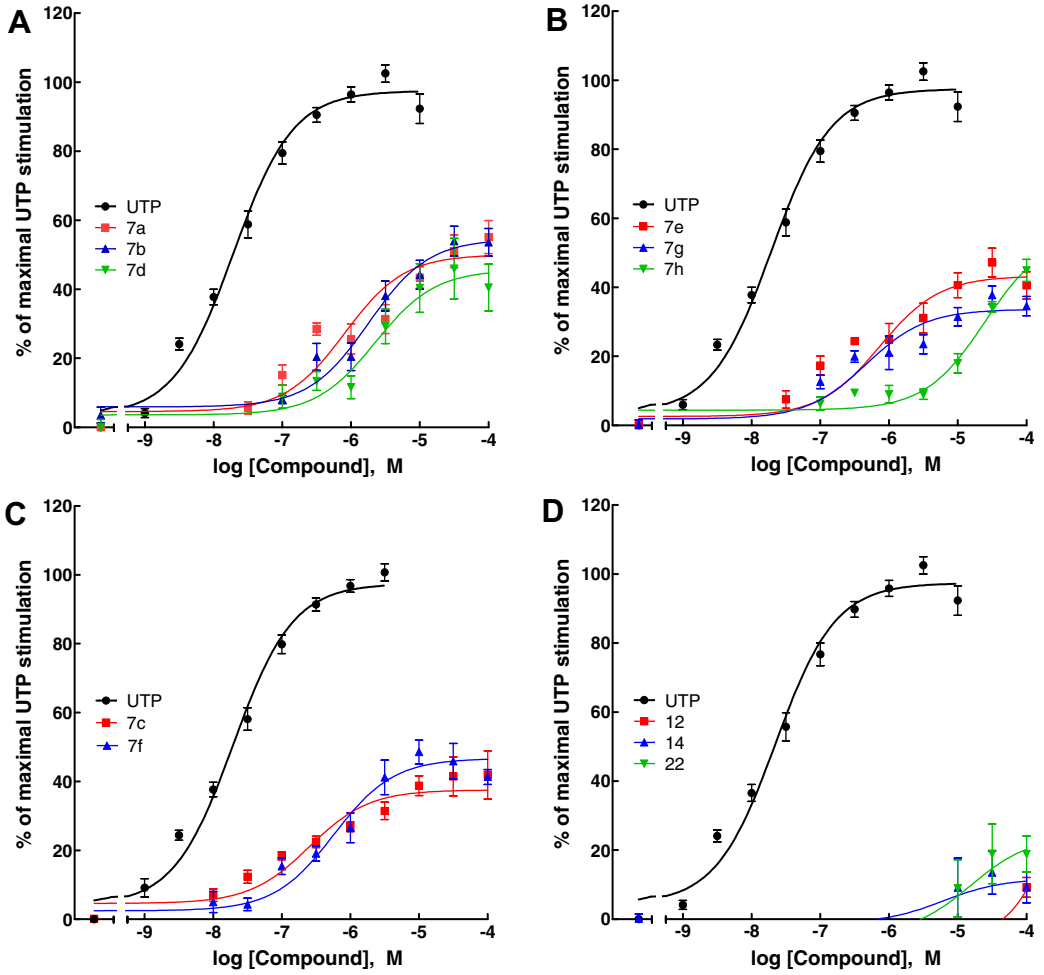



**Figure 3.** Concentration dependent activation of the $P2Y_2$ receptor by phosphonate nucleotide derivatives. Accumulation of [$^3$H]inositol phosphates was quantified as described under Methods. The data are plotted as percent of the maximal response observed with UTP, are presented as the mean ± SEM, and are representative of results obtained in three or more separate experiments.

**Table 1**
Relative potencies and maximal effects of UTP and phosphonate analogues at the human $P2Y_2$ receptor

| Compound | $EC_{50}$ (μM) | Max response |
|---|---|---|
| **1** | 0.02 ± 0.003 | 100 |
| **3** | 5.1 ± 0.9 | 32 ± 9 |
| **7a** | 1.5 ± 0.5 | 53 ± 7 |
| **7b** | 2.1 ± 0.6 | 54 ± 7 |
| **7c** | 0.4 ± 0.2 | 43 ± 5 |
| **7d** | 3.1 ± 1.3 | 41 ± 12 |
| **7e** | 1.0 ± 0.8 | 41 ± 7 |
| **7f** | 0.7 ± 0.3 | 45 ± 5 |
| **7g** | 0.5 ± 0.3 | 35 ± 5 |
| **7h** | >20 | 45 ± 5 |
| **12** | >20 | 9 ± 5 |
| **14** | >20 | 9 ± 8 |
| **17** | 4.0 ± 0.7 | nd |
| **22** | >20 | 19 ± 9 |

Agonist potency ($EC_{50}$ value) and maximal response relative to UTP was determined for each analogue in $P2Y_2$-1321N1 cells as described under Methods. The data are presented as the mean ± SEM of values obtained from 3 to 6 separate experiments with each analogue.

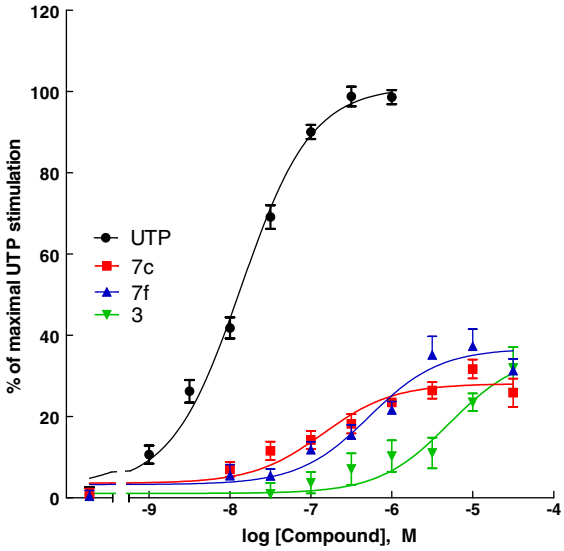



**Figure 4.** Comparison of the concentration dependence of the most potent phosphonates for activation of the $P2Y_2$ receptor. Accumulation of [$^3$H]inositol phosphates was quantified as described under Methods. The data are plotted as percent of the maximal response observed with UTP, are presented as the mean ± SEM, and are representative of results obtained in three separate experiments.

also were carried out in the absence and presence of a high (100 μM) concentration of **7c** or **7f** to determine whether the activity of these molecules was consistent with that of agonists acting at the orthosteric or an allosteric site of the $P2Y_2$ receptor. As illustrated in Figure 5, neither **7c** nor **7f** affected the potency of

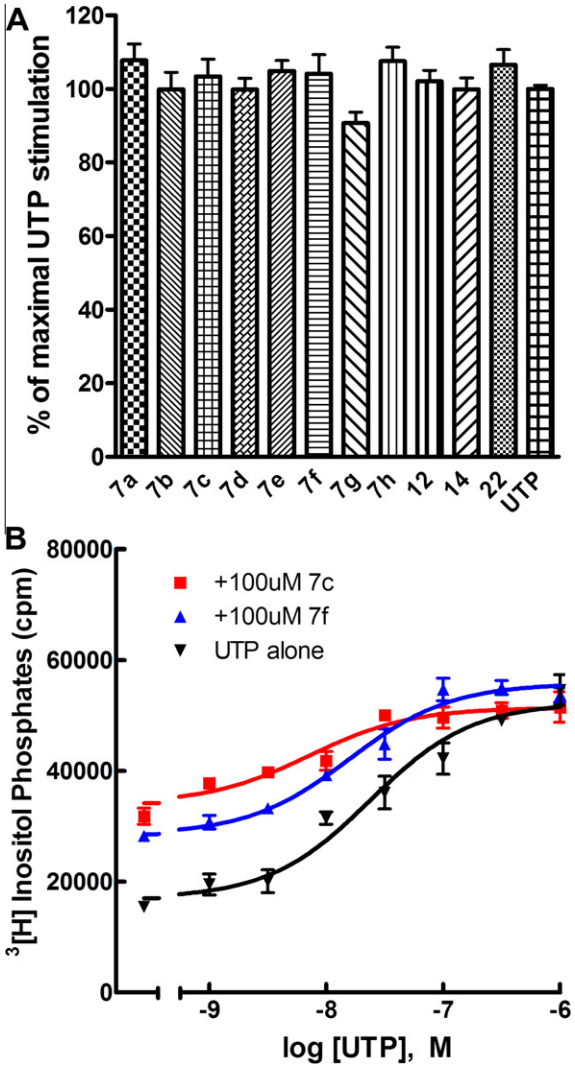



**Figure 5.** Effect of high concentrations of phosphonate analogues on the $P2Y_2$ receptor-mediated activity of UTP. (A) The effects of 100 μM concentrations of the indicated phosphonates were tested in the presence of 100 nM UTP in $P2Y_2$-1321N1 cells as described in Methods. (B) Concentration response curves for UTP were generated in $P2Y_2$-1321N1 cells in the absence or presence of 100 μM **7c** or **7f**. [$^3$H]inositol phosphate accumulation was quantified as described in Methods. The data are presented as the mean ± SEM and are representative of results obtained in three separate experiments.

UTP. These results are consistent with the idea that the uridine 5′-phosphonate analogues act at an allosteric site rather than the orthosteric binding pocket to produce their effect on $P2Y_2$ receptor activity.

### 2.3. Conformational analysis

The furanose ring of nucleotides displays a characteristic equilibrium between two favored puckering conformations, that is the (N) and South (S) state.[29] Davies et al.[30] derived the following equations that relate the fraction of the (N) conformer $X_N$ with the values of the vicinal three scalar couplings that can be measured between the protons of ring:

$$J_{H'_1,H'_2} = 9.3(1 - X_N)$$

$$J_{H'_2,H'_3} = 4.6X_N + 5.3(1 - X_N)$$

$$J_{H'_3,H'_4} = 9.3X_N$$

**Table 2**
$^1$H NMR derived mole fraction of the (N)-type conformer of **3** and **7a** at different temperatures

| *T* (°C) | $X_N$ | |
|---|---|---|
| | **3** | **7a** |
| 20 | 55.5 | 52.9 |
| 27 | 54.8 | 53.1 |
| 35 | 54.4 | 54.1 |
| 42 | 54.1 | 53.8 |
| 50 | 54.0 | 53.0 |

To assess the impact of the presence of a substituent at the C-5 position of the uracil base on the relative proportions of (N) and (S) conformers, these scalar couplings were measured at five different temperatures for the 5-phenyl substituted uridine phosphonate **7a**, while the same was done for the non-C-5 substituted uridine phosphonate **3**. The fraction of the (N)-type conformer was obtained by minimizing the sum of square difference between the experimental couplings and those calculated from the above equations. The results are shown in Table 2.

For compound **3**, the (N) and (S) conformers are nearly equally present, with a small preference for the (N). From Table 1, it appears that compound **7a** has similar (N)/(S) populations as **3**. It can therefore be concluded that the incorporation of a phenyl group at the C-5 position appears to have no effect on the conformational state of the ribofuranose ring. Interestingly, this is in contrast to the effect of implanting a phenyl ring at C-6 of the base as was published recently by Nencka et al.,[31] that clearly shows an enhanced preference for the (N) conformation. A second aspect of the conformation is the orientation of the base relative to the ring structure, which is here assumed to be either *syn* (with the substituent pointing away from the ring) or *anti* (with the substituent pointing towards the ring). The NOESY spectrum of **3** confirmed that the *anti* rotamer could be adopted. However, when a bulky group is implanted at the C-5 position, it orients away from the ribose ring, meaning that in this case only the *syn* rotamer will be available.

## 3. Conclusions

In this study we explored the influence of modifications of uridine 5′-methylenephosphonate (**3**) on activity at the human $P2Y_2$ receptor. A Suzuki–Miyaura coupling of a suitable 5-bromo precursor with aryl and heteroaryl boronic acids allowed generation of a small series of C-5 substituted analogues of **3**. All 5-modified analogues caused $P2Y_2$ receptor-dependent inositol phosphate accumulation. Within this series, 5-(2-furanyl) (**7f**), 5-(4-fluorophenyl) (**7c**), and thien-2-yl (**7g**) substitutions afforded the highest potencies. Interestingly, none of the phosphonate analogues exhibited the same maximal activity as UTP, and these analogues failed to inhibit activity of UTP down to the level of activity observed with the 5-modified analogues alone as would be expected for classical partial agonists acting at the orthosteric binding pocket. Indeed, no change in the concentration effect curve to UTP occurred in the presence of supramaximal concentrations of **7c** or **7f**. These results are consistent with the concept that these analogues allosterically activate the $P2Y_2$ receptor in a manner that has no obvious influence on the effects of UTP mediated through its orthosteric binding site. Although our observations strongly suggest allosteric regulation by the phosphonate analogues, they do not prove it, and the lack of availability of a radioligand for direct assessment of the orthosteric binding site prevents more confident conclusions about their mechanism of action. By altering the sugar part of **3**, we showed that the SAR in the phosphonate series does not fully parallel that observed in the triphosphate class, fur-

ther suggesting that the current phosphonates may bind at a site that differs from the cognate agonist binding pocket.

## 4. Experimental section

### 4.1. Chemical synthesis

All reagents were from standard commercial sources and of analytic grade. Precoated Merck Silica Gel F254 plates were used for TLC, spots were examined under ultraviolet light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (63–200 μm, 60 Å, Biosolve, Valkenswaard, The Netherlands). $^1$H, $^{13}$C, and $^{31}$P NMR spectra were recorded in $CDCl_3$, DMSO-$d_6$, $CD_3OD$ or $D_2O$ on a Varian Mercury 300 MHz and a Bruker 700 MHz spectrometer. Chemical shifts are given in parts per million (ppm), $\delta$ relative to residual solvent peak for $^1$H and $^{13}$C and to external $D_3PO_4$ for $^{31}$P. Structural assignment was confirmed with COSY and DEPT. All signals assigned to hydroxyl groups were exchangeable with $D_2O$. Exact mass measurements were performed on a Waters LCT Premier XETM Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a $CH_3CN$–$H_2O$ (1:1) mixture at 10 μL min$^{-1}$.

#### 4.1.1. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-bromouracil (5)

To a solution of compound **4**[9] (399 mg, 0.95 mmol) in DMF (8 mL) was added *N*-bromosuccinimide (NBS, 187 mg, 1.05 mmol) under $N_2$. The reaction mixture was stirred at room temperature for 16 h. DMF was removed in vacuo and the residue was purified by column chromatography ($CH_2Cl_2$/MeOH 95:5) to afford compound **5** (376 mg, 79%) as a colorless oil. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.20–1.28 (m, 9H, $OCH_2CH_3$, $CH_3$), 1.47 (s, 3H, $CH_3$), 1.69–1.92 (m, 4H, H-5′, H-6′), 3.90–4.04 (m, 5H, $OCH_2CH_3$, H-4′), 4.64–4.67 (m, H-3′), 5.04 (dd, $J$ = 2.1, 6.6 Hz, H-2′), 5.73 (d, $J$ = 2.1 Hz, H-1′), 8.23 (s, H-6), 11.91 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.53. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.25, 16.32 ($OCH_2CH_3$), 20.83 (d, $J$ = 140 Hz, C-6′), 25.24, 27.00 ($C(CH_3)_2$), 25.70 (C-5′), 60.91, 61.00 ($OCH_2CH_3$), 82.61 (C-3′), 83.38 (C-2′), 85.65 (d, $J$ = 17.9 Hz, C-4′), 91.77 (C-1′), 96.12 (C-5), 113.49 ($C(CH_3)_2$), 142.53 (C-6), 149.56 (C-2), 159.23 (C-4). HRMS (ESI) for $C_{17}H_{27}BrN_2O_8P$ [M+H]$^+$ found, 497.0697; calcd, 497.0683.

### 4.2. General procedure for the synthesis of 5-modified nucleoside phosphonates via Suzuki–Miyaura coupling (6a–h)

A mixture of compound **5** (1 equiv), aryl boronic acid (2 equiv), $Pd(PPh_3)_4$ (0.1 equiv) and $Na_2CO_3$ (3.3 equiv) in DMF and degassed $H_2O$ was heated (±130 °C, oil bath) under argon for 6 h or until TLC indicated consumption of all starting material. The mixture was then concentrated and co-distilled with toluene. The residue was purified by column chromatography affording 5-modified analogues **6a–h** in moderate yield.

#### 4.2.1. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-phenyluracil (6a)

Reaction of compound **5** (78 mg, 0.16 mmol) with phenylboronic acid (39 mg, 0.32 mmol), $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) and $Na_2CO_3$ (55 mg, 0.52 mmol) in DMF (4 mL) and degassed $H_2O$ (0.5 mL) was performed as described in the general procedure. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 95:5) afforded compound **6a** as a colorless oil (33 mg, 43%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18–1.23 (m, 6H, $OCH_2CH_3$), 1.30 (s, 3H, $CH_3$), 1.49 (s, 3H, $CH_3$), 1.74–1.89 (m, 4H, H-5′, H-6′), 3.91–4.02 (m, 5H, $OCH_2CH_3$, H-4′), 4.67–4.71 (m, H-3′), 5.14 (dd, $J$ = 2.4, 6.6 Hz, H-2′), 5.85 (d, $J$ = 2.1 Hz, H-1′), 7.29–7.41 (m, 3H, Ph), 7.52–7.55 (m, 2H, Ph), 7.91 (s, H-6), 11.62 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.55. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.22, 16.30 ($OCH_2CH_3$), 20.81 (d, $J$ = 140 Hz, C-6′), 25.26, 27.03 ($C(CH_3)_2$), 25.80 (C-5′), 60.94, 60.98 (d, $J$ = 6.3 Hz, $OCH_2CH_3$), 82.76 (C-3′), 83.42 (C-2′), 85.55 (d, $J$ = 17.9 Hz, C-4′), 91.96 (C-1′), 113.44, 113.85 (C-5, ($C(CH_3)_2$)), 127.39, 128.05, 128.27, 132.69, 140.40 (Ph,C-6), 149.70 (C-2), 162.21 (C-4). HRMS (ESI) for $C_{23}H_{32}N_2O_8P$ [M+H]$^+$ found, 495.1891; calcd, 495.1891.

#### 4.2.2. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-(naphthalen-2-yl)uracil (6b)

Reaction of compound **5** (63 mg, 0.13 mmol) with naphthalene-1-boronic acid (45 mg, 0.25 mmol), $Pd(PPh_3)_4$ (15 mg, 0.013 mmol) and $Na_2CO_3$ (44 mg, 0.42 mmol) in DMF (3 mL) and degassed $H_2O$ (0.4 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 96:4) afforded compound **6b** as a colorless solid (42 mg, 61%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.19 (dt, 6H, $J$ = 1.5, 7.2 Hz, $OCH_2CH_3$), 1.31 (s, 3H, $CH_3$), 1.50 (s, 3H, $CH_3$), 1.74–1.91 (m, 4H, H-5′, H-6′), 3.90–4.04 (m, 5H, $OCH_2CH_3$, H-4′), 4.71 (dd, $J$ = 4.5 Hz, $J$ = 6.3 Hz, H-3′), 5.18 (dd, $J$ = 2.4, 6.6 Hz, H-2′), 5.87 (d, $J$ = 2.1 Hz, H-1′), 7.49–7.55 (m, 2H, naphthyl), 7.69 (dd, $J$ = 1.8, 8.4 Hz, naphthyl), 7.90–7.95 (m, 3H, naphthyl), 8.06 (s, H-6), 8.12 (d, $J$ = 1.2 Hz, naphthyl), 11.69 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 16.21, 16.28 ($OCH_2CH_3$), 20.81 (d, $J$ = 140 Hz, C-6′), 25.30, 27.05 ($C(CH_3)_2$), 25.88 (C-5′), 61.01 ($OCH_2CH_3$), 82.80 (C-3′), 83.47 (C-2′), 85.56 (C-4′), 92.28 (C-1′), 113.40, 113.66 (C-5, ($C(CH_3)_2$)), 126.09, 126.19, 126.49, 126.82, 127.28, 127.42, 127.94, 130.42, 132.12, 132.78, 140.89 (naphthyl, C-6), 149.80 (C-2), 162.51 (C-4). HRMS (ESI) for $C_{27}H_{34}N_2O_8P$ [M+H]$^+$ found, 545.2076; calcd, 545.2047.

#### 4.2.3. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-(4-fluorophenyl)uracil (6c)

Reaction of compound **5** (78 mg, 0.16 mmol) with 4-fluorophenyl boronic acid (44 mg, 0.31 mmol), $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) and $Na_2CO_3$ (54 mg, 0.51 mmol) in DMF (4 mL) and degassed $H_2O$ (0.5 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 95:5) afforded compound **6c** as a colorless solid (56 mg, 70%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18 (dt, 6H, $J$ = 0.9, 7.2 Hz, $OCH_2CH_3$), 1.27 (s, 3H, $CH_3$), 1.47 (s, 3H, $CH_3$), 1.72–1.89 (m, 4H, H-5′, H-6′), 3.92–4.02 (m, 5H, $OCH_2CH_3$, H-4′), 4.69 (dd, $J$ = 4.8, 6.6 Hz, H-3′), 5.14 (dd, $J$ = 2.4, 6.9 Hz, H-2′), 5.83 (d, $J$ = 2.4 Hz, H-1′), 7.19–7.25 (m, 2H, Ph), 7.57–7.63 (m, 2H, Ph), 7.92 (s, H-6), 11.64 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.56. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.22, 16.29 ($OCH_2CH_3$), 20.82 (d, $J$ = 139 Hz, C-6′), 25.27, 27.03 ($C(CH_3)_2$), 25.85 (C-5′), 60.95, 60.99 (d, $J$ = 6.0 Hz, $OCH_2CH_3$), 82.77 (C-3′), 83.40 (C-2′), 85.56 (d, $J$ = 17.9 Hz, C-4′), 92.01 (C-1′), 112.89, 113.46, 114.74, 115.02 (Ph, C-5, ($C(CH_3)_2$)), 129.08, 130.24, 130.35 (Ph), 140.45 (C-6), 149.71 (C-2), 159.94 (Ph), 162.26 (C-4). HRMS (ESI) for $C_{23}H_{31}FN_2O_8P$ [M+H]$^+$ found, 513.1815; calcd, 513.1797.

#### 4.2.4. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-styryluracil (6d)

Reaction of compound **5** (91 mg, 0.18 mmol) with trans-2-phenylvinyl boronic acid (56 mg, 0.37 mmol), $Pd(PPh_3)_4$ (21 mg, 0.018 mmol) and $Na_2CO_3$ (64 mg, 0.60 mmol) in DMF (4 mL) and degassed $H_2O$ (0.5 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH

95:5) afforded compound **6d** as a colorless solid (72 mg, 76%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18 (app dt, 6H, $J$ = 1.2, 6.9 Hz, $OCH_2CH_3$), 1.28 (s, 3H, $CH_3$), 1.48 (s, 3H, $CH_3$), 1.71–1.88 (m, 4H, H-5′, H-6′), 3.90–4.01 (m, 5H, $OCH_2CH_3$, H-4′), 4.68 (dd, $J$ = 4.8, 6.6 Hz, H-3′), 5.06 (dd, $J$ = 2.4, 6.6 Hz, H-2′), 5.80 (d, $J$ = 2.4 Hz, H-1′), 6.88 (d, $J$ = 16.2 Hz, styryl), 7.20–7.25 (m, styryl), 7.33 (d, 2H, $J$ = 7.8 Hz, styryl), 7.43–7.48 (m, 3H, styryl), 7.94 (H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.51. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.22, 16.30 ($OCH_2CH_3$), 20.83 (d, $J$ = 140 Hz, C-6′), 25.29, 27.02 ($C(CH_3)_2$), 25.89 (C-5′), 60.97, 61.01 (d, $J$ = 6.3 Hz, $OCH_2CH_3$), 82.64 (C-3′), 83.49 (C-2′), 85.41 (d, $J$ = 17.6 Hz, C-4′), 91.34 (C-1′), 111.22 (C-5), 113.54 ($C(CH_3)_2$), 120.83, 125.99, 127.42, 128.32, 128.74, 137.43 (styryl), 140.09 (C-6), 149.26 (C-2), 162.21 (C-4). HRMS (ESI) for $C_{25}H_{34}N_2O_8P$ $[M+H]^+$ found, 521.2049; calcd, 521.2047.

#### 4.2.5. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-(*p*-tolyl)uracil (6e)

Reaction of compound **5** (63 mg, 0.13 mmol) with 4-methylphenylboronic acid (36 mg, 0.25 mmol), $Pd(PPh_3)_4$ (15 mg, 0.013 mmol) and $Na_2CO_3$ (45 mg, 0.42 mmol) in DMF (3 mL) and degassed $H_2O$ (0.4 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 97:3) afforded compound **6e** as a colorless solid (29 mg, 46%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.24–1.33 (m, 9H, $OCH_2CH_3$, $CH_3$), 1.54 (s, 3H, $CH_3$), 1.72–2.07 (m, 4H, H-5′, H-6′), 2.71 (s, 3H, *p*-tolyl), 4.01–4.13 (m, 5H, $OCH_2CH_3$, H-4′), 4.64 (dd, $J$ = 4.5, 6.3 Hz, H-3′), 5.04 (dd, $J$ = 2.1, 6.3 Hz, H-2′), 5.67 (d, $J$ = 2.4 Hz, H-1′), 7.16–7.79 (m, 4H, *p*-tolyl), 8.00 (H-6). $^{31}$P NMR ($CDCl_3$): $\delta$ 31.27. HRMS (ESI) for $C_{24}H_{34}N_2O_8P$ $[M+H]^+$ found, 509.2076; calcd, 509.2047.

#### 4.2.6. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-(furan-2-yl)uracil (6f)

Reaction of compound **5** (93 mg, 0.19 mmol) with furan-2-boronic acid (42 mg, 0.37 mmol), $Pd(PPh_3)_4$ (22 mg, 0.019 mmol) and $Na_2CO_3$ (65 mg, 0.62 mmol) in DMF (4.5 mL) and degassed $H_2O$ (0.6 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 95:5) afforded compound **6f** as a brown solid (36 mg, 40%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.16–1.28 (m, 6H, $OCH_2CH_3$) 1.28 (s, 3H, $CH_3$), 1.48 (s, 3H, $CH_3$), 1.76–1.81 (m, 4H, H-5′, H-6′), 3.82–4.07 (m, 5H, $OCH_2CH_3$, H-4′), 4.58–4.68 (m, H-3′), 5.03–5.13 (m, H-2′), 5.84–5.86 (m, H-1′), 6.50–6.54 (m, furanyl), 6.83–6.88 (m, furanyl), 7.61–7.66 (m, furanyl), 8.02 (app d, $J$ = 4.8 Hz, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.63. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.29, 16.37 ($OCH_2CH_3$), 20.99 (d, $J$ = 148 Hz, C-6′), 25.31, 27.07 ($C(CH_3)_2$), 60.99, 61.11 ($OCH_2CH_3$), 82.83 (C-3′), 83.74 (C-2′), 85.95 (d, $J$ = 7.5 Hz, C-4′), 92.37 (C-1′), 105.72, 108.40 (furanyl), 111.73 (C-5), 113.46 (($C(CH_3)_2$)), 128.83, 131.55 (furanyl), 141.79 (C-6), 149.24 (C-2), 160.37 (C-4). HRMS (ESI) for $C_{21}H_{30}N_2O_9P$ $[M+H]^+$ found, 485.1708; calcd, 485.1683.

#### 4.2.7. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-(thien-2-yl)uracil (6g)

Reaction of compound **5** (81 mg, 0.16 mmol) with thiophene-2-boronic acid (42 mg, 0.33 mmol), $Pd(PPh_3)_4$ (19 mg, 0.016 mmol) and $Na_2CO_3$ (57 mg, 0.54 mmol) in DMF (4 mL) and degassed $H_2O$ (0.5 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 95:5) afforded compound **6g** as a colorless solid (32 mg, 39%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.1–1.22 (m, 6H, $OCH_2CH_3$) 1.28 (s, 3H, $CH_3$), 1.48 (s, 3H, $CH_3$), 1.68–1.91 (m, 4H, H-5′, H-6′), 3.90–3.97 (m, 5H, $OCH_2CH_3$, H-4′), 4.67–4.69 (m, H-3′), 5.13–5.15 (m, H-2′), 5.82 (m, H-1′), 7.05–7.07 (m, thienyl), 7.46–7.47 (m, 2H, m, thienyl), 8.20 (s, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.64. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.25, 16.33 ($OCH_2CH_3$), 20.85 (d, $J$ = 140 Hz, C-6′), 25.31, 27.07 ($C(CH_3)_2$), 60.99 ($OCH_2CH_3$), 82.87 (C-3′), 83.57 (C-2′), 85.86 (C-4′), 93.79 (C-1′), 108.56 (C-5), 113.40 ($C(CH_3)_2$), 128.73–138.33 (thienyl). HRMS (ESI) for $C_{21}H_{30}N_2O_8PS$ $[M+H]^+$ found, 501.1453; calcd, 501.1455.

#### 4.2.8. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-2′,3′-*O*-isopropylidene-β-D-*ribo*-hexofuranosyl]-5-(benzothiophen-3-yl)uracil (6h)

Reaction of compound **5** (105 mg, 0.21 mmol) with 1-benzothiophen-3-ylboronic acid (77 mg, 0.42 mmol), $Pd(PPh_3)_4$ (24 mg, 0.021 mmol) and $Na_2CO_3$ (74 mg, 0.70 mmol) in DMF (5 mL) and degassed $H_2O$ (0.7 mL) was performed as described in the general procedure for the synthesis of 5-modified phosphonates. Purification of the crude mixture on a silica gel column ($CH_2Cl_2$/MeOH 95:5) afforded compound **6h** as a brown solid (49 mg, 42%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.25–1.32 (m, 9H, $OCH_2CH_3$, $CH_3$), 1.55 (s, 3H, $CH_3$), 1.77–1.93 (m, 2H, H-6′), 1.98–2.08 (m, 2H, H-5′), 4.02–4.12 (m, 5H, $OCH_2CH_3$, H-4′), 4.61 (t, $J$ = 5.7 Hz, H-3′), 5.00 (app d, $J$ = 6.6 Hz, H-2′), 5.70 (app s, H-1′), 7.32–7.41 (m, 2H, benzothiophenyl), 7.46–7.48 (m, benzothiophenyl), 7.54 (s, H-6), 7.67 (d, $J$ = 7.8 Hz, benzothiophenyl), 7.86 (d, $J$ = 7.8 Hz, benzothiophenyl), 9.53 (br s, 3-NH). $^{31}$P NMR ($CDCl_3$): $\delta$ 31.01. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 16.52, 16.60 ($OCH_2CH_3$), 21.99 (d, $J$ = 142 Hz, C-6′), 25.48, 27.31 ($C(CH_3)_2$), 26.41 (C-5′), 61.81 ($OCH_2CH_3$), 83.43 (C-3′), 84.41 (C-2′), 86.64 (d, $J$ = 17.9 Hz, C-4′), 93.79 (C-1′), 110.69, 115.11 (C-5, ($C(CH_3)_2$)), 122.48, 123.08, 124.65, 124.71, 126.86, 126.94, 137.70 (benzothiophenyl), 140.15, 140.39 (C-6, benzothiophenyl), 149.54 (C-2), 162.12 (C-4). HRMS (ESI) for $C_{25}H_{32}N_2O_8PS$ $[M+H]^+$ found, 551.1620; calcd, 551.1612.

#### 4.2.9. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-phenyluracil (7a)

Phosphonic ester **6a** (32 mg, 0.068 mmol) was dissolved in 1.4 mL of $CH_2Cl_2$ under argon and treated with TMSBr (18 μL, 1.35 mmol) and the solution was stirred overnight. The solvents were evaporated and the residue was co-distilled with toluene. Then, 0.7 mL of $H_2O$ was added followed by 1.4 mL of a 50% aqueous TFA solution and the mixture was stirred for 4 h. The solvent was evaporated and subsequently portioned between EtOAc/$Et_2O$ (1:1) and $H_2O$. The organic phase was washed with $H_2O$ and the aqueous layers were combined and lyophilized. Purification of the crude mixture using RP high-performance liquid chromatography (HPLC, Phenomenex Luna C-18, $H_2O$/0.1% HCOOH in $CH_3CN$, 90:10→0:100 in 23 min, flow 17.5 mL/min) afforded compound **7a** as a white powder (14.1 mg, 55%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.40–1.43 (m, 2H, H-6′), 1.80–1.84 (m, 2H, H-5′), 3.82–3.83 (m, 2H, H-3′, H-4′), 4.19 (t, $J$ = 5.1 Hz, H-2′), 5.72 (d, $J$ = 4.5 Hz, H-1′), 7.26–7.40 (m, 3H, Ph), 7.51–7.54 (m, 2H, Ph), 7.64 (s, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 25.89. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 26.89 (C-5′), 72.46 (C-3′), 72.52 (C-2′), 83.59 (d, $J$ = 15.9 Hz, C-4′), 89.54 (C-1′), 113.97 (C-5), 127.36, 128.16, 128.22, 132.80 (Ph), 138.34 (C-6), 150.11 (C-2), 162.07 (C-4). HRMS (ESI) for $C_{16}H_{18}N_2O_8P$ $[M-H]^-$ found, 397.0815; calcd, 397.0806.

#### 4.2.10. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-(naphthalen-2-yl)uracil (7b)

Reaction of compound **6b** (70 mg, 0.073 mmol) with TMSBr (19 μL, 1.46 mmol), work-up and purification as described for **7a**, afforded compound **7b** as a white powder (22.6 mg, 39%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.60 (br s, 2H, H-6′), 1.78–1.84 (m, 2H, H-5′), 3.77–3.85 (m, 2H, H-3′, H-4′), 4.25 (t, $J$ = 5.4 Hz, H-2′), 5.73 (d, $J$ = 4.8 Hz, H-1′), 7.29–7.41 (m, 3H, naphthyl), 7.52–7.69 (m, 2H, naphthyl), 7.69 (s, H-6), 11.57 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 25.71. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 23.80 (C-6′),

27.04 (C-5′), 72.56 (C-3′), 72.62 (C-2′), 83.52 (C-4′), 89.83 (C-1′), 113.94 (C-5), 126.19, 126.27, 126.58, 126.78, 127.50, 128.22, 130.51, 132.23, 132.91 (naphthyl), 138.95 (C-6), 150.21 (C-2), 162.32 (C-4). HRMS (ESI) for $C_{20}H_{20}N_2O_8P$ $[M-H]^-$ found, 447.0969; calcd, 447.0963.

#### 4.2.11. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-(4-fluorophenyl)uracil (7c)

Reaction of compound **6c** (56 mg, 0.11 mmol) with TMSBr (29 μL, 2.20 mmol), work-up and purification as described for **7a**, afforded compound **7c** as a white powder (26.1 mg, 57%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.62 (br s, 2H, H-6′), 1.79 (br s, 2H, H-5′), 3.77–3.84 (m, 2H, H-3′, H-4′), 4.26 (t, $J$ = 5.1 Hz, H-2′), 5.73 (d, $J$ = 4.8 Hz, H-1′), 7.21 (t, 2H, $J$ = 8.7 Hz, Ph), 7.56–7.60 (m, 2H, Ph), 7.70 (s, H-6), 11.59 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 26.17. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 27.05 (C-5′), 72.57 (C-3′), 72.60 (C-2′), 83.65 (C-4′), 89.67 (C-1′), 113.11 (C-5), 114.92, 115.20, 129.21, 129.25, 130.29, 130.40 (Ph), 138.44 (C-6), 150.18 (C-2), 160.01 (Ph), 162.17 (C-4). HRMS (ESI) for $C_{16}H_{17}FN_2O_8P$ $[M-H]^-$ found, 415.0722; calcd, 415.0712.

#### 4.2.12. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-styryluracil (7d)

Reaction of compound **6d** (72 mg, 0.14 mmol) with TMSBr (36 μL, 2.75 mmol), work-up and purification as described for **7a**, afforded compound **7d** as a white powder (6.5 mg, 11%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.60 (br s, 2H, H-6′), 1.87 (br s, 2H, H-5′), 3.78–3.86 (m, 2H, H-3′, H-4′), 4.15 (app s, H-2′), 5.75–5.76 (m, H-1′), 6.98 (d, $J$ = 16.2 Hz, styryl), 7.15–7.57 (m, 6H, styryl), 7.78 (s, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 24.42. $^{13}$C NMR (175 MHz, DMSO-$d_6$): $\delta$ 24.35 (C-6′), 27.07 (C-5′), 72.35 (C-3′), 72.72 (C-2′), 83.48 (C-4′), 88.68 (C-1′), 111.27 (C-5), 121.28 (5-*C*H=CH–), 125.97 ($C_{ortho}$), 127.27 ($C_{para}$), 128.20 (5-CH=*C*H–), 128.57 ($C_{meta}$), 137.57 ($C_{ipso}$), 138.78 (C-6), 149.62 (C-2), 162.04 (C-4). HRMS (ESI) for $C_{18}H_{20}N_2O_8P$ $[M-H]^-$ found, 423.0954; calcd, 423.0963.

#### 4.2.13. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-(*p*-tolyl)uracil (7e)

Reaction of compound **6e** (29 mg, 0.058 mmol) with TMSBr (15 μL, 1.15 mmol) work-up and purification as described for **7a**, afforded compound **7e** as a white powder (4.3 mg, 18%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.40–1.99 (m, 4H, H-5′, H-6′), 3.77–3.82 (m, 2H, H-3′, H-4′), 4.22 (m, H-2′), 5.73 (d, $J$ = 4.5 Hz, H-1′), 7.19 (d, 2H, $J$ = 7.5 Hz, *p*-tolyl), 7.42 (d, 2H, $J$ = 7.8 Hz, *p*-tolyl), 7.62 (s, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 25.55. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 20.61 ($CH_3$), 27.00 (C-6′), 72.33 (C-3′), 72.42 (C-2′), 83.64 (C-4′), 89.28 (C-1′), 113.74 (C-5), 127.85, 128.58, 129.71, 136.47 (*p*-tolyl), 137.58 (C-6), 149.92 (C-2), 161.95 (C-4). HRMS (ESI) for $C_{17}H_{20}N_2O_8P$ $[M-H]^-$ found, 411.0970; calcd, 411.0963.

#### 4.2.14. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-(furan-2-yl)uracil (7f)

Reaction of compound **6f** (32 mg, 0.067 mmol) with TMSBr (18 μL, 1.34 mmol), work-up and purification as described for **7a**, afforded compound **7f** as a white powder (5.8 mg, 22%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.61 (br s, 2H, H-6′), 1.83–1.91 (m, 2H, H-5′), 3.80–3.82 (m, 2H, H-3′, H-4′), 4.12 (s, H-2′), 5.73 (d, $J$ = 3.9 Hz, H-1′), 6.51 (d, $J$ = 1.8 Hz, furanyl), 6.87 (d, $J$ = 3.3 Hz, furanyl), 7.66 (s, furanyl), 7.82 (s, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 25.08. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 27.12 (C-5′), 72.58, 73.34 (C-2′, C-3′), 83.56 (C-4′), 89.56 (C-1′), 105.69, 108.10, 111.56 (C-5, furanyl), 134.25 (C-6), 141.81, 146.08 (furanyl), 149.42 (C-2), 160.07 (C-4). HRMS (ESI) for $C_{14}H_{16}N_2O_9P$ $[M-H]^-$ found, 387.0604; calcd, 387.0599.

#### 4.2.15. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-(thien-2-yl)uracil (7g)

Reaction of compound **6g** (32 mg, 0.063 mmol) with TMSBr (17 μL, 1.26 mmol), work-up and purification as described for **7a**, afforded compound **7g** as a white powder (9.3 mg, 37%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.61 (br s, 2H, H-6′), 1.75–1.91 (m, 2H, H-5′), 3.80–3.85 (m, 2H, m, H-3′, H-4′), 4.22–4.28 (m, H-2′), 5.71 (d, $J$ = 4.5 Hz, H-1′), 7.02–7.10 (m, thienyl), 7.46 (d, $J$ = 5.1 Hz, thienyl), 7.50 (s, thienyl), 7.96 (s, H-6). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 25.42. $^{13}$C NMR (175 MHz, DMSO-$d_6$): $\delta$ 24.21 (C-6′), 26.99 (C-5′), 72.36 (C-3′), 72.73 (C-2′), 83.68 (C-4′),89.80 (C-1′), 108.63 (C-5), 123.20 (C-3″), 125.80 (C-5″), 126.54 (C-4″), 133.39 (C-2″), 135.82 (C-6), 149.48 (C-2), 161.30 (C-4). HRMS (ESI) for $C_{14}H_{16}N_2O_8PS$ $[M-H]^-$ found, 403.0343; calcd, 403.0371.

#### 4.2.16. 1-[5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]-5-(benzothiophen-3-yl)uracil (7h)

Reaction of compound **6h** (49 mg, 0.088 mmol) with TMSBr (23 μL, 1.76 mmol), work-up and purification as described for **7a**, afforded compound **7h** as a white powder (31.5 mg, 78%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.59 (br s, 2H, H-6′), 1.76–1.80 (m, 2H, H-5′), 3.76–3.83 (m, 2H, H-3′, H-4′), 4.21–4.4.24 (m, H-2′), 5.77 (d, $J$ = 4.8 Hz, H-1′), 7.36–7.44 (m, 2H, benzothiophenyl), 7.63–7.66 (m, benzothiophenyl), 7.78 (app d, 2H, $J$ = 7.2 Hz, benzothiophenyl, H-6), 7.98–8.02 (m, benzothiophenyl), 11.66 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 26.13. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 26.65 (C-5′), 72.49 (C-3′), 72.44 (C-2′), 83.47 (C-4′), 89.37 (C-1′), 109.30 (C-5), 123.05, 124.28, 124.39, 126.65, 128.23, 138.02, 139.14, 139.68 (benzothiophenyl, C-6), 150.29 (C-2), 162.00 (C-4). HRMS (ESI) for $C_{18}H_{18}N_2O_8PS$ $[M-H]^-$ found, 453.0531; calcd, 453.0527.

#### 4.2.17. 1-[5′,6′-Dideoxy-6′-(diethoxyphosphinyl)-β-D-*ribo*-hexofuranosyl]uracil (8)

A solution of compound **4** (428 mg, 1.02 mmol) and 50% aq HCOOH (10 mL) was stirred for 4.5 h at room temperature. The mixture was evaporated in vacuo and purified on a silica column using $CH_2Cl_2$/MeOH (90:10) as a solvent. Compound **8** was obtained as a colorless solid (220 mg, 57%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.23 (t, 6H, $J$ = 6.9 Hz, $OCH_2CH_3$), 1.72–1.86 (m, 4H, H-5′, H-6′), 3.32–3.83 (m, 2H, H-3′, H-4′), 3.93–4.11 (m, 5H, $OCH_2CH_3$, H-2′), 5.12 (d, $J$ = 5.7 Hz, 3′-OH), 5.35 (d, $J$ = 5.7 Hz, 2′-OH), 5.63 (d, $J$ = 8.1 Hz, CH=CH), 5.68 (d, $J$ = 5.1 Hz, H-1′), 7.59 (d, $J$ = 8.1 Hz, CH=CH), 11.34 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 31.86. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.26, 16.33 ($OCH_2CH_3$), 20.92 (d, $J$ = 140 Hz, C-6′), 25.93 (C-5′), 60.93, 60.96 (d, $J$ = 6 Hz, $OCH_2CH_3$), 72.48, 72.57 (C-2′, C-3′), 82.65 (d, $J$ = 17 Hz, C-4′), 88.93 (C-1′), 102.06 (C-5), 141.42 (C-6), 150.61 (C-2), 163.03 (C-4). HRMS (ESI) for $C_{14}H_{24}N_2O_8P$ $[M+H]^+$ found, 379.1262; calcd, 379.1265.

#### 4.2.18. 1-[2,2′-*O*-Anhydro-5′,6′-dideoxy-6′-(diethoxyphosphinyl)-β-D-arabinofuranosyl]uracil (9)

Compound **8** (205 mg, 0.54 mmol) was dissolved into a mixture of thionyl chloride (0.17 mL) and $CH_3CN$ (1.4 mL) with vigorously stirring and then the reaction was maintained at room temperature. After 2 h, the mixture was poured into $H_2O$ and extracted with $CH_2Cl_2$. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. Without further purification, the 2′,3′-sulfonyl derivative of **8** and sodium acetate (223 mg, 2.72 mmol) were heated in DMF at about 85 °C. After stirring for 3 h, solvents were removed in vacuo and the crude mixture purified on a silica gel column ($CH_2Cl_2$/MeOH (95:5→80:20)) yielding 180 mg (92%) of **9** as a white foam. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.14–1.23 (m, 7H, $OCH_2CH_3$, H-5′a), 1.59–1.68 (m, 3H, H-5′b, H-6′), 3.84–3.94 (m, 4H, $OCH_2CH_3$), 4.00–4.10 (m, H-4′), 4.32 (app s, H-3′), 5.19 (d, $J$ = 5.7 Hz, H-2′), 5.87 (d, $J$ = 7.5 Hz, CH=CH), 6.30 (d, $J$ = 5.7 Hz, H-1′), 7.91 (d, $J$ = 7.5 Hz, CH=CH). $^{31}$P NMR

(DMSO-$d_6$): $\delta$ 31.02. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 16.45, 16.53 ($OCH_2CH_3$), 22.04 (d, $J$ = 143 Hz, C-6′), 26.42 (C-5′), 62.14, 62.17 (d, $J$ = 6.6 Hz, $OCH_2CH_3$), 87.72 (d, $J$ = 16.7 Hz, C-4′), 89.49 (C-2′), 90.31 (C-1′), 109.94 (C-5), 136.16 (C-6), 160.19 (C-2), 172.90 (C-4). HRMS (ESI) for $C_{14}H_{22}N_2O_7P$ $[M+H]^+$ found, 361.1157; calcd, 361.1159.

#### 4.2.19. 1-[2′-Azido-2′-deoxy-5′,6′-dideoxy-6′-(ethoxyhydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]uracil (10)

A suspension of compound **9** (33 mg, 0.092 mmol) and $NaN_3$ (37 mg, 0.56 mmol) in 0.7 mL DMF was heated overnight at 150 °C. The mixture was cooled to room temperature and evaporated in vacuo. The residue was dissolved in $CH_2Cl_2$/MeOH (8:2) and filtrated. Solvents were removed to afford compound **10** which was used in the next reaction without further purification (30 mg, 88%, yellow oil). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.08–1.20 (m, 3H, $OCH_2CH_3$), 1.48–1.70 (m, 2H, H-6′), 1.76–1.91 (m, 2H, H-5′), 3.75–3.89 (m, 3H, $OCH_2CH_3$, H-2′), 4.13–4.17 (m, H-4′), 4.24–4.26 (m, H-3′), 5.66–5.74 (m, 2H, H-1′, CH=CH), 7.94–8.02 (m, CH=CH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 25.36. HRMS (ESI) for $C_{12}H_{19}N_5O_7P$ $[M+H]^+$ found, 376.0997; calcd, 376.1017.

#### 4.2.20. 1-[2′-Amino-2′-deoxy-5′,6′-dideoxy-6′-(ethoxyhydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]uracil (11)

Compound **10** (24 mg, 0.065 mmol) and $PPh_3$ (34 mg, 0.13 mmol) were dissolved in THF (1 mL). After stirring for 10 minutes, $H_2O$ was added (17 μL). After stirring overnight, the volatiles were removed in vacuo. The residue dissolved in $H_2O$, washed with EtOAc/$Et_2O$ (1:1) and lyophilized to yield 24 mg of crude **11** as a light yellow solid. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.02–1.15 (m, 3H, $OCH_2CH_3$), 1.21–1.52 (m, 2H, H-6′), 1.76 (br s, 2H, H-5′), 3.56–3.77 (m, 3H, $OCH_2CH_3$, H-2′), 4.04–4.10 (m, H-4′), 4.22 (app s, H-3′), 5.83–5.88 (m, CH=CH), 6.27 (app d, $J$ = 5.4 Hz, H-1′), 7.91–7.93 (d, $J$ = 7.5 Hz, CH=CH). $^{31}$P NMR ($D_2O$-$d_6$): $\delta$ 26.76. HRMS (ESI) for $C_{12}H_{19}N_3O_7P$ $[M-H]^-$ found, 348.0954; calcd, 348.0966.

#### 4.2.21. 1-[2′-Amino-2′-deoxy-5′,6′-dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]uracil (12)

To a solution of compound **11** (23.6 mg, 0.067 mmol) in $CH_2Cl_2$ (2.0 mL) was added TMSBr (90 μL, 0.68 mmol). After stirring overnight, the volatiles were removed in vacuo. The residue was dissolved in $H_2O$, washed with EtOAc/$Et_2O$ (1:1) and lyophilized. The residue was purified using flash chromatography (*i*PrOH/$NH_4OH$/$H_2O$ 6:3:1) yielding compound **12** (18.6 mg, 78%) as a light yellow powder. Compound **12** was isolated in the ammonium salt form. $^1$H NMR (300 MHz, $D_2O$): $\delta$ 1.61–1.78 (m, 2H, H-6′), 1.89–1.95 (m, 2H, H-5′), 4.00–4.01 (m, H-2′), 4.12–4.18 (m, H-4′), 4.34–4.37 (m, H-3′), 5.93 (d, $J$ = 7.8 Hz, CH=CH), 6.06 (app d, $J$ = 6.6 Hz, H-1′), 7.73 (d, $J$ = 8.4 Hz, CH=CH). $^{31}$P NMR ($D_2O$-$d_6$): $\delta$ 24.03. $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 24.10 (d, $J$ = 134 Hz, C-6′), 27.20 (d, $J$ = 3.5 Hz, C-5′), 55.79 (C-2′), 71.65 (C-3′), 86.20 (d, $J$ = 17.3 Hz, C-4′), 87.27 (C-1′), 102.90 (C-5), 141.67 (C-6), 151.88 (C-2), 166.21 (C-4). HRMS (ESI) for $C_{10}H_{15}N_3O_7P$ $[M-H]^-$ found, 320.0646; calcd, 320.0653.

#### 4.2.22. 1-[2′-Chloro-2′-deoxy-5′,6′-dideoxy-6′-(diethoxyhydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]uracil (13)

To a solution of compound **9** (76 mg, 0.21 mmol) in dry dioxane (0.5 mL) was added 2 N HCl in diethylether (1.4 mL). After stirring overnight, the mixture was concentrated in vacuo and the residue purified on a silica gel column ($CH_2Cl_2$/MeOH 90:10) affording compound **13** as a colorless solid (45 mg, 54%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.23 (t, 6H, $J$ = 7.2 Hz, $OCH_2CH_3$), 1.65–1.98 (m, 4H, H-5′, H-6′), 3.87–3.89 (m, H-4′), 3.94–4.02 (m, 5H, $OCH_2CH_3$, H-3′), 4.73 (app t, $J$ = 6.0 Hz, H-2′), 5.69 (d, $J$ = 8.1 Hz, CH=CH), 5.89 (d, $J$ = 5.1 Hz, 3′-OH), 5.96 (d, $J$ = 6 Hz, H-1′), 7.63 (d, $J$ = 8.4 Hz, CH=CH), 11.45 (s, 3-NH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 32.03. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.43, 16.50 ($OCH_2CH_3$), 20.99 (d, $J$ = 140 Hz, C-6′), 25.82 (d, $J$ = 3.9 Hz, C-5′), 61.12 (C-2′), 61.20 ($OCH_2CH_3$), 71.93 (C-3′), 83.57 (d, $J$ = 17 Hz, C-4′), 88.83 (C-1′), 102.62 (C-5), 140.71 (C-6), 150.69 (C-2), 163.07 (C-4). HRMS (ESI) for $C_{14}H_{23}ClN_2O_7P$ $[M+H]^+$ found, 397.0942; calcd, 397.0926.

#### 4.2.23. 1-[2′-Chloro-2′-deoxy-5′,6′-dideoxy-6′-(dihydroxyphosphinyl)-β-D-*ribo*-hexofuranosyl]uracil (14)

To a solution of compound **13** (41 mg, 0.10 mmol) in $CH_2Cl_2$ (1.5 mL) was added TMSBr (27 μL, 0.20 mmol). After stirring overnight, the volatiles were removed in vacuo. The residue was dissolved in $H_2O$, washed with EtOAc/$Et_2O$ (1:1) and lyophilized. The residue was purified using flash chromatography (*i*PrOH/$NH_4OH$/$H_2O$ 6:3:1) yielding 58% of compound **14** (21.8 mg, white powder). Compound **14** was isolated in the ammonium salt form. $^1$H NMR (300 MHz, $D_2O$): $\delta$ 1.60–1.82 (m, 2H, H-6′), 1.94–2.05 (m, 2H, H-5′), 4.13–4.19 (m, H-4′), 4.27 (app t, $J$ = 5.4 Hz, H-3′), 4.66 (app t, $J$ = 5.1 Hz, H-2′), 5.92 (d, $J$ = 8.4 Hz, CH=CH), 6.08 (d, $J$ = 4.8 Hz, H-1′), 7.71 (d, $J$ = 8.1 Hz, CH=CH). $^{31}$P NMR ($D_2O$): $\delta$ 24.24. $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 24.17 (d, $J$ = 134 Hz, C-6′), 27.10 (d, $J$ = 3.8 Hz C-5′), 61.39 (C-2′), 72.56 (C-3′), 84.25 (d, $J$ = 17.9 Hz C-4′), 90.27 (C-1′), 102.73 (C-5), 141.52 (C-6), 151.69 (C-2), 166.25 (C-4). HRMS (ESI) for $C_{10}H_{13}ClN_2O_7P$ $[M-H]^-$ found, 339.0154; calcd, 339.0154.

#### 4.2.24. (1′*S*,2′*R*,3′*S*,4′*R*,5′*S*)-1′-(Diisopropyl-phosphonoethenyl)-2′,3′-*O*-(isopropylidine)-4′-(uracil-1-yl)-bicyclo[3.1.0]hexane (16)

Diisopropyl azodicarboxylate (93 μL, 0.47 mmol) was added at rt to a mixture of triphenylphosphine (123 mg, 0.47 mmol) and 3-*N*-benzoyluracil (101 mg, 0.47 mmol) in anhydrous THF (3 mL). After stirring for 40 min, a solution of compound **15**[22] (85 mg, 0.23 mmol) in anhydrous THF (3 mL) was added to the mixture. After stirring for 36 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–6% MeOH in EtOAc) to afford *N*-3-benzoyl protected uracil nucleoside as a white solid material ($R_f$ = 0.4, EtOAc/MeOH 94:6), which was treated with 6 N $NH_3$/MeOH (6 mL) and heated up to 50 °C. After stirring for 19 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0–8% MeOH in $CH_2Cl_2$) to afford phosphonate diester **16** (25 mg, 24%) as a white solid material. $R_f$ = 0.3 ($CH_2Cl_2$/MeOH 92:8). $^1$H NMR ($CDCl_3$): $\delta$ 0.72–0.80 (m), 0.89–0.95 (m), 1.24 (s, 3H), 1.28–1.33 (m, 12 H), 1.35–1.39 (m), 1.48 (s, 3H), 1.60–2.04 (m, 4H), 4.33 (s), 4.64–4.72 (m, 3H), 4.91 (d, $J$ = 7.1 Hz), 5.70 (d, $J$ = 7.69 Hz), 7.14 (d, $J$ = 7.69 Hz), 8.25 (s, NH). HRMS (ESI) for $C_{21}H_{34}N_2O_7P$ $[M+H]^+$ found, 457.2121; calcd, 457.2104.

#### 4.2.25. (1′*S*,2′*R*,3′*S*,4′*R*,5′*S*)-2′,3′-(Dihydroxy)-1′-(phosphonoethenyl)-4′-(uracil-1-yl)-bicyclo[3.1.0]hexane (17)

Nucleoside **16** (22 mg, 0.048 mmol) was co-evaporated with anhydrous toluene (3 × 2 mL) and dissolved in anhydrous $CH_2Cl_2$ (2 mL). Iodotrimethylsilane (96 μL, 0.48 mmol) was added. After stirring for 15 h, the reaction mixture was cooled to 0 °C followed by the addition of ice-cold $H_2O$ (15 mL) and $CH_2Cl_2$ (15 mL). The phases were separated, and the aqueous phase was washed with $CH_2Cl_2$ (25 mL) and diethylether (3 × 35 mL). The resulting aqueous phase was evaporated to dryness and purified by RP-HPLC (Phenomenex Luna C-18, 10 mM triethylammonium acetate (TEAA)–$CH_3CN$, 100:0→70:30 in 30 min, flow 3 mL/min) to afford compound **17** (2.89 mg, 19%) as a white solid material. Compound **17** was isolated in the triethylammonium salt form. $^1$H NMR ($D_2O$, 300 MHz): $\delta$ 0.72–0.80 (m, 1H), 1.12–1.17 (m), 1.36–1.46 (m), 1.54–2.03 (m, 4H), 4.00 (d, $J$ = 6.60 Hz), 4.46 (d, $J$ = 6.60 Hz), 4.62

(s), 5.86 (d, $J$ = 8.25 Hz), 7.61 (d, $J$ = 8.25 Hz). $^{31}$P NMR ($D_2O$): $\delta$ 26.82. HRMS (ESI) for $C_{12}H_{16}N_2O_7P$ $[M-H]^-$ found, 331.0682; calcd, 331.0695.

#### 4.2.26. 2′,3′-Dideoxy-3′-phenylselenyl-5′-[(diethoxyphosphinyl)-methoxy]-β-D-uridine (19)

A solution of phenylselenyl chloride (44 mg, 0.23 mmol) in $CH_2Cl_2$ (0.1 mL) was added dropwise to a solution of glycal **18**[25] (41 mg, 0.23 mmol) in $CH_2Cl_2$ (0.7 mL). After the mixture was stirred at −70 °C for 1 h, the solvent was removed under reduced pressure and the residue redissolved in $CH_2Cl_2$ (0.5 mL). Dimethyl(hydroxylmethyl)phosphonate (74 μL, 0.50 mmol) was added and the solution was cooled to −70 °C. Over 3 min a solution of silver perchlorate (52 mg, 0.25 mmol) in $CH_3CN$ (0.1 mL) was added and the mixture was allowed to warm to 0 °C and poured into an aq $NaHCO_3$ solution. The organic phase was separated, dried over $MgSO_4$ and evaporated in vacuo. The residual oil was chromatographed on silica ($CH_2Cl_2$/MeOH 96:4) yielding compound **19** (40 mg, 35%) as a colorless solid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.26 (app dt, 6H, $J$ = 1.5, 7.2 Hz, $OCH_2CH_3$), 2.41–2.45 (m, 2H, H-2′), 3.59–3.67 (m, H-5′a), 3.79–3.90 (m, 2H, H-5′b, H-3′), 4.03–4.13 (m, 4H, $OCH_2CH_3$), 5.09 (app s, H-4′), 5.74 (app dd, $J$ = 2.4, 8.4 Hz, CH=CH), 6.48 (app t, $J$ = 7.2 Hz, H-1′), 7.23–7.33 (m, 3H, Ph), 7.49–7.55 (m, 2H, Ph), 7.61 (d, $J$ = 8.4 Hz, CH=CH), 8.51 (s, 3-NH). $^{31}$P NMR ($CDCl_3$): $\delta$ 20.12. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 16.65, 16.72 ($OCH_2CH_3$), 36.27 (C-2′), 43.79 (C-3′), 60.47 (C-5′), 62.82, 62.91 ($OCH_2CH_3$), 86.53 (C-1′), 103.84 (C-4′), 110.04 (C-5), 127.36, 128.89, 129.73, 135.12 (Ph), 140.41 (C-6), 150.51 (C-2), 162.78 (C-4). HRMS (ESI) for $C_{19}H_{26}N_2O_7PSe$ $[M+H]^+$ found, 505.0630; calcd, 505.0637.

#### 4.2.27. 2′,3′-Dihydro-5′-[(diethoxyphosphinyl)methoxy]-β-D-uridine (20)

To a solution of compound **19** (41 mg, 0.082 mmol) in MeOH (0.7 mL) was added dropwise a suspended solution of sodium bicarbonate (12 mg, 0.14 mmol) and sodium periodate (22 mg, 0.10 mmol) in $H_2O$ (0.7 mL). After being stirred at rt for 1 h, the mixture was heated at 80 °C for 75 min. Volatiles were removed in vacuo and the residue was suspended in $CH_2Cl_2$, filtered through celite and dried over $MgSO_4$. After evaporation under reduced pressure, the residue was purified on a silica gel column ($CH_2Cl_2$/MeOH 96:4) to give compound **20** (19 mg, 68%) as a yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.31–1.36 (m, 6H, $OCH_2CH_3$), 3.87–3.94 (m, H-5′a), 4.03–4.22 (m, 5H, H-5′b, $OCH_2CH_3$), 5.71 (d, $J$ = 7.8 Hz, CH=CH), 5.80 (app s, H-4′), 6.10 (app d, $J$ = 6.0 Hz, H-2′), 6.28–6.31 (m, H-3′), 6.97 (app s, H-1′), 7.39 (d, $J$ = 8.1 Hz, CH=CH), 9.21 (s, 3-NH). $^{31}$P NMR ($CDCl_3$): $\delta$ 20.21. $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 16.18, 16.25 ($OCH_2CH_3$), 61.17, 62.36, 63.41 ($OCH_2CH_3$, C-5′), 87.77 (C-1′), 102.92 (C-5), 108.30, 108.46 (C-4′), 130.11 (C-2′), 131.73 (C-3′), 139.77 (C-6), 150.21 (C-2), 162.78 (C-4). HRMS (ESI) for $C_{13}H_{20}N_2O_7P$ $[M+H]^+$ found, 347.0999; calcd, 347.1003.

#### 4.2.28. 5′-[(Diethoxyphosphinyl)methoxy]-β-D-uridine (21)

Compound **20** (37 mg, 0.11 mmol) was dissolved in a 5:1 acetone–$H_2O$ mixture (4 mL) and treated with osmium tetraoxide (0.19 mL [4 wt % solution], 0.032 mmol) and *N*-methylmorpholine-*N*-oxide (32 mg, 0.26 mmol). After stirring for 48 h, the mixture was quenched with a saturated solution of $Na_2S_2O_3$ (4 mL) and stirred for 30 min. The black mixture was extracted with EtOAc three times and the combined organic layers were dried over $MgSO_4$ and evaporated. Chromatography of the residue on silica gel ($CH_2Cl_2$/MeOH 90:10) yielded diol **21** as a colorless solid (24 mg, 60%). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.23 (t, 6H, $J$ = 6.9 Hz, $OCH_2CH_3$), 3.88–3.91 (m, 3H, H-3′, H-5′a, H-5′b), 4.02–4.10 (m, 4H, $OCH_2CH_3$), 4.93 (app s, H-4′), 5.52 (d, $J$ = 6.0 Hz, 2′-OH), 5.60 (d, $J$ = 4.2 Hz, 3′-OH), 5.65 (d, $J$ = 8.1 Hz, CH=CH), 6.06 (d, $J$ = 6.9 Hz, H-1′), 7.49 (d, $J$ = 8.1 Hz, CH=CH). $^{31}$P NMR (DMSO-$d_6$): $\delta$ 20.55. $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.44, 16.51 ($OCH_2CH_3$), 59.50 (C-5′), 62.35, 62.43 ($OCH_2CH_3$), 73.07 (C-2′), 73.53 (C-3′), 87.78 (C-1′), 103.17 (C-5), 107.87, 108.03 (C-4′), 140.47 (C-6), 151.13 (C-2), 163.12 (C-4). HRMS (ESI) for $C_{13}H_{22}N_2O_9P$ $[M+H]^+$ found, 381.1035; calcd, 381.1057.

#### 4.2.29. 5′-[(Dihydroxyphosphinyl)methoxy]-β-D-uridine (22)

Compound **18** (23 mg, 0.061 mmol) was dissolved in dry DMF (3.8 mL) and cooled to 0 °C. The solution was then treated with 2,6-lutidine (71 μL, 0.61 mmol) followed by the dropwise addition of TMSBr (41 μL, 0.30 mmol). The mixture was allowed to warm to rt and stirred for 16 h. The solvent was evaporated and subsequently portioned between EtOAc/$Et_2O$ (1:1) and $H_2O$. The organic phase was washed with $H_2O$ and the aqueous layers were combined and lyophilized. The residue was purified using flash chromatography (*i*PrOH/$NH_4OH$/$H_2O$ 6:3:1) yielding compound **22** (9.0 mg, 50%) as a white powder. $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 3.47–3.55 (m, H-5′a), 3.80–3.87 (m, H-5′b), 4.09 (app d, $J$ = 4.2 Hz, H-3′), 4.40–4.44 (m, H-2′), 4.96 (H-4′), 5.84 (d, $J$ = 8.1 Hz, CH=CH), 6.25 (d, $J$ = 6.6 Hz, H-1′), 7.91 (d, $J$ = 7.8 Hz, CH=CH). $^{31}$P NMR ($CD_3OD$): $\delta$ 14.09. $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 64.88 (C-5′), 66.99 (C-2′), 73.62 (C-3′), 89.86 (C-1′), 104.15 (C-5), 110.31, 110.49 (C-4′), 143.28 (C-6), 152.96 (C-2), 166.08 (C-4). HRMS (ESI) for $C_9H_{12}N_2O_9P$ $[M-H]^-$ found, 323.0296; calcd, 323.0286.

### 4.3. Assay of PLC activity

Stable cell lines for study of the human $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors were generated by retroviral expression of the receptor in 1321N1 human astrocytoma cells, which do not natively express P2Y receptors.[24] Agonist-induced [$^3$H]inositol phosphate production was measured in cells plated at 20,000 cells/well on 96-well plates two days prior to assay. Sixteen hours before the assay, the inositol lipid pool of the cells was radiolabeled by incubation in 100 μL of serum-free inositol-free Dulbecco's modified Eagle's medium, containing 1.0 μCi of [$^3$H]*myo*-inositol. No changes of medium were made subsequent to the addition of [$^3$H]inositol. On the day of the assay, cells were challenged with 25 μL of a five-fold concentrated solution of receptor agonists in 100 mM Hepes (*N*-(2-hydroxyethyl)-piperazine-N-2-ethanesulfonic acid), pH 7.3 in HBSS, containing 50 mM LiCl for 30 min at 37 °C. Incubations were terminated by aspiration of the drug-containing medium and addition of 90 μL of ice-cold 50 mM formic acid. After 30 min, supernatants were neutralized with 30 μL of 150 mM $NH_4OH$ and applied to Dowex AG1-X8 anion exchange columns. Total inositol phosphates were eluted and radioactivity was measured using a liquid scintillation counter.[27]

### 4.4. Data analysis

Agonist potencies ($EC_{50}$ values) were determined from concentration-response curves by non-linear regression analysis using the GraphPad software package Prism (GraphPad, San Diego, CA). Each concentration of drug was tested in triplicate assays, and concentration effect curves for each test drug were repeated in at least three separate experiments with freshly diluted molecule. The results are presented as mean ± SEM from multiple experiments or in the case of concentration effect curves from a single experiment carried out with triplicate assays that were representative of results from multiple experiments.

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## Supplementary data

Supplementary data (NMR spectra and HPLC traces for selected derivatives) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.02.012.

## References and notes

1. Burnstock, G.; Kennedy, C. *Gen. Pharmacol.* **1985**, *16*, 433.
2. Abbracchio, M. P.; Burnstock, G. *Pharmacol. Ther.* **1994**, *64*, 445.
3. Barnard, E. A. *Trends Biochem. Sci.* **1992**, *17*, 368.
4. Barnard, E. A.; Burnstock, G.; Webb, T. E. *Trends Pharmacol. Sci.* **1994**, *15*, 67.
5. Brunschweiger, A.; Müller, C. E. *Curr. Med. Chem.* **2006**, *13*, 289.
6. Lustig, K. D.; Shiau, A. K.; Brake, A. J.; Julius, D. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5113.
7. Parr, C. E.; Sullivan, D. M.; Paradiso, A. M.; Lazarowski, E. R.; Burch, L. H.; Olsen, J. C.; Erb, L.; Weisman, G. A.; Boucher, R. C.; Turner, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3275.
8. Kellerman, D.; Evans, R.; Mathew, D.; Shaffer, C. *Adv. Drug Deliv. Rev.* **2002**, *54*, 1463.
9. Cosyn, L.; Van Calenbergh, S.; Joshi, B. V.; Ko, H.; Carter, R. L.; Harden, T. K.; Jacobson, K. A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3002.
10. Morse, D. M.; Smullen, J. L.; Davis, C. W. *Am. J. Physiol. Cell. Physiol.* **2001**, *280*, 1485.
11. Volonte, C.; Ciotti, M. T.; D' Ambrosi, N.; Lockhart, B.; Spedding, M. *Neuropharmacology* **1999**, *38*, 1335.
12. Astra Pharm. Ltd. et al. Exp. Opin. Ther. Patents. 1999, 9, 205–207 Astra Pharm. Ltd. Int. Pat. Appl. No. WO98/9845309.
13. Ivanov, A. A.; Ko, H.; Cosyn, L.; Maddileti, S.; Besada, P.; Fricks, I.; Costanzi, S.; Harden, T. K.; Van Calenbergh, S.; Jacobson, K. A. *J. Med. Chem.* **2007**, *50*, 1166.
14. De Clercq, E.; Holý, A. *Nat. Rev. Drug Disc.* **2005**, *4*, 928.
15. Jones, G. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1968**, *90*, 5337.
16. Padyukova, N. S.; Karpeisky, M. Y.; Kolobushkina, L. I.; Mikhailov, S. N. *Tetrahedron Lett.* **1987**, *28*, 3623.
17. Barton, D. H. R.; Stephane, D. G.; Quiclet-Sire, B.; Samadi, M. *Tetrahedron* **1992**, *48*, 1627.
18. Xu, Y.; Flavin, M. T.; Xu, Z. *J. Org. Chem.* **1996**, *61*, 7697.
19. Pomeisl, K.; Holy, A.; Pohl, R. *Tetrahedron Lett.* **2007**, *48*, 3065.
20. Verheyden, J. P. H.; Wagner, D.; Moffatt, J. G. *J. Org. Chem.* **1971**, *36*, 250.
21. Holy, A. *Synthesis-Stuttgart* **1998**, 381.
22. Kumar, T. S.; Zhou, S. Y.; Joshi, B. V.; Balasubramanian, R.; Yang, T.; Liang, B. T.; Jacobson, K. A. *J. Med. Chem.* **2010**, *53*, 2562.
23. Shatila, R. S.; Bouhadir, K. H. *Tetrahedron Lett.* **2006**, *47*, 1767.
24. Blackburn, G. M.; Ingleson, D. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1150.
25. Zemlicka, J.; Gasser, R.; Freisler, J. V.; Horwitz, J. P. *J. Am. Chem. Soc.* **1972**, *94*, 3213.
26. Kim, C. U.; Luh, B. Y.; Martin, J. C. *J. Org. Chem.* **1991**, *56*, 2642.
27. Nicholas, R. A.; Lazarowski, E. R.; Watt, W. C.; Li, Q.; Harden, T. K. *Mol. Pharmacol.* **1996**, *50*, 224.
28. Bourdon, D. M.; Wing, M. R.; Edwards, E. B.; Sondek, J.; Harden, T. K. *Methods Enzymol.* **2006**, *406*, 489.
29. (a) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205; (b) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333.
30. Davies, D. B.; Danyluk, S. S. *Biochemistry* **1974**, *13*, 4417.
31. Nencka, R.; Sinnaeve, D.; Karalic, I.; Martins, J. C.; Van Calenbergh, S. *Org. Biomol. Chem.* **2010**, *8*, 5234.