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Structural Modifications of UMP, UDP, and UTP Leading to Subtype-Selective Agonists for $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors[||]

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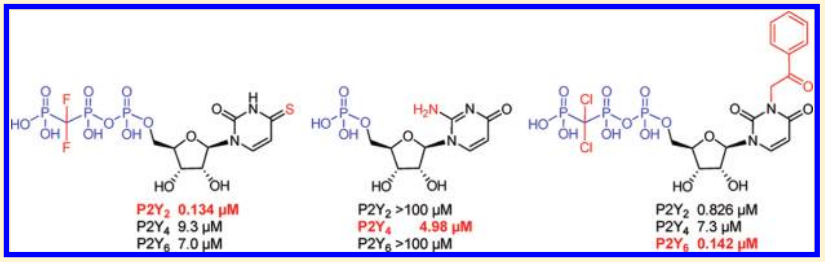
Supporting Information

ABSTRACT: A large series of derivatives and analogues of the uracil nucleotides UMP, UDP, and UTP with modifications in various positions of the uracil moiety and/or the phosphate groups were synthesized and evaluated at human $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors. 2--(Ar)alkylthio substitution of UMP and UDP was best tolerated by the $P2Y_2$ receptor. 2-Phenethylthio-UMP (**13e**) showed an EC_{50} value of 1.3 μM at $P2Y_2$ and >70-fold selectivity versus $P2Y_4$ and $P2Y_6$ receptors. Substitution of the 2-keto group in UMP by NH (**13g**, iso-CMP) resulted in the first potent and selective $P2Y_4$ agonist (EC_{50} 4.98 μM, >20-fold selective vs $P2Y_2$ and $P2Y_6$). In contrast, replacement of the 2-keto function in UDP by NH yielded a potent $P2Y_2$ agonist (**12g**, iso-CDP, EC_{50} = 0.604 μM, >100-fold selective). In an attempt to obtain metabolically stable UTP analogues, β,γ-dichloro- and β,γ-difluoro-methylene-UTP derivatives were synthesized. The triphosphate modifications were much better tolerated by $P2Y_2$, and in some cases also by $P2Y_6$, than by $P2Y_4$ receptors. 4-Thio-β,γ-difluoromethylene-UTP (**14g**) was a potent $P2Y_2$ agonist with an EC_{50} value of 0.134 μM and >50-fold selectivity. N3-Phenacyl-β,γ-dichloromethylene-UTP (**14b**) proved to be a potent $P2Y_6$ receptor agonist (EC_{50} 0.142 μM) with high selectivity versus $P2Y_4$ (50-fold) and moderate selectivity versus $P2Y_2$ receptors (6-fold).



INTRODUCTION

Nucleotide receptors, also termed P2 receptors, are a group of receptors belonging to the family of purinergic receptors, which consists of three receptor subfamilies, P0, P1, and P2.[1−5] P2 receptors are subdivided into two classes of receptors, P2X ion channel receptors containing seven members ($P2X_{1,2,3,4,5,6,7}$), and G protein-coupled P2Y receptors containing eight members ($P2Y_{1,2,4,6,11,12,13,14}$ receptors).[4−7] The P2Y receptor family comprises two distinct clusters: the G_q-coupled $P2Y_1$-like receptors, which include $P2Y_{1,2,4,6,11}$, and the G_i-coupled $P2Y_{12}$-like receptors, which include $P2Y_{12,13,14}$ subtypes.[6] The human $P2Y_{1,11,12,13}$ receptors are activated by adenine nucleotides, while the $P2Y_{4,6,14}$ receptors are activated by pyrimidine nucleotides. The $P2Y_2$ receptor is activated by both adenine and pyrimidine nucleotides. Of the four P2Y receptors that can be activated by pyrimidine nucleotides, the $P2Y_2$ and $P2Y_4$ receptors are activated by UTP (**I**, Figure 1) and the $P2Y_6$ receptor is activated by UDP (**II**, Figure 1), while the $P2Y_{14}$ receptor can be activated by UDP as well as UDP-glucose. Species differences exist, for example, in contrast to the human $P2Y_4$ receptor (a purely UTP-activated receptor), the rat orthologue is potently activated by UTP as well as ATP.[6,8]

The pyrimidine nucleotides UTP and UDP participate in the regulation of a broad range of physiological functions in numerous cells and tissues through their action on the $P2Y_2$, $P2Y_4$, and/or $P2Y_6$ receptors.[9] The $P2Y_2$ receptors are mainly distributed in spleen, testes, brain, liver, kidney, lung, heart, skeletal muscle, in vascular and epithelial cells, and in the immune system including T-cells and monocytes, while the $P2Y_4$ receptors are expressed in gastrointestinal cells, placenta, and cochlear cells. $P2Y_6$ receptors are distributed in spleen, small intestine, thymus, placenta, aorta, lung, stomach, leukocytes, vascular smooth muscle cells, microglial cells, and neutrophils.[6,8]

Synthetic nucleotides that interact with P2Y receptors are currently in clinical trials as new drugs for the treatment of various diseases. For example, the short-acting $P2Y_{12}$ receptor antagonist cangrelor (**III**, Figure 1) is in phase III clinical trials as iv anticoagulant applied before surgery.[10] The $P2Y_2$ receptor agonist denufosol (**IV**, Figure 1; EC_{50} values: 220 nM at $P2Y_2$, 800 nM at $P2Y_4$, and >1 μM at $P2Y_6$ receptors)[11a] is currently evaluated in phase III clinical studies for the treatment of cystic fibrosis, and the $P2Y_2$ agonist diquafosol (**V**, Figure 1; EC_{50} values: 100 nM at $P2Y_2$, 400 nM at $P2Y_4$, and 2 μM at $P2Y_6$ receptors)[11b] has recently been approved in Japan[15a,b] for the treatment of dry eye syndrome; the compound is still under further investigation to be approved by the FDA.[11−15]

Previously published structure−activity relationships (SAR) of agonists for the pyrimidine nucleotide-activated $P2Y_2$, $P2Y_4$, and

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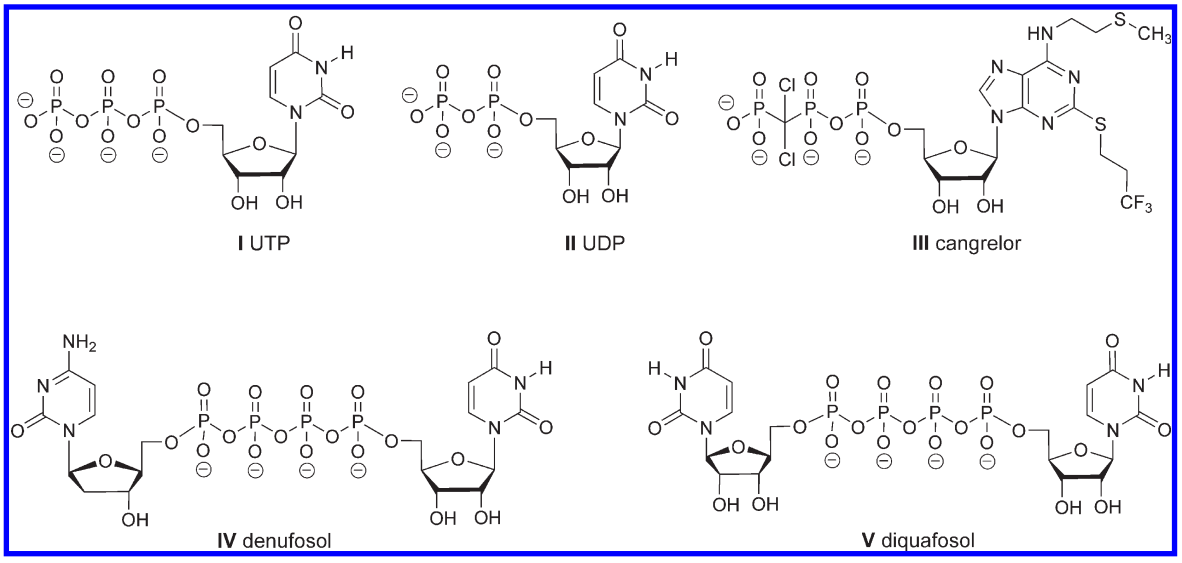

Figure 1. Selection of natural and synthetic P2Y receptor ligands.

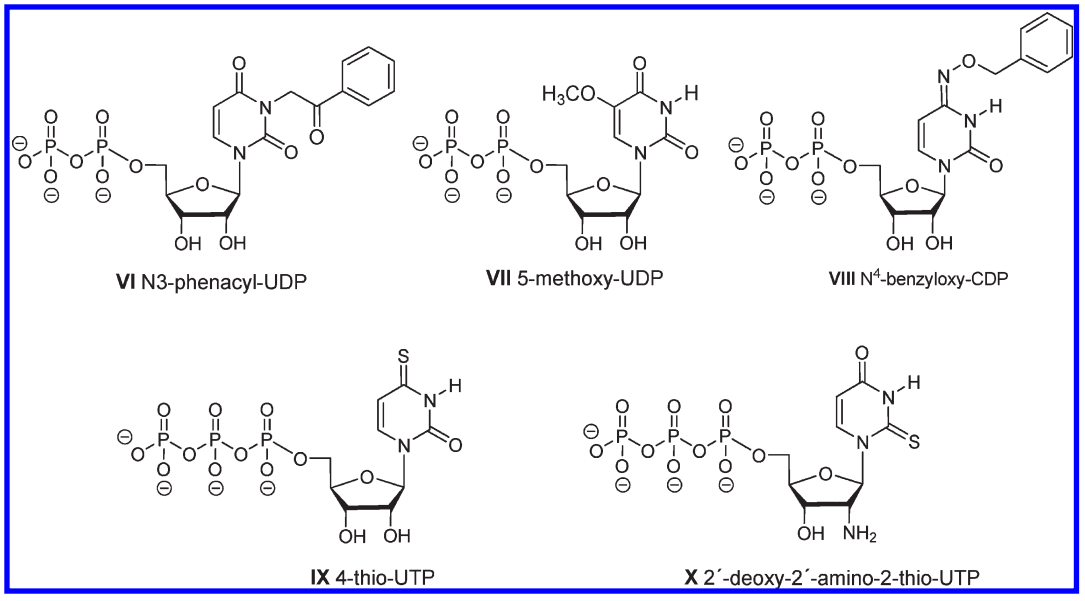

Figure 2. Selected potent $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptor agonists.

$P2Y_6$ receptors were based on modifications of the three structural parts of the physiological agonists UTP (**I**) and UDP (**II**): the phosphate chain, the ribose moiety, and/or the uracil residue. The di/triphosphate residue[6,8] was modified to obtain stability versus *ecto*-nucleotidases, for example, in UTPγS, UDPβS, nucleoside-5′-phosphonates, and β,γ- (or α,β-) dichloro- (or difluoro-) methylene phosphonate analogues of UTP (or UDP),[16−18] or through formation of dinucleotides.[11,12] Modifications at the ribose moiety[19] showed the importance of the two hydroxyl groups in the 2′- and 3′-positions, which are thought to be involved in hydrogen bond formation with the receptors.[8] Modifications at the uracil moiety[18,20,21] through introduction of various groups at different positions led to the discovery of potent agonists, such as N3-phenacyl-UDP (**VI**, Figure 2), a potent and selective $P2Y_6$ receptor agonist exhibiting an EC_{50} of 70 nM,[18] 5-methoxy-UDP (**VII**, Figure 2; EC_{50} = 80 nM at $P2Y_6$),[22] and N^4-benzyloxy-CDP (**VIII**, Figure 2; EC_{50} = 26 nM at $P2Y_6$).[23] 4-Thio-UTP (**IX**, Figure 2) showed high potency at $P2Y_4$ receptors (EC_{50} = 23 nM),[21] but the compound was comparably active at $P2Y_2$ receptors and is therefore nonselective. Currently, no selective agonist for the $P2Y_4$ receptor is known. One of the most potent and selective $P2Y_2$ receptor agonists is 2′-deoxy-2′-amino-2-thio-UTP (**X**, Figure 2), exhibiting an EC_{50} value of 8 nM at $P2Y_2$ receptors with good selectivity versus the $P2Y_4$ and $P2Y_6$ receptors.[24] In our previous work, we studied the SARs of UTP and UDP derivatives and analogues modified at the phosphate chain at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors, and we presented 5-bromo-β,γ-dichloromethylene-UTP exhibiting an EC_{50} value of 120 nM at $P2Y_6$ receptors.[18] In a further project, we modified the ribose moiety via replacement by acyclic alkyl chains linked with phosphates or phosphonates, and some of the synthesized compounds showed antagonistic activity at $P2Y_2$ receptors.[16]

Despite intensive research efforts in recent years on the (patho)physiological roles of P2Y receptors, only a very limited number of SAR studies[6,7,11−24] on agonists or antagonists have

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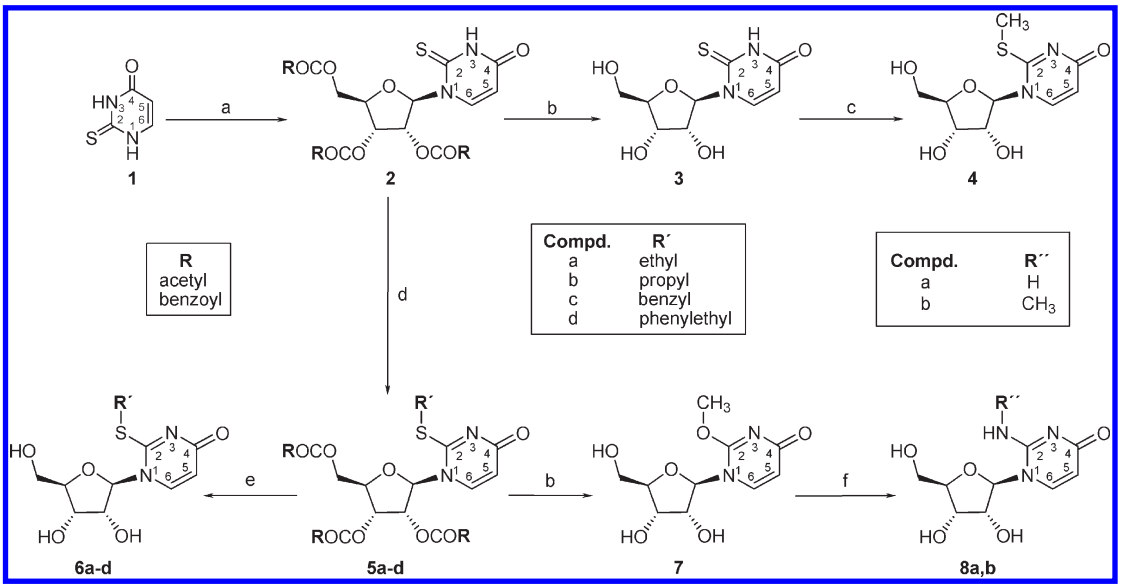
Scheme 1. Synthesis of 2-Substituted Uridine Nucleosides[a]



[a] Reagents and conditions: (a) Three steps: (i) HMDS, reflux, 5 h; (ii) $SnCl_4$, 1,2-dichloroethane, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- (or acetyl-) β-D-ribofuranose, room temperature, 5 h; (iii) $NaHCO_3$, H_2O. (b) Two steps: (i) $NaOCH_3$, CH_3OH, room temperature, 3 h; (ii) DOWEX 50 × 8 proton form. (c) CH_3I, triethylamine (TEA), C_2H_5OH:H_2O (1:1), 1 h, room temperature. (d) R′−X (X = Br, I), CH_2Cl_2, TEA, reflux, 24−36 h. (e) NH_3, CH_3OH, 30 min to 1 h. (f) NH_3 or CH_3−NH_2, CH_3OH, 0−4 °C, 8 h.

been published. Therefore, SAR of pyrimidine nucleotides at P2Y receptors are only incompletely known, and the interaction between nucleotide agonists and P2Y receptors is not fully understood. Furthermore, many of the discovered potent agonists are characterized by the lacking of *ecto*-nucleotidase stability and therefore cannot be used in in vivo studies. In case of the UTP-activated $P2Y_4$ receptor subtype, no selective agonists or antagonists are known to date. To gain better knowledge about the pyrimidine nucleotide binding sites at the receptors and to fully understand the SARs at these receptors, our goal was to design and synthesize novel UTP and UDP analogues with broad structural modifications. Selective and stable agonists are needed as tools and potential drugs. Beside metabolic stability, the new compounds should be modified in such a way that they cannot be integrated into DNA or RNA. This could, for example, be achieved by introduction of large substituents at the pyrimidine ring or at the phosphate chain. Therefore, we planned to synthesize and investigate a series of base-modified nucleotides substituted at the 2-position of the uracil moiety linked via a thioether, ether, or amino group. In a second series, and to reach *ecto*-nucleotidase stability, we combined base modification with alteration of the phosphate chain by preparing β,γ-dichloro- and β,γ-difluoro-methylene-UTP analogues. Finally, we studied whether bulky substituents at the γ-position of the phosphate chain would be tolerated by the receptors.

SYNTHESES

Nucleoside derivatives related to uridine with modifications in the pyrimidine structure were synthesized starting from the appropriate uracil derivatives by a standard silyl−Hilbert−Johnson procedure with minor modifications. 2-Thiouracil (**1**) was silylated with hexamethyldisilazane (HMDS) in the presence of a catalytic amount of trimethylsilyl chloride or ammonium sulfate, respectively. Silylated **1** was condensed with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl- or 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in dichloroethane catalyzed by tin(IV) chloride. After removal of the silyl groups using saturated aqueous sodium hydrogen carbonate solution, products **2** were obtained (Scheme 1). Nucleosides **2** were deprotected by transesterification using sodium methylate solution in methanol to yield 2-thiouridine (**3**).[25,26] 2-Methylthiouridine (**4**) was obtained by methylation of **3** with methyl iodide in an aqueous ethanolic solution in 95% yield (for details, see the Supporting Information). Alkylation of **3** with other alkylating agents resulted in the formation of mixtures of the products with many side products, mostly resulting from additional alkylation of ribose hydroxy functions. Therefore, we successfully alkylated the protected 2-thiouridine derivatives **2** with different bromoalk(ar)alkyl derivatives to afford **5a**−**d** (Scheme 1). Deprotection of **5a**−**d** with sodium methylate solution in methanol resulted in nucleophilic substitution and formation of 2-methoxyuridine (**7**).[27] Controlled deprotection of **5a**−**d** using ammonia in methanol afforded, after purification, 2-(ar)alkylthiouridine derivatives **6a**−**d** in 20−25% yield. Substitution of **7** with ammonia or methylamine in methanol yielded 2-aminouridine derivatives **8a,b** (Scheme 1). A crystal structure of 2-propylthio-1-β-D-ribofuranosylpyrimidine-2,4-dione (**6b**) was obtained (see the Supporting Information) showing that the nucleoside adopted an *anti*-conformation like the natural nucleosides.[28]

4-Thiouridine (**10**) was synthesized starting from uridine (**9**), which was acetylated[29] by acetic anhydride in pyridine to give 1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-(3*H*)-pyrimidine-2,4-dione, followed by thioation by heating it under reflux with Lawesson's reagent in benzene to yield 4-thio-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-(3*H*)-pyrimidine-2,4-dione.[30] Deprotection using ammonia in methanol afforded, after purification, **10** in a good yield (Scheme 2 in the Supporting Information). N3-Phenacyluridine (**11**, Scheme 2 in the Supporting Information) was synthesized via alkylation of uridine (**9**) with phenacyl bromide in the presence of potassium carbonate as a base using a mixture of equal amounts of acetone and *N*,*N*-dimethylformamide (DMF) as a solvent yielding 82% of isolated product.[31]

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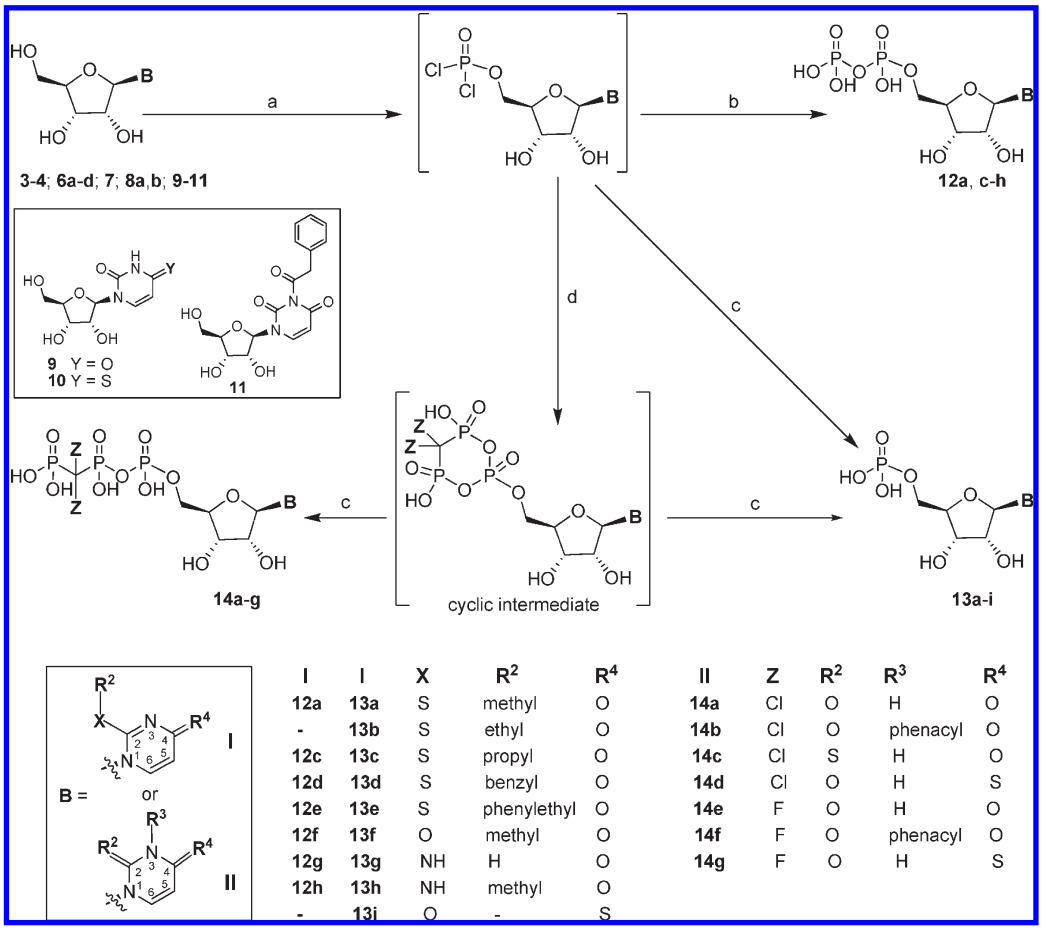
Scheme 2. Synthesis of Nucleotide Derivatives and Analogues[a]



I	I	X	R^2	R^4
12a	13a	S	methyl	O
-	13b	S	ethyl	O
12c	13c	S	propyl	O
12d	13d	S	benzyl	O
12e	13e	S	phenylethyl	O
12f	13f	O	methyl	O
12g	13g	NH	H	O
12h	13h	NH	methyl	O
-	13i	O	-	S

II	Z	R^2	R^3	R^4
14a	Cl	O	H	O
14b	Cl	O	phenacyl	O
14c	Cl	S	H	O
14d	Cl	O	H	S
14e	F	O	H	O
14f	F	O	phenacyl	O
14g	F	O	H	S

[a] Reagents and conditions: (a) $POCl_3$, $(C_2H_5O)_3PO$, 1,8-bis-(dimethylamino)naphthaline, 0 °C, 5 h. (b) Two steps: (i) $(HNBu_3)_2HPO_4$, Bu_3N; (ii) (Et_3NHHCO_3), pH 7.4−7.6, H_2O, room temperature, 1 h. (c) (Et_3NHHCO_3), pH 7.4−7.6, H_2O, room temperature, 1 h. (d) $(Bu_3NH)_2CH_2P_2Z_2O_6$ (Z = Cl, F), Bu_3N, 5 min, 0 °C.

The synthesized 2-substituted pyrimidine nucleosides (compounds **3**, **4**, **6a**−**d**, **7**, and **8a**,**b**) were subjected to phosphorylation according to the Ludwig procedure[32] with minor modifications (Scheme 2). The lyophilized nucleosides (**3**, **4**, **6a**−**d**, **7**, and **8a**,**b**) were dissolved in triethyl phosphate and reacted with phosphorus oxychloride in the presence of 1,8-bis-(dimethylamino)naphthaline, yielding the reactive 5′-dichlorophosphate intermediates (Scheme 2). Those were immediately reacted with a mixture of 1 equiv of tri-*n*-butylamine and a 5-fold excess of bis(tri-*n*-butylammonium) phosphate in DMF to afford, after hydrolysis with triethylammonium hydrogencarbonate (TEAC), the desired nucleoside diphosphates **12a**,**c**−**h**. Nucleoside monophosphates (**13a**−**i**, Scheme 2) were obtained by hydrolyzing the nucleoside 5′-dichlorophosphates by treatment with TEAC.

Pyrimidine triphosphate analogues containing a $P_\beta P_\gamma$-difluoro- or dichloromethylene bridge were obtained from the nucleosides uridine (**9**), 2-thiouridine (**3**), 4-thiouridine (**10**), and N3-phenacyluridine (**11**). The nucleosides were subjected to phosphorylation using phosphorus oxychloride followed by reaction with bis-(tri-*n*-butylammonium) difluoro- or dichloromethylenediphosphonate in DMF to form a cyclic intermediate, which was hydrolyzed with TEAC to give the nucleotide analogues **14a**−**g** (Scheme 2).[18,33]

In a one-pot, four-step reaction, 5-bromouridine (**15**) was reacted with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one to yield the activated phosphite, which was condensed with diphosphate to form the cyclic triphosphite intermediates (Scheme 3). The latter was oxidized by iodine, followed by subsequent reaction with aniline as a nucleophile yielding, after hydrolysis with water, the γ-substituted 5-bromouridine triphosphate **16** (Scheme 3).[34]

Phosphorylation reactions generally yielded mixtures of charged nucleotides (mono-, di-, and triphosphates) and uncharged starting compounds. Therefore, purification was performed by anion exchange chromatography. In addition to various nucleotides, the products also contained large amounts of inorganic salts, such as inorganic phosphates and some buffer components. Therefore, the products were further purified by reverse phase high-performance liquid chromatography (HPLC).

The structures of the synthesized nucleotides and nucleotide analogues were confirmed by ^1H, ^{13}C, and ^{31}P NMR spectroscopy, in addition to HPLC analysis coupled to electrospray ionization mass spectrometry (LC/ESI-MS). Spectroscopic data for all new compounds were in agreement with the proposed structures (for details, see the Supporting Information).

The stability of the synthesized pyrimidine triphosphate analogues containing a $P_\beta P\gamma$-difluoro- or dichloromethylene bridge is expected to be high as in case of the competitive $P2Y_{12}$ antagonist cangrelor (**III**, Figure 1), which is in clinical development as an antithrombotic drug.[10,35] The hydrolytic stability of other similar

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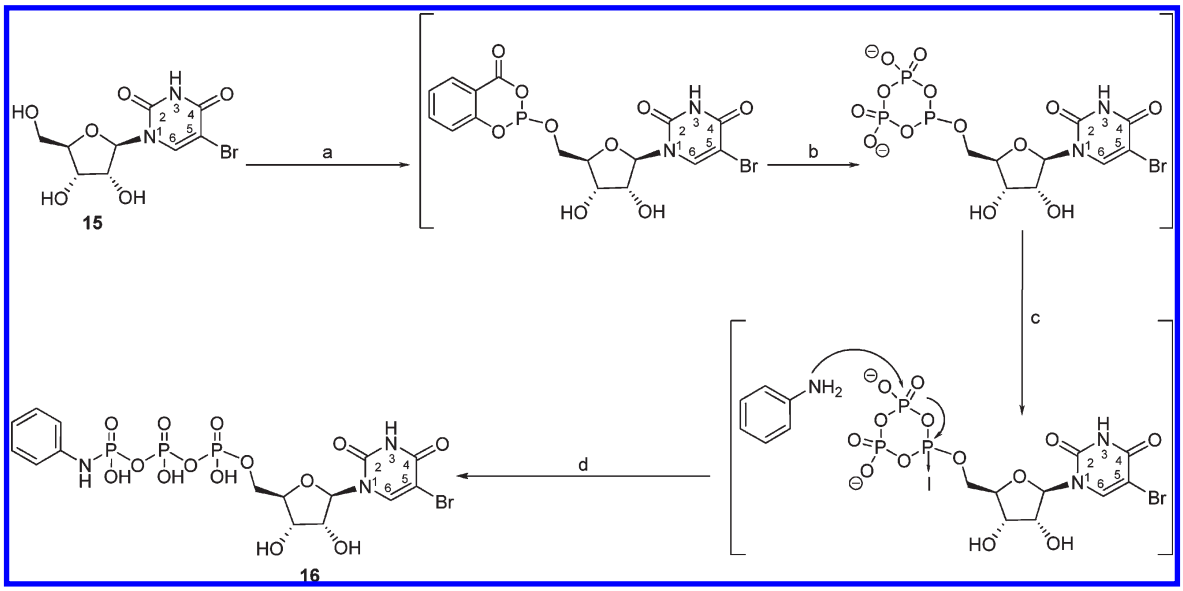
Scheme 3. Synthesis of γ-Phenylamino-5-bromouridine-5′-triphosphate[a]



[a] Reagents and conditions: (a) 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, pyridine, DMF, 0 °C, 1 h. (b) $(HNBu_3)_2H_2P_2O_7$, Bu_3N, DMF, room temperature, 1 h. (c) Two steps: (i) I_2, 0 °C, 1 h; (ii) aniline, DMF, room temperature, 2 h. (d) H_2O, room temperature, 30 min.

compounds, for example, the ATP analogue 2-methylthio-5′-adenylic acid (1-phosphonomethyl-1-phosphonyl)anhydride, was recently evaluated, and the compound was found to be stable at physiological pH.[36] At acidic pH mimicking gastric juice (pH 1.4, 37 °C), the determined half-life was 65 h as monitored by ^{31}P NMR. In addition, the compound was found to be quite stable in the presence of nucleoside triphosphate diphosphohydrolases (NTPDase1,2,3,8) and nucleotide pyrophosphatases (NPP1,3).[36]

In the present study, the stability of stock solutions of the synthesized pyrimidine di- and triphosphate derivatives and analogues, which had been repeatedly frozen and thawed again to keep them at room temperature for some time, was monitored by LC/MS about 1.5 years after the biological evaluations had been completed. The results confirmed published data since most compounds showed still more than 80% purity (the initial purity upon testing had been >95%). The measured stability was higher in case of P_β,P_γ-dihalomethylene-UTP analogues (e.g., **14f**, 86% purity) and the P_γ-phenylamino-UTP analogue (**16**, 84% purity) than the diphosphate derivatives (e.g., **12e**, 72% purity). For LC/MS results of stability measurement, see the Supporting Information.

BIOLOGICAL ACTIVITY

The nucleotides and nucleotide analogues were investigated at human $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors stably expressed in astrocytoma 1321N1 cells as previously described.[18,37] The intracellular accumulation of [^{3}H]inositol phosphates (IP) induced by the activation of G_q-coupled receptors was quantified. The compounds were initially tested at 1 and 100 μM concentration; for selected, potent compounds, EC_{50} values were determined by measuring full concentration−response curves. For additional compounds, EC_{50} values were estimated based on four data points. All data were confirmed by three independent experiments. All nucleotides featuring an intact ribose ring, which have been investigated so far at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors, have been found to be agonists rather than antagonists. Only nucleotide analogues, in which the ribose was replaced with acyclic structures, were characterized as antagonists.[16] Because the ribose moiety was not modified in the present study, and thus, antagonism was not to be expected, compounds were only tested for agonistic activity.

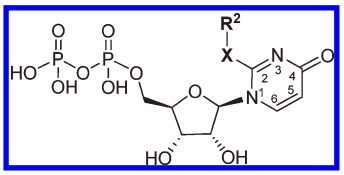
STRUCTURE−ACTIVITY RELATIONSHIPS

UDP Derivatives and Analogues. UDP is the physiological agonist at $P2Y_6$ receptors (EC_{50} = 48 nM), and it is selective for that receptor (see Table 1). All of the synthesized 2-substituted UDP derivatives showed lower potency at $P2Y_6$ receptors than UDP. Small modification of 2-thio-UDP, which is about 4-fold less potent than UDP (EC_{50} of 239 nM at $P2Y_6$ receptors)[18] by methylation (2-methylthio-UDP, **12a**), resulted in a decrease in activity (EC_{50} of **12a** = 4.7 μM). Introduction of larger groups led to a complete loss of activity as in the case of 2-propylthio-UDP (**12c**). Substitution with aromatic residues was better tolerated, 2-phenylethylthio-UDP (**12e**, EC_{50} = 2.5 μM) showing higher potency than the corresponding benzylthio analogue **12d** (EC_{50} = 6.8 μM). The 2-amino analogues of UDP (exchange of O by NH_2 or $NHCH_3$ at C2) leading to isocytidine diphosphate (isoCDP) **12g** and its methylated derivative **12h** were completely inactive at $P2Y_6$ receptors. 2-Methoxy-UDP (**12f**) was the most potent compound of the present series exhibiting an EC_{50} of 1.37 μM (see Table 1 and Figure 3C). Similarly, a methoxy group in the 5-position of UDP has recently been shown to be also well tolerated (5-methoxy-UDP **VII**, Figure 2; EC_{50} = 80 nM at $P2Y_6$ receptors).[22]

At $P2Y_4$ receptors, the (ar)alkyl 2-thio-substituted UDP derivatives were tolerated, and the rank order of potency was methyl (**12a**, EC_{50} = 1.52 μM) > propyl (**12c**, EC_{50} = 4.9 μM) > benzyl (**12d**, EC_{50} = 5.98 μM) ≫ phenylethyl (**12e**, EC_{50} > 100 μM), indicating that large substituents in that position were unfavorable. 2-Methoxy-UDP (**12f**) was also tolerated by the $P2Y_4$ receptors with an EC_{50} value of 4.49 μM, while 2-amino derivatives (**12g,h**) were inactive like at the $P2Y_6$ receptor (see Table 1 and Figure 3B).

Surprisingly, the 2-substituted-UDP derivatives, which had originally been designed for $P2Y_6$ receptors, showed higher

Table 1. Potency of Uridine Diphosphate Derivatives as Agonists at Human $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors



			$EC_{50} \pm$ SEM (μM) IP accumulation human recombinant P2Y receptors expressed in 1321N1 astrocytoma cells ($n \geq 3$)[a]		
compd	X	R^2	$P2Y_2$	$P2Y_4$	$P2Y_6$
UDP	O		ND,[b] 16.5[38]	ND,[b] 14.0[39]	0.048 ± 0.007
UTP			0.0179 ± 0.005	0.0884 ± 0.027	ND,[b] 6.0, >10.0[6,41]
2-thio-UDP	S		~5.0[18]	~4.0[18]	0.239 ± 20[18]
12a	S	methyl	0.523 ± 0.12	1.52 ± 0.17	4.7[c]
12c	S	propyl	1.2 ± 0.22	4.9[c]	≥100
12d	S	benzyl	6.2[c]	5.98 ± 1.5	6.8[c]
12e	S	phenylethyl	0.544 ± 0.23	≥100	2.5[c]
12f	O	methyl	0.907 ± 0.04	4.49 ± 1.03	1.37 ± 0.12
12g (iso-CDP)	NH	H	0.604 ± 0.12	≥100	≥100
12h	NH	methyl	7.6[c]	>100	>100

[a] Data calculated from full dose−response curve unless otherwise noted. [b] ND, not determined. [c] Estimated EC_{50} value based on four data points ($n = 3$), SEM < 10%.

potency at the $P2Y_2$ receptors. This may be explained by a different binding mode due to the bulky 2-substitutent. 2-Methylthio-UDP (**12a**) was 33-fold more potent than UDP at $P2Y_2$ receptors with an EC_{50} value of 0.523 μM. Extension of the alkyl group by two atoms as in case of propyl derivative **12c** led to a decrease in the activity by 2-fold, compound **12c** exhibiting an EC_{50} value of 1.2 μM. Introduction of a benzyl moiety (**12d**, 6.2 μM) was less well tolerated than phenylethyl (**12e**, 0.544 μM). Compound **12e** had nearly the same potency as the methylthio-UDP (**12a**) at $P2Y_2$ receptors, but compound **12e** possessed higher selectivity versus $P2Y_4$ receptors (EC_{50} >100 μM). 2-Methoxy-UDP (**12f**) was also tolerated by the $P2Y_2$ receptor as by the other pyrimidine nucleotide-activated P2Y receptor subtypes with an EC_{50} value at $P2Y_2$ of 0.907 μM. 2-Methylamino-UDP (**12h**, 7.6 μM) was active at $P2Y_2$ but weaker than **12f**, while 2-amino-UDP (**12g**, EC_{50} 0.604 μM) was more potent at $P2Y_2$ receptors and highly subtype-selective (>100-fold) versus $P2Y_4$ and $P2Y_6$ receptors (see Table 1 and Figure 3A).

UMP Derivatives and Analogues. In our previous work,[18] we had synthesized and investigated base-modified UMP derivatives and had found that 5-bromo-UMP was well tolerated by $P2Y_2$ ($EC_{50} \sim 4.0$ μM) and even better by $P2Y_6$ receptors (EC_{50} = 2 μM).[18] The enzymatic stability of nucleotide monophosphates is much higher than that of the corresponding di- and triphosphates since monophosphates are—in contrast to the other nucleotides—not hydrolyzed by NTPDases and NPPs. Thus, derivatives and analogues of UMP may be of interest as P2Y agonists with increased metabolic stability in comparison with nucleoside di- or triphosphates. Therefore, we synthesized and investigated base-modified UMP derivatives (Table 2). The investigated compounds showed lower activity at the $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors in comparison with the physiological agonists UTP and UDP.

At $P2Y_6$ receptors, only two compounds exhibited micromolar activity, 2-propylthio-UMP (**13c**, EC_{50} = 6.2 μM) and 2-methoxy-UMP (**13f**, EC_{50} = 9.8 μM), but both were nonselective. At $P2Y_4$ receptors, 2-ethylthio-UMP (**13b**) showed an EC_{50} value of 4.33 μM, and 2-amino-UMP (**13g**) exhibited an EC_{50} = 4.98 μM, while the other tested compounds were inactive at a concentration of 100 μM at this receptor. Compound **13g** is highly selective versus $P2Y_2$ and $P2Y_6$ receptors. In fact, **13g** is the first $P2Y_4$-selective ligand described so far.

2-Amino-UMP derivatives (**13g** and **13h**) and 4-thio-UMP (**13i**) were inactive at $P2Y_2$ receptors (EC_{50} > 100 μM), while 2-methoxy-UMP (**13f**) was tolerated by $P2Y_2$ receptors (EC_{50} = 9.9 μM). 2-Ar(alkyl)thio-substituted UMP derivatives were the best tolerated derivatives of the present series at $P2Y_2$ receptors. The rank order of potency was 2-phenylethylthio-UMP (**13e**; EC_{50} = 1.3 μM) ≥ 2-ethylthio-UMP (**13b**; EC_{50} = 1.5 μM) ≥ 2-methylthio-UMP (**13a**; EC_{50} = 2.9 μM) ≥ 2-propylthio-UMP (**13c**; EC_{50} = 4.8 μM) ≫ 2-benzylthio-UMP (**13d**; EC_{50} ≥ 100 μM). 2-Phenylethylthio-UMP (**13e**; EC_{50} = 1.3 μM) was the most active UMP derivative in the present series at $P2Y_2$ receptors with more than 100-fold selectivity versus $P2Y_4$ and $P2Y_6$ receptors. Future optimization of **13e** may lead to more potent, enzymatically stable $P2Y_2$ receptor agonists.

***β,γ*-Di(halo)methylene-Bridged UTP Analogues.** In a previous study, we showed that a 5-bromo-UTP analogue in which the P_β,P_γ-oxygen bridge was replaced by dichloromethylene linker showed nanomolar affinity at $P2Y_2$ receptors (0.354 μM) and was even 3-fold more potent at $P2Y_6$ receptors (0.120 μM).[18] These promising results led us to synthesize further UTP derivatives (modified at the 2-, 3-, and 4-positions of the uracil moiety), in which the P_β,P_γ-oxygen bridge was replaced by a dichloro- or difluoromethylene moiety. The obtained compounds **14a**−**g** were tested as agonists at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors, and the results are collected in Table 3. *β,γ*-Dichloromethylene-UTP (**14a**) was less potent at $P2Y_2$ receptors (EC_{50} = 0.612 μM) than the parent compound UTP (EC_{50} = 0.0179 μM), but **14a** showed very high selectivity versus $P2Y_4$ receptors (EC_{50} ≥ 100 μM); the compound showed an EC_{50} value of 6.2 μM at $P2Y_6$ receptors.

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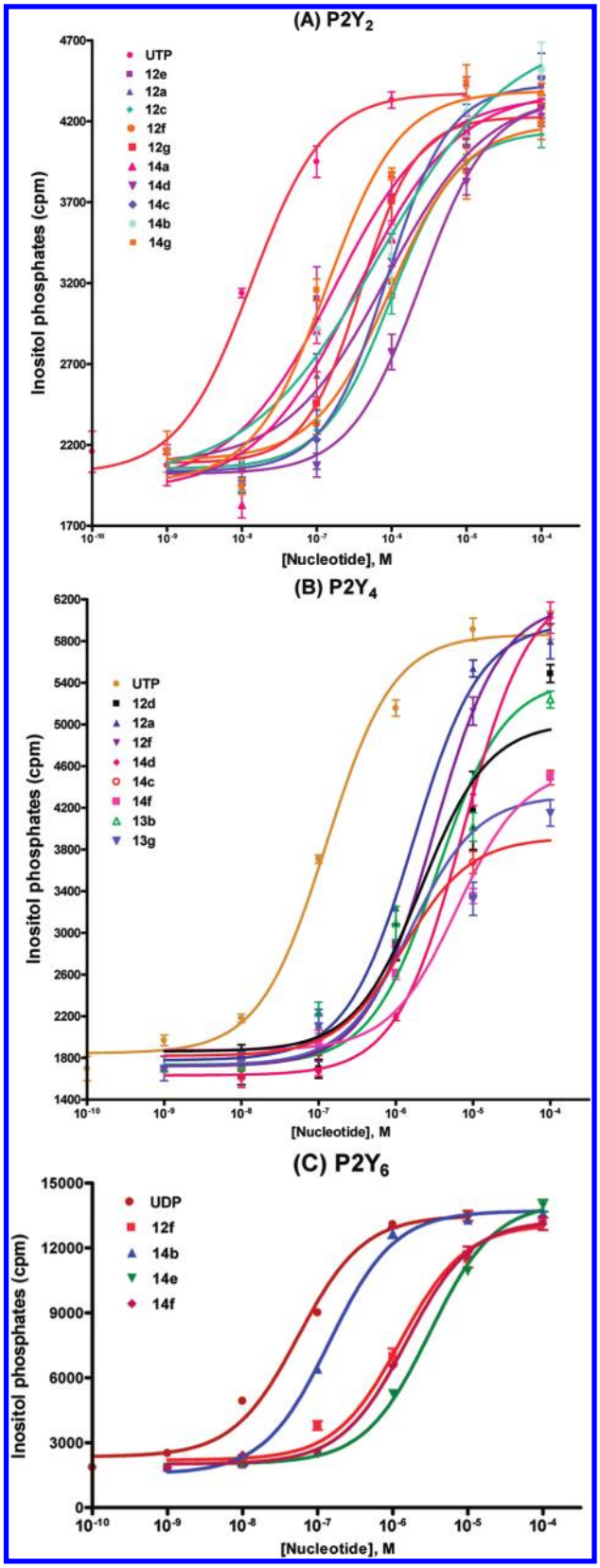


Figure 3. Results of concentration−response curves of selected pyrimidine nucleotide derivatives and analogues as agonists at $P2Y_2$ (A), $P2Y_4$ (B), and $P2Y_6$ (C) receptors. Dose-dependent stimulation of IP accumulation was determined in 1321N1 human astrocytoma cells stably transfected with the relevant human receptor subtype. Data points represent means of three independent experiments performed in triplicate.

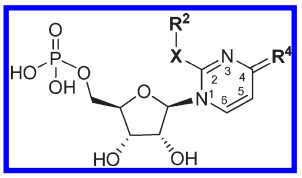
β,γ-Difluoromethylene-UTP (**14e**) had been investigated at $P2Y_2$ receptors only in an early study and was found to have an EC_{50} value of 8.92 μM.[7] The affinity was higher in our results (EC_{50} = 4.9 μM), and we provide additional information on the activity of **14e** at $P2Y_4$ and $P2Y_6$ receptors. Compound **14e** was 2-fold more potent at $P2Y_6$ than at $P2Y_2$ receptors, while it showed no activity at $P2Y_4$ receptors (EC_{50} > 100 μM), similarly as its dichloromethylene analogue **14a**. The dichloromethylene modified-UTP (**14a**) was 8-fold more potent at $P2Y_2$ receptors than the difluoromethylene-modified compound (**14e**), while their activity at $P2Y_6$ receptors was similar (see Table 3). 2-Thio-β,γ-dichloromethylene-UTP (**14c**) had recently been investigated at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors by Jacobson and co-workers.[40] In their study, the compound was reported to show an EC_{50} value of 2.51 μM at $P2Y_2$ receptors, >10 μM at $P2Y_4$ and $P2Y_6$ receptors. However, in our hands, **14c** showed somewhat higher potency at $P2Y_2$ (EC_{50} = 0.794 μM) and moderate potency at $P2Y_4$ (EC_{50} 3.85 μM) and $P2Y_6$ receptors (EC_{50} = 7.7 μM). This discrepancy cannot be explained at present.

Substitution of the uracil base of UDP (**II**) at N3 with the bulky phenacyl moiety (compound **VI** in Figure 2) had previously been found to yield a potent (EC_{50} = 0.070 μM) and subtype-selective $P2Y_6$ receptor agonist.[18] To obtain enzymatically stable analogues of compound **VI**, we synthesized N3-phenacyl-β,γ-dichloromethylene-UTP (**14b**) and its corresponding difluoro- substituted analogue (**14f**), since we had previously found that the dichlormethylene modification, for example, in 5-bromo-β,γ-dichloromethylene-UTP, was well tolerated by $P2Y_6$ receptors (see Table 3). The compounds were investigated at $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors, and the results showed that both modifications (dichloromethylene in **14b** and difluoromethylene in **14f**) were tolerated by the $P2Y_2$ receptors. The EC_{50} values were almost identical (EC_{50} = 0.826 μM for **14b** and 1.2 μM for **14f**). Both **14b** and **14f** also showed moderate potency at $P2Y_4$ receptors (EC_{50} = 7.3 μM for **14b** and 3.93 μM for **14f**). The difluoromethylene modification in **14f** was also tolerated by $P2Y_6$ (EC_{50} = 1.46 μM). The best result was obtained with the dichloromethylene modification in **14b** at the $P2Y_6$ receptors. Compound **14b** was only 3-fold less potent than the physiological agonist UDP with an EC_{50} = 0.142 μM; it was 6-fold selective versus $P2Y_2$ and more than 50-fold selective versus $P2Y_4$ receptors.

4-Thio-UTP (**IX**, Figure 2) was reported to be a very potent $P2Y_2$ receptor agonist, but the compound showed the same potency at $P2Y_4$ receptors[21] and is thus nonselective. In the present study, we modified compound **IX** by replacement of the β,γ-oxygen bridge with a β,γ-dichloromethylene (**14d**) or a β,γ-difluoromethylene (**14g**) moiety. Both modifications resulted in low potency at $P2Y_4$ receptors (EC_{50} = 7.87 μM for **14d** and 9.3 μM for **14g**) and at $P2Y_6$ receptors (EC_{50} = 8.1 μM for **14d** and 7.0 μM for **14g**). At $P2Y_2$ receptors, the dichloromethylene modification (in compound **14d**) was better tolerated than at the other subtypes and yielded an EC_{50} value of 1.81 μM. The best result was obtained with the difluoromethylene modification (in compound **14g**) exhibiting an EC_{50} value at $P2Y_2$ receptors of 0.134 μM (Table 3 and Figure 3C) and showing more than 60-fold selectivity versus $P2Y_4$ and $P2Y_6$ receptors. Compound **14g** is a potent, enzymatically stable, and subtype-selective $P2Y_2$ receptor agonist. Further base modifications of **14g** may lead to even more potent $P2Y_2$ receptor agonists.

γ-Phenylamino-UTP Derivative. The relatively small modification of the phosphate chain via replacement of one of the P_γ oxygen atoms in UTP or of the P_β oxygen atoms of UDP by a sulfur atom was reported to produce potent and enzymatically stable agonists for $P2Y_2$, $P2Y_4$, or $P2Y_6$ receptors.[42] In the

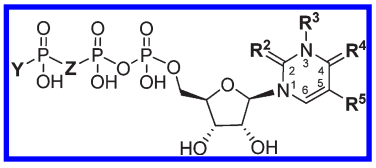
Table 2. Potency of Uridine Monophosphate Derivatives as Agonists at Human $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors



				EC_{50} ± SEM (μM) IP accumulation human recombinant P2Y receptors expressed in 1321N1 astrocytoma cells ($n \geq 3$)[a]		
compd	X	R^2	R^4	$P2Y_2$	$P2Y_4$	$P2Y_6$
UMP	O		O	>500[18]	>500[18]	>500[18]
13a	S	methyl	O	2.9[b]	>100	>100
13b	S	ethyl	O	1.5[b]	4.33 ± 1.17	>100
13c	S	propyl	O	4.8[b]	>100	6.2[b]
13d	S	benzyl	O	≥100[b]	>100	>100
13e	S	phenylethyl	O	1.3[b]	>100	>100
13f	O	methyl	O	9.9[b]	>100	9.8
13g	NH	H	O	>100	4.98 ± 1.17	>100
13h	NH	methyl	O	>100	>100	>100
13i	O		S	>100	>100	>100

[a] Data calculated from full dose−response curve unless otherwise noted. [b] Estimated EC_{50} value based on four data points ($n = 3$), SEM < 10%.

Table 3. Potency of Uridine Triphosphate Analogs as Agonists at Human $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors



							EC_{50} ± SEM (μM) IP accumulation human recombinant P2Y receptors expressed in 1321N1 astrocytoma cells ($n \geq 3$)[a]		
compd	R^2	R^3	R^4	R^5	Z	Y	$P2Y_2$	$P2Y_4$	$P2Y_6$
UTP	O	H	O	H	O	OH	0.0179 ± 0.005	0.0884 ± 0.027	ND,[b] 6.0, >10.0[6,41]
5-Br-UTP	O	H	O	Br	O	OH	0.347[18]	3.46[18]	0.291[18]
5-Br-β,γ-CCl_2-UTP	O	H	O	Br	CCl_2	OH	0.354[18]	3.99[18]	0.120[18]
2-thio-UTP	S	H	O	H	O	OH	0.050[18]	1.77[18]	~1.5[18]
4-thio-UTP	O	H	S	H	O	OH	0.026[21]	0.023[42]	ND[b]
14a	O	H	O	H	CCl_2	OH	0.612 ± 0.17	≥100[c]	6.2[c]
14b	O	phenacyl	O	H	CCl_2	OH	0.826 ± 013	7.3[c]	0.142 ± 0.001
14c	S	H	O	H	CCl_2	OH	0.794 ± 0.080 (2.51)[40]	3.85 ± 1.31 (≫10)[40]	7.7[c] (>10)[40]
14d	O	H	S	H	CCl_2	OH	1.81 ± 0.39	7.87 ± 0.35	8.1[c]
14e	O	H	O	H	CF_2	OH	4.9[c] (8.92)[7]	> 100	2.63 ± 0.47
14f	O	phenacyl	O	H	CF_2	OH	1.2[c]	3.93 ± 0.42	1.46 ± 0.06
14g	O	H	S	H	CF_2	OH	0.134 ± 0.02	9.3[c]	7.0[c]
16	O	H	O	Br	O	NH-phenyl	1.8[c]	> 100	6.1[c]

[a] Data calculated from full dose−response curve unless otherwise noted. [b] ND, not determined. [c] Estimated EC_{50} value based on four data points ($n = 3$), SEM < 10%.

present study, the replacement of one of the P_γ oxygen atoms by a bulky substituent was attempted to obtain an enzymatically stable compound and at the same time to prevent its incorporation into nucleic acids (RNA). Hillmann et al.[43] recently investigated the binding sites of the agonists UTP, ATP, and dinucleotides, such as diadenosine tetraphosphate ($A_{P4}A$) and inosine uridine tetraphosphate ($I_{P4}U$), at $P2Y_2$ receptors. They localized a binding pocket for larger ligands like dinucleotides in

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the upper third part of the receptor molecule in an entry channel between TM5 and TM6. Therefore, we selected to introduce a (bulky) phenylamino moiety at the P_γ of 5-bromo-UTP **16**, which is known to be a potent agonist at the P2Y receptors under investigation, yielding phenylamino-UTP. The results showed that this modification was not tolerated by $P2Y_4$ receptors (EC_{50} > 100 μM), while it was moderately tolerated by $P2Y_6$ receptors (EC_{50} = 6.1 μM). The highest activity of **16** was obtained at $P2Y_2$ receptors, where it exhibited an EC_{50} value of 1.8 μM (Table 3). Compound **16** may serve as a starting point, which may together with further modifications in the uracil moiety, eventually lead to the discovery of more potent, stable, and subtype-selective $P2Y_2$ receptor agonists.

All investigated nucleotides were found to be full agonists at $P2Y_2$ and $P2Y_6$ receptors (Figure 3). While compounds **12a**, **12f**, and **14d** were also full agonists at $P2Y_4$ receptors, **12d**, **13b**, **13g**, **14d**, and **14f** appeared to exhibit somewhat lower efficacy indicating partial agonistic activity at the $P2Y_4$ receptor.

CONCLUSIONS

We have synthesized a large series of uracil nucleotide derivatives and analogues with substitutions or modifications in various positions of the uracil moiety and/or the phosphate groups. The new compounds were investigated for their ability to activate UDP- or UTP-activated human $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors recombinantly expressed in 1321N1 astrocytoma cells.

While UMP is inactive at all uracil nucleotide-activated P2Y receptor subtypes, several UMP derivatives substituted in the 2-position showed activity at one or more receptor subtypes. 2-(Ar)alkylthio substitution was best tolerated by the $P2Y_2$ receptor. The most potent and $P2Y_2$-selective compound was 2-phenethylthio derivative **13e** showing an EC_{50} value of 1.3 μM at $P2Y_2$ and >100-fold selectivity versus $P2Y_4$ and $P2Y_6$ receptors. Substitution of the 2-keto group in UMP by NH in **13g** (iso-CMP) resulted in a relatively potent $P2Y_4$ agonist exhibiting an EC_{50} value of 4.98 μM and >20-fold selectivity versus $P2Y_2$ ad $P2Y_6$.

In a series of modified UDP derivatives, 2-(ar)alkylthio substitution was again well tolerated by $P2Y_2$ receptors but less so by the $P2Y_4$ and particularly by the UDP-activated $P2Y_6$ receptors. In contrast to the findings with UMP analogues, replacement of the 2-keto function by NH in UDP yielded a potent and selective $P2Y_2$ agonist (**12g**, iso-CDP, EC_{50} = 0.604 μM, >100-fold selective vs $P2Y_4$ and $P2Y_6$).

In an attempt to obtain metabolically stable UTP analogues, a series of β,γ-dichloro- and β,γ-difluoro-methylene-UTP derivatives were synthesized, which contained substitutions in the uracil moiety. The triphosphate modifications were generally much better tolerated by $P2Y_2$ than by $P2Y_4$ receptors, both of which are UTP-activated receptors (e.g., compare UTP with **14a** and **14e**). Some of the phosphate-modified UTP analogues were also very potent agonists at the UDP-activated $P2Y_6$ receptor. 4-Thio-β,γ-difluoromethylene-UTP (**14g**) was the most potent $P2Y_2$ agonist of this series with an EC_{50} value of 0.134 μM and >60-fold selectivity versus $P2Y_4$ and $P2Y_6$ receptors. Because of its high potency, selectivity, and expected metabolic stability toward ectonucleotidases, in particular NTPDases, **14g** may be a useful pharmacological tool for studying $P2Y_2$ receptors. N3-Phenacyl-β,γ-dichloromethylene-UTP (**14b**) proved to be a potent $P2Y_6$ receptor agonist with high selectivity versus $P2Y_4$ (50-fold) and some selectivity versus $P2Y_2$ receptors (6-fold). Compound **14b** may be a new lead structure for the development of potent, metabolically stable, $P2Y_6$-selective agonists.

Substitution of 5-bromo-UTP with a phenylamino residue in the γ-position of the phosphate chain reduced potency at all three receptor subtypes but was best tolerated by the $P2Y_2$ receptor. Such modifications may offer a new way to obtain $P2Y_2$ receptor agonists with enhanced stability toward ectonucleotidases.

EXPERIMENTAL SECTION

All commercially available reagents were obtained from various producers (Acros, Aldrich, Fluka, Merck, and Sigma) and used without further purification. Solvents were used without additional purification or drying, unless otherwise noted. The reactions were monitored by thin layer chromatography (TLC) using aluminum sheets with silica gel 60 F_{254} (Merck). Column chromatography was carried out with silica gel 0.060−0.200 mm, pore diameter ca. 6 nm. Mass spectra were recorded on an API 2000 (Applied Biosystems, Darmstadt, Germany) mass spectrometer (turbo ion spray ion source) coupled with a Waters HPLC system (Agilent 1100) using a Phenomenex Luna 3 μ C18 column. ^{1}H, ^{31}P, and ^{13}C NMR spectra were performed on a Bruker Avance 500 MHz spectrometer. $CDCl_3$, DMSO-d_6, MeOD-d_4, or D_2O were used as solvents as indicated below. ^{31}P NMR spectra were recorded at room temperature; orthophosphoric acid (85%) was used as an external standard. Shifts are given in ppm relative to the external standard (^{31}P NMR) or relative to the remaining protons of the deuterated solvents used as internal standard (^{1}H, ^{13}C). Elemental microanalyses were performed on a VarioEL apparatus at the Pharmaceutical Institute, University of Bonn. Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. The purity of the prepared nucleosides was checked by TLC on silica gel 60 F_{254} (Merck) aluminum plates, using chloroform:methanol (20:1) or dichloromethane:methanol (9:1, 5:1, or 3:1) as the mobile phase. The purity of the prepared nucleotides was confirmed by HPLC on an RP-HPLC column (Knauer 20 mm i.d., Eurospher-100 C18). The column was eluted with a solvent gradient of 0−10% of acetonitrile in 50 mM aqueous NH_4HCO_3 buffer for 30 min at a flow rate of 5 mL/min. UV absorption was detected at 254 nm. The purity of the nucleotide samples was controlled in a second chromatography system by dissolving 1 mg/mL of compound in H_2O:MeOH (1:1) containing 2 mM ammonium acetate. A sample of 10 μL was injected into an HPLC instrument (Agilent 1100) using a Phenomenex Luna 3 μ C18 column. Elution was performed with a gradient of water:methanol (containing 2 mM ammonium acetate) from 90:10 to 0:100 for 30 min at a flow rate of 250 μL/min, starting the gradient after 10 min. UV absorption was detected from 190 to 400 nm using a diode array detector. The purity of the products was generally ≥95%.

The syntheses of nucleosides **2**,[18] **3**,[18] **4**,[27] **5a**,[27] **6a**,[27] **7**,[27] **8a**,[44] **8b**,[45,46] **10**,[29,30] and **11**[31] have previously been described and were prepared according to published procedures with minor modifications (for details of the synthesis, see the Supporting Information), while the nucleosides **5b**−**d** and **6b**−**d** are new.

General Procedure for the Synthesis of Nucleosides 5a−d. 2-Thio-1-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)-(3*H*)-pyrimidine-2,4-dione (**2**,[18] 3 mmol) was dissolved in 40 mL of dichloromethane, and 7.24 mmol of triethylamine was added followed by the addition of 7 mmol of the corresponding alk(ar)yl halide. The reaction mixture was refluxed for 24−36 h, and the completion of the reaction was determined by TLC (chloroform:methanol 20:1). The solvent was evaporated under reduced pressure, and the residue was extracted with ethyl acetate and washed with water. The organic layer was separated, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was washed twice with 30 mL of tertiary butylmethyl ether each, followed by dissolving it in 3 mL of acetone. Excess *n*-hexane (ca. 20 mL) was added, and the formed precipitate was separated and washed by petroleum ether (bp 40−60 °C) to give the pure product.

General Procedure for the Synthesis of Nucleosides 6a−d. To a stirred solution of 2 mmol of nucleoside **5a**−**d** in 5 mL of methanol,

10 mL of a previously prepared solution of methanol saturated with ammonia was added, and the reaction mixture was stirred for 30 min to 1 h at room temperature. The completion of the reaction was controlled by TLC (dichloromethane:methanol 9:1). The methanol was removed by evaporation, and the formed benzoic acid methyl ester was extracted with diethyl ether. The residue was taken up in distilled water, and the water was evaporated under reduced pressure. The residue was subsequently purified by column chromatography (dichloromethane:methanol 5:1). The appropriate fractions were collected, pooled, and evaporated under reduced pressure. The product was recrystallized by dissolving it in 2 mL of acetone and addition of excess *n*-hexane to yield the pure product.

General Procedure for the Synthesis of Nucleotides (12a, c−h and 14a−g). Lyophilized nucleoside (1 mmol) was dissolved in 5 mL of triethyl phosphate (dried over 10 Å molecular sieves). The mixture was stirred at room temperature under argon and then cooled to 4 °C. Dry 1,8-bis(dimethylamino)naphthaline (proton sponge, 1.5 mmol) was added, followed by 1.3 mmol of $POCl_3$ 5 min later. After several hours of stirring at 0 °C, tri-*n*-butylamine (0.72 mmol) was added to the solution followed by 10 mL (5 mmol) of 0.5 M bis-(tri-*n*-butylammonium) phosphate solution in DMF (for compounds **12a,c−h**) or 0.5 M bis-(tri-*n*-butylammonium) di(halo)methylene bisphosphonate solution in DMF (for compounds **14a−g**). After 2−5 min, the mixture was poured into a cold 0.5 M aqueous TEAC solution (30 mL, pH 7.5) and stirred at 0 °C for several minutes. The solution was allowed to reach room temperature upon stirring and then left standing for 1 h. Triethyl phosphate was extracted with tertiary butylmethyl ether, and the aqueous solution was evaporated and lyophilized to yield glassy colorless oils. The reactions were controlled by TLC using a freshly prepared solvent system [2-propanol:NH_4OH (25% NH_3 in water):water = 6:3:1]. TLC plates were dried before UV absorption was detected, and the plates were subsequently sprayed with a phosphate reagent.[16,18,20,33]

Synthesis of γ-Phenylamino-5-bromouridine 5′-triphosphate (16). To a stirred solution of 5-bromouridine (0.5 mmol) in a mixture of anhydrous DMF (1.0 mL) and anhydrous pyridine (0.25 mL) at 0 °C under argon, a freshly prepared solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (0.5 mmol) in anhydrous DMF (1.0 mL) was added. After the solution was stirred at room temperature for 1 h, tributylamine (0.4 mL) was added, followed by the addition of 0.5 M bis(tri-*n*-butylammonium)diphosphate (3 mmol) in anhydrous DMF. The resulting mixture was stirred for 1 h and cooled with ice, and iodine (1.0 mmol) was subsequently added. After the mixture was stirred at room temperature for 1 h, aniline (0.5 mmol in 1.0 mL of DMF) was added. The reaction mixture was stirred at room temperature for 2 h, cooled with ice, quenched with water, and stirred at room temperature for 30 min and subsequently lyophilized.

Purification of Nucleotides. *Ion Exchange Chromatography.* The crude nucleotides were purified by ion exchange chromatography on an fast protein liquid chromatography (FPLC) instrument (ÄKTA FPLC, from Amersham Biosciences) with an XK 26 mm/20 cm length column (Pharmacia) using Sephadex DEAE A-25 gel HCO_3^- form swelled in a 1 M solution of TEAC at 4 °C. After equilibration of the column with deionized water, the crude product was dissolved in 2 mL of aqueous TEAC. The column was washed with deionized water, followed by a solvent gradient of 0−800 mM TEAC buffer using approximately 2000 mL of solvent to elute the mono-, di-, and triphosphates. Fractions were collected, and appropriate fractions were pooled, diluted in water, and lyophilized.

Preparative HPLC. Lyophilized nucleotides obtained from FPLC purification were dissolved in 5 mL of deionized water and injected into an RP-HPLC column (Knauer 20 mm ID, Eurospher-100 C18). The column was eluted with a solvent gradient of 0−20% of acetonitrile in 50 mM aqueous NH_4HCO_3 buffer for 25−35 min at a flow rate of 5 mL/min. The UV absorption was detected at 254 nm. Fractions were collected, and appropriate fractions were pooled, diluted with water, and lyophilized several times to remove the NH_4HCO_3, yielding the pure nucleotides as white powders.

Most nucleotides were stored and tested as tributylammonium salts due to enhanced stability in comparison with the sodium salts. The tributylammonium cation was previously shown to have no effect on the receptors.[18]

2-Propylthio-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidine-4-one (***5b***). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.81−0.84 (t, 3H, *J* = 7.36 Hz), 1.50−1.55 (m, 2H), 3.04−3.07 (t, 2H, *J* = 6.97 Hz), 4.75−4.77 (m, 2H), 4.86−4.88 (q, 1H, *J* = 4.20 Hz), 5.84−5.85 (m, 2H), 5.94−5.95 (d, 1H, *J* = 7.89 Hz), 6.33−6.34 (d, 1H, *J* = 4.99 Hz), 7.4−8.0 (m, 16H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 12.96, 21.73, 33.19, 63.71, 70.35, 73.60, 79.94, 89.55, 109.59, 128.25−134.19, 139.58, 161.35, 164.46, 164.73, 165.57, 166.20. mp 130−132 °C. Yield, 77.7%.

2-Benzylthio-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidine-4-one (***5c***). ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.3−4.4 (m, 2H), 4.74−4.75 (m, 2H), 4.83−4.86 (q, 1H, *J* = 4.85 Hz), 5.82−5.83 (m, 2H), 5.98−5.99 (d, 1H, *J* = 7.62 Hz), 6.30−6.31 (d, 1H, *J* = 4.99 Hz), 7.21−8.0 (m, 21H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.27, 63.79, 70.34, 73.64, 80.05, 89.48, 109.84, 127.56−136.23, 139.55, 160.94, 164.48, 164.73, 165.57, 166.14. mp 183−186 °C. Yield, 62.9%.

2-Phenylethylthio-1-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)pyrimidine-4-one (***5d***). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.7−2.8 (m, 2H), 3.31−3.34 (t, 2H, *J* = 7.62 Hz), 4.73−4.78 (m, 2H), 4.84−4.87 (q, 1H, *J* = 5.08 Hz), 5.82−5.87 (m, 2H), 5.96−5.98 (d, 1H, *J* = 7.89 Hz), 6.30−6.32 (d, 1H, *J* = 4.73 Hz), 7.15−8.0 (m, 21H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 32.64, 34.41, 63.68, 70.29, 73.61, 79.86, 89.59, 109.66, 126.50−136.23, 139.74, 161.14, 164.49, 164.70, 165.56, 166.21. mp 137−140 °C. Yield, 54.2%.

2-Propylthio-1-β-D-ribofuranosylpyrimidine-4-one (***6b***). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.95−0.98 (t, 3H, *J* = 7.36 Hz), 1.61−1.68 (six, 2H, *J* = 7.19 Hz), 3.04−3.10 (m, 2H), 3.55−3.65 (m, 2H), 3.91−3.93 (q, 1H, *J* = 3.15 Hz), 3.97−4.00 (q, 1H, *J* = 3.85 Hz), 4.07−4.10 (q, 1H, *J* = 5.43 Hz), 5.15−5.17 (t, 1H, *J* = 4.99 Hz), 5.19−5.20 (d, 1H, *J* = 4.73 Hz), 5.55−5.56 (d, 1H, *J* = 6.05 Hz), 5.70−5.71 (d, 1H, *J* = 5.52 Hz), 5.92−5.94 (d, 1H, *J* = 7.89 Hz), 8.04−8.05 (d, 1H, *J* = 7.89 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 13.26, 21.75, 33.08, 60.88, 70.16, 74.70, 86.06, 91.40, 109.11, 139.51, 162.01, 166.69. mp 138−141 °C. Yield, 21.52%.

2-Benzylthio-1-β-D-ribofuranosylpyrimidine-4-one (***6c***). ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.54−3.63 (m, 2H), 3.89−3.91 (q, 1H, *J* = 3.06 Hz), 3.96−3.98 (q, 1H, *J* = 3.59 Hz), 4.06−4.09 (q, 1H, *J* = 5.43 Hz), 4.37−4.44 (m, 2H), 5.15−5.17 (t, 1H, *J* = 4.99 Hz), 5.18−5.19 (d, 1H, *J* = 4.99 Hz), 5.54−5.56 (d, 1H, *J* = 6.05 Hz), 5.64−5.65 (d, 1H, *J* = 5.52 Hz), 5.96−5.98 (d, 1H, *J* = 7.62 Hz), 7.25−7.44 (m, 5H), 8.05−8.07 (d, 1H, *J* = 7.89 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.18, 60.87, 70.15, 74.69, 86.16, 91.49, 109.37, 127.61, 128.63, 129.42, 136.49, 139.52, 161.50, 166.61. mp 134−137 °C. Yield, 21.5%.

2-Phenylethylthio-1-β-D-ribofuranosylpyrimidine-4-one (***6d***). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.93−2.96 (t, 2H, *J* = 7.56 Hz), 3.31−3.43 (m, 2H), 3.55−3.64 (m, 2H), 3.90−3.92 (q, 1H, *J* = 3.15 Hz), 3.98−3.99 (q, 1H, *J* = 3.62 Hz), 4.07−4.10 (q, 1H, *J* = 5.56 Hz), 5.16−5.18 (t, 1H, *J* = 4.88 Hz), 5.19−5.20 (d, 1H, *J* = 4.72 Hz), 5.54−5.55 (d, 1H, *J* = 5.99 Hz), 5.65−5.66 (d, 1H, *J* = 5.35 Hz), 5.95−5.97 (d, 1H, *J* = 7.88 Hz), 7.20−7.31 (m, 5H), 8.05−8.07 (d, 1H, *J* = 7.56 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ 32.51, 34.34, 60.90, 70.19, 74.71, 86.12, 91.42, 109.20, 126.55, 128.54, 128.70, 139.55, 139.96, 161.79, 166.69. mp 150−153 °C. Yield, 27.54%.

2-Methylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (***12a***). ^{1}H NMR (500 MHz, D_2O): δ 2.22 (s, 3H), 3.97−4.09 (m, 2H), 4.29 (m, 1H), 4.41−4.45 (m, 2H), 5.93 (d, 1H, *J* = 4.41 Hz), 6.61 (m, 1H), 7.85 (d, 1H, *J* = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O): δ −8.37. LC/ESI-MS: negative mode 433 ($[M - H]^-$).

2-Propylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (***12c***). ^{1}H NMR (500 MHz, D_2O + MeOD): δ 0.90 (t, 3H, *J* = 7.40 Hz), 1.64 (m, 2H), 3.03 (t, 2H, *J* = 7.40 Hz), 4.27 (m, 3H), 4.44 (m, 2H), 5.48 (d, 1H, *J* = 5.67 Hz), 6.06−6.10 (m, 1H), 8.06 (d, 1H, *J* = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O + MeOD): δ −9.38. LC/ESI-MS: negative mode 461 ($[M - H]^-$), positive mode 463 ($[M + H]^+$).

2-Benzylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (**12d**). ^{1}H NMR (500 MHz, D_2O): δ 3.25 (m, 2H), 4.43 (m, 1H), 4.49 (m, 1H), 4.58 (m, 1H), 4.74 (m, 2H), 6.19 (m, 1H), 6.53 (m, 1H), 7.53−7.72 (m, 5H), 8.43 (s, 1H). ^{31}P NMR (202 MHz, D_2O): δ −8.63 (m), −11.11 (m). ^{13}C NMR (125 MHz, D_2O): δ 38.64, 67.01, 73.54, 77.83, 86.78, 94.56, 101.32, 130.76, 131.66, 132.15, 139.13, 144.35, 150.93, 164.05, 174.63. LC/ESI-MS: negative mode 509 ($[M - H]^-$), positive mode 511 ($[M + H]^+$).

2-Phenylethylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (**12e**). ^{1}H NMR (500 MHz, D_2O): δ 3.06−3.10 (m, 2H), 3.53−3.58 (m, 1H), 3.61−3.67 (m, 1H), 4.26−4.32 (m, 2H), 4.34 (t, 2H, J = 4.57 Hz), 4.41 (t, 1H, J = 4.88 Hz), 6.0 (d, 1H, J = 4.41 Hz), 6.28 (d, 1H, J = 7.56 Hz), 7.25−7.35 (m, 5H), 8.20 (d, 1H, J = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O) δ −8.78 (m), −11.07 (m). ^{13}C NMR (125 MHz, D_2O): δ 13.38, 37.19, 66.96, 71.88, 77.83, 86.63, 94.53, 111.76, 129.63, 131.42, 132.01, 142.43, 144.23, 167.22, 174.52. LC/ESI-MS: negative mode 523 ($[M - H]^-$), positive mode 525 ($[M + H]^+$).

2-Methoxy-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (**12f**). ^{1}H NMR (500 MHz, D_2O + MeOD): δ 2.20 (s, 3H), 4.19−4.23 (m, 3H), 4.36 (t, 1H, J = 4.72 Hz), 4.41 (t, 1H, J = 5.04 Hz), 5.93 (m, 1H), 5.94 (d, 1H, J = 2.83 Hz), 7.98 (d, 1H, J = 8.19 Hz). ^{31}P NMR (202 MHz, D_2O + MeOD): δ −6.63 (m), −7.43 (m). ^{13}C NMR (125 MHz, D_2O + MeOD): δ 33.05, 60.25, 71.94, 76.72, 83.04, 91.51, 105.33, 136.82, 144.65, 169.17. LC/ESI-MS: negative mode 417 ($[M - H]^-$), positive mode 419 ($[M + H]^+$).

2-Amino-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (**12g**). ^{1}H NMR (500 MHz, D_2O): δ 4.05−4.13 (m, 2H), 4.22 (m, 1H), 4.25 (m, 1H), 4.37 (m, 1H), 5.63 (d, 1H, J = 6.62 Hz), 6.01 (m, 1H), 8.10 (d, 1H, J = 7.88 Hz). ^{31}P NMR (202 MHz, D_2O): δ −9.79 (m). LC/ESI-MS: negative mode 402 ($[M - H]^-$), positive mode 404 ($[M + H]^+$).

2-Methylamino-1-β-D-ribofuranosylpyrimidine-4-one-5′-diphosphate (**12h**). ^{1}H NMR (500 MHz, D_2O): δ 2.93 (s, 3H), 3.99 (m, 3H), 4.19 (m, 1H), 4.34 (m, 1H), 5.57 (d, 1H, J = 6.62 Hz), 5.95 (d, 1H, J = 7.56 Hz), 7.78 (d, 1H, J = 7.88 Hz). ^{31}P NMR (202 MHz, D_2O): δ −9.21, −9.83. LC/ESI-MS: negative mode 416 ($[M - H]^-$), positive mode 418 ($[M + H]^+$).

2-Methylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13a**). ^{1}H NMR (500 MHz, D_2O): δ 2.22 (s, 3H), 4.05−4.09 (m, 2H), 4.32 (m, 1H), 4.37 (m, 1H), 4.44 (m, 1H), 6.03 (d, 1H, J = 4.09 Hz), 6.33 (d, 1H, J = 7.56 Hz), 8.34 (d, 1H, J = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O): δ 1.06. LC/ESI-MS: negative mode 353 ($[M - H]^-$), positive mode 355 ($[M + H]^+$).

2-Ethylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13b**). ^{1}H NMR (500 MHz, D_2O): δ 1.41 (t, 3H, J = 7.40 Hz), 3.35 (q, 2H, J = 7.35 Hz), 4.10 (m, 2H), 4.27 (m, 1H), 4.34 (t, 1H, J = 4.88 Hz), 4.38 (t, 1H, J = 5.20 Hz), 5.96 (d, 1H, J = 8.19 Hz), 5.98 (d, 1H, J = 5.04 Hz), 8.02 (d, 1H, J = 7.88 Hz). ^{31}P NMR (202 MHz, D_2O): δ 0.63. LC/ESI-MS: negative mode 367 ($[M - H]^-$), positive mode 369 ($[M + H]^+$).

2-Propylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13c**). ^{1}H NMR (500 MHz, D_2O): δ ^{1}H NMR (500 MHz, D_2O + MeOD): δ 0.99 (t, 3H, J = 7.40 Hz), 1.72 (m, 2H), 3.02−3.06 (m, 2H), 4.06 (m, 2H), 4.25 (m, 1H), 4.42 (m, 2H), 5.49 (d, 1H, J = 4.41 Hz), 6.02−6.10 (m, 1H), 8.06 (d, 1H, J = 8.19 Hz). ^{31}P NMR (202 MHz, D_2O + MeOD): δ 1.97. LC/ESI-MS: negative mode 381 ($[M - H]^-$), positive mode 383 ($[M + H]^+$).

2-Benzylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13d**). ^{1}H NMR (500 MHz, D_2O + MeOD): δ 3.88−3.97 (m, 2H), 4.21 (m, 1H), 4.29−4.32 (m, 1H), 4.55 (m, 1H), 4.84 (m, 2H), 5.94 (d, 1H, J = 5.35 Hz), 6.28 (d, 1H, J = 7.88 Hz), 7.30−7.51 (m, 5H), 8.38 (d, 1H, J = 7.88 Hz). ^{31}P NMR (202 MHz, D_2O): δ 0.27. LC/ESI-MS: negative mode 429 ($[M - H]^-$), positive mode 431 ($[M + H]^+$).

2-Phenylethylthio-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13e**). ^{1}H NMR (500 MHz, D_2O): δ 3.06−3.10 (m, 2H), 3.53−3.64 (m, 2H), 3.96−4.06 (m, 2H), 4.28 (m, 1H), 4.34 (t, 1H, J = 4.72 Hz), 4.37 (t, 1H, J = 4.57 Hz), 6.0 (d, 1H, J = 4.72 Hz), 6.29 (d, 1H, J = 7.88 Hz), 7.24−7.34 (m, 5H), 8.35 (d, 1H, J = 7.88 Hz). ^{31}P NMR (202 MHz, D_2O) δ 2.84. ^{13}C NMR (125 MHz, D_2O): δ 13.39, 37.17, 65.54, 72.53, 77.99, 94.62, 100.58, 111.83, 129.62, 131.42, 132.0, 142.45, 144.70, 163.24, 173.56. LC/ESI-MS: negative mode 443 ($[M - H]^-$), positive mode 445 ($[M + H]^+$).

2-Methoxy-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13f**). ^{1}H NMR (500 MHz, D_2O): δ 2.30 (s, 3H), 3.90−3.98 (m, 2H), 4.23 (m, 1H), 4.33 (t, 1H, J = 4.57 Hz), 4.40 (t, 1H, J = 5.04 Hz), 5.97−5.98 (m, 2H), 8.09 (d, 1H, J = 8.19 Hz). ^{31}P NMR (202 MHz, D_2O): δ 2.79. LC/ESI-MS: negative mode 337 ($[M - H]^-$), positive mode 339 ($[M + H]^+$).

2-Methylamino-1-β-D-ribofuranosylpyrimidine-4-one-5′-monophosphate (**13h**). ^{1}H NMR (500 MHz, D_2O): δ 2.95 (s, 3H), 3.94−3.99 (m, 2H), 4.31 (t, 1H, J = 2.20 Hz), 4.36−4.38 (m, 1H), 4.58−4.61 (m, 1H), 5.53 (d, 1H, J = 7.25 Hz), 5.98 (d, 1H, J = 7.88 Hz), 7.80 (d, 1H, J = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O): δ 1.33. LC/ESI-MS: negative mode 336 ($[M - H]^-$), positive mode 338 ($[M + H]^+$).

3-Phenacyl-5′-uridylic Acid (1,1-Chloro-1-phosphonomethyl-1-phosphonyl)anhydride (**14b**). ^{1}H NMR (500 MHz, MeOD): δ 4.18 (m, 1H), 4.29−4.37 (m, 3H), 4.44 (q, 1H, J = 4.30 Hz), 5.44 (s, 2H), 6.02−6.05 (m, 2H), 7.57−8.22 (m, 5H), 8.10 (d, 1H, J = 8.19 Hz). ^{31}P NMR (202 MHz, MeOD): δ 6.38, −1.89, −10.72. ^{13}C NMR (125 MHz, MeOD): δ 8.70, 43.30, 60.30, 66.37, 71.35, 76.11, 85.37, 91.15, 102.65, 129.42, 130.27, 135.29, 136.60, 141.76, 152.98, 159.32. LC/ESI-MS: negative mode 670, 670 ($[M - H]^-$), positive mode 686, 689 ($[M + NH_4^+ + H]^+$).

4-Thio-5′-uridylic Acid (1,1-Chloro-1-phosphonomethyl-1-phosphonyl)anhydride (**14d**). ^{1}H NMR (500 MHz, D_2O): δ 3.98−4.11 (m, 2H), 4.26 (q, 1H, J = 2.31 Hz), 4.33 (t, 1H, J = 4.88 Hz), 4.38 (t, 1H, J = 4.88 Hz), 5.93 (d, 1H, J = 4.41 Hz), 6.65 (d, 1H, J = 7.56 Hz), 7.97 (d, 1H, J = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O): δ 10.09, 1.21, −7.98. LC/ESI-MS: negative mode 565 ($[M - H]^-$).

3-Phenacyl-5′-uridylic Acid (1,1-Difluoro-1-phosphonomethyl-1-phosphonyl)anhydride (**14f**). ^{1}H NMR (500 MHz, MeOD): δ 4.18 (m, 1H), 4.31−4.34 (m, 3H), 4.41 (t, 1H, J = 4.41 Hz), 5.44 (d, 2H, J = 1.26 Hz), 6.02−6.08 (m, 2H), 7.57−8.09 (m, 5H), 8.10 (d, 1H, J = 7.25 Hz). ^{31}P NMR (202 MHz, MeOD): δ 2.66, −5.51, −10.99. ^{13}C NMR (125 MHz, MeOD): δ 11.82, 43.41, 66.44, 71.72, 76.11, 85.40, 88.26, 91.15, 102.73, 129.43, 130.28, 135.29, 136.59, 141.69, 152.99, 164.94. LC/ESI-MS: negative mode 635 ($[M - H]^-$).

4-Thio-5′-uridylic Acid (1,1-Difluoro-1-phosphonomethyl-1-phosphonyl)anhydride (**14g**). ^{1}H NMR (500 MHz, D_2O): δ 4.25−4.30 (m, 3H), 4.37−4.40 (m, 2H), 5.95 (d, 1H, J = 4.09 Hz), 6.66 (d, 1H, J = 7.88 Hz), 7.85 (d, 1H, J = 7.56 Hz). ^{31}P NMR (202 MHz, D_2O): δ 3.13, −2.07, −10.90 (d, J = 29.53 Hz). ^{13}C NMR (125 MHz, D_2O): δ 67.56, 72.21, 76.95, 82.87, 86.25, 91.74, 116.79, 139.57, 152.20, 193.66. LC/ESI-MS: negative mode 533 ($[M - H]^-$).

5-Bromo-1-β-D-ribofuranosyl(3H)pyrimidine-2,4-dione 5′-(γ-phenylamino)triphosphate (**16**). ^{1}H NMR (500 MHz, D_2O + MeOD + NaOD): δ 3.34−3.36 (m, 2H), 3.94 (t, 1H, J = 5.20 Hz), 4.05−4.08 (m, 2H), 5.80 (d, 1H, J = 5.04 Hz), 6.92−7.31 (m, 5H), 7.92 (s, 1H). ^{31}P NMR (202 MHz, D_2O + MeOD + NaOD): δ −9.38 (d, J = 19.68 Hz), −10.58 (d, J = 17.22 Hz), −21.74 (t, J = 18.45 Hz). ^{13}C NMR (125 MHz, D_2O + MeOD + NaOD): δ 68.72, 73.43, 78.05, 86.10, 93.09, 101.50, 120.26, 120.32, 123.13, 131.99, 142.72, 161.90, 174.17. LC/ESI-MS: negative mode 636 and 639 ($[M - H]^-$), positive mode 655 ($[M + NH_4^+ + H]^+$).

Determination of IP Accumulation at $P2Y_2$, $P2Y_4$, and $P2Y_6$ Receptors. Assays were performed using 1321N1 astrocytoma cells recombinantly expressing the human $P2Y_2$, $P2Y_4$, or $P2Y_6$ receptor subtype, respectively, as previously described.[18,37] A scintillation proximity assay in a 96-well plate format was applied[18] determining IP production induced by activation of the G_q-coupled receptors by the test compounds. Data were analyzed with PRISM 4.0 (GraphPad Software Inc., San Diego, CA). Three separate experiments were performed, and each data point was determined in triplicate.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic procedures, ^{1}H and ^{13}C NMR spectral data for compounds **2**−**4**, **5a**, **6a**, **7**, **8a**,**b**, **10**, **11**, **13g**,**i**, and **14a**,**c**,**e**, and elemental analyses for nucleosides **4**, **5a**−**d**, **6a**−**d**, and **7**, as well as results of initial screening of the test compounds at the P2Y receptor subtypes, and HPLC purity data for selected nucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

Notes

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Notes

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■ ABBREVIATIONS USED

DMF, *N*,*N*-dimethylformamide; HMDS, hexamethyldisilazane; isoCDP, isocytidine diphosphate; IP, inositol phosphates; NPP, nucleotide pyrophosphatase; NTPDase, nucleoside triphosphate diphosphohydrolase; SAR, structure−activity relationships; TEA, triethylamine; TEAC, triethylammonium hydrogencarbonate

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