Structure−Activity Relationship of Uridine 5′-Diphosphoglucose Analogues as Agonists of the Human $P2Y_{14}$ Receptor

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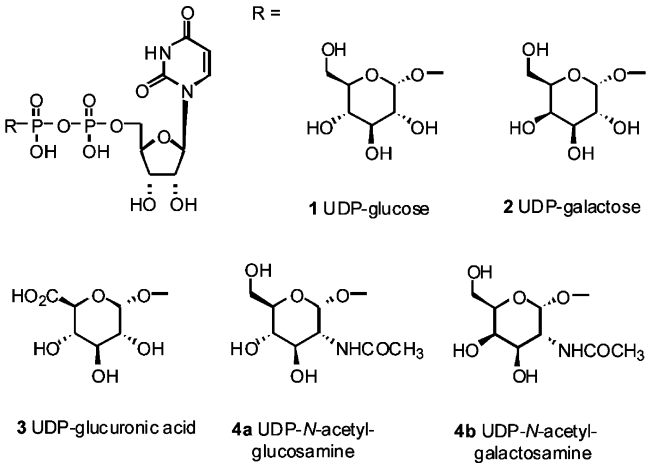
UDP-glucose (UDPG) and derivatives are naturally occurring agonists of the G_i protein-coupled $P2Y_{14}$ receptor, which occurs in the immune system. We synthesized and characterized pharmacologically novel analogues of UDPG modified on the nucleobase, ribose, and glucose moieties, as the basis for designing novel ligands in conjunction with modeling. The recombinant human $P2Y_{14}$ receptor expressed in COS-7 cells was coupled to phospholipase C through an engineered Gα-q/i protein. Most modifications of the uracil or ribose moieties abolished activity; this is among the least permissive P2Y receptors. However, a 2-thiouracil modification in **15** (EC_{50} 49 ± 2 nM) enhanced the potency of UDPG (but not UDP-glucuronic acid) by 7-fold. 4-Thio analogue **13** was equipotent to UDPG, but *S*-alkylation was detrimental. Compound **15** was docked in a rhodposin-based receptor homology model, which correctly predicted potent agonism of UDP-fructose, UDP-mannose, and UDP-inositol. The hexose moiety of UDPG interacts with multiple H-bonding and charged residues and provides a fertile region for agonist modification.

Introduction

The nucleotide-activated P2 receptors occur in two subfamilies of plasma membrane receptors. P2Y receptor subtypes include eight members, the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors, which couple to G proteins. The seven subtypes of ligand-gated cation channels that comprise the P2X receptor subfamily are denoted $P2X_1$−$P2X_7$. All of these subtypes have been cloned and functionally characterized.[1−10] The family of P2Y receptors can be divided into two subgroups. A $P2Y_1$-like subgroup ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$) is G_q-coupled and stimulates phospholipase C (PLC^a), and a $P2Y_{12}$-like subgroup ($P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) is G_i coupled and inhibits adenylyl cyclase.[11] P2Y receptors are distributed in a broad range of tissues and are of interest as therapeutic targets, including antithrombotic therapy, modulation of the immune system and cardiovascular system, inflammation, pain, diabetes, and treatment of cystic fibrosis and other pulmonary diseases.[12−15] P2X receptors are activated principally by adenine nucleotides, and P2Y receptors are activated by adenine and/or uracil nucleotides. For example, the $P2Y_2$ receptor is activated by both uridine 5′-triphosphate (UTP) and adenosine 5′-triphosphate (ATP), the $P2Y_4$ receptor by UTP, and the $P2Y_6$ receptor by uridine 5′-diphosphate (UDP).[16]

The $P2Y_{14}$ receptor is distributed in various tissues including placenta, adipose, stomach, intestine, brain, spleen, thymus, lung, and heart.[3] Potent and selective ligands, currently lacking, are needed for pharmacological studies. UDP-glucose (**1**, UDPG), UDP-galactose (**2**), and UDP-*N*-acetylglucosamine (**4a**) are naturally occurring agonists of the $P2Y_{14}$ receptor (Chart 1). The precise physiological function of the $P2Y_{14}$ receptor has not yet been clearly established, although it appears to be involved in immune function.[17] In contrast to other P2Y receptors, the $P2Y_{14}$ receptor is not activated by uridine 5′-di- or triphosphates or by adenine nucleotides.[21] Extracellular release of UDPG has been demonstrated, and UDPG does not activate any other P2Y receptor.[17−23] The SAR (structure−activity relationship) of synthetic nucleotides for activation of the human $P2Y_{14}$ receptor has not previously been probed in a systematic fashion. Thus, we synthesized novel analogues of UDPG and characterized their pharmacological activities at the recombinant human $P2Y_{14}$ receptor. The nucleobase, ribose, and glucose moieties were modified in this study. Molecular modeling was carried out to predict sites of interaction of the ligands with the $P2Y_{14}$ receptor and to provide recognition hypotheses to be used in the design of additional analogues.

Chart 1. Structures of Three Naturally Occurring UDP-Sugars (**1**, **2**, and **4a**) and Related Derivatives That Activate the $P2Y_{14}$ Receptor



Results and Discussion

Chemical Synthesis. Analogues of UDPG (**1**) with modifications in the ribose ring, uracil moiety, and sugar moiety, as well as dinucleotides (Table 1), were synthesized. The ribose ring

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[a] Abbreviations: DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; MCMM, Monte Carlo multiple minimum; PLC, phospholipase C; SAR, structure−activity relationship; UDPG, UDP-glucose; MD, molecular dynamics.

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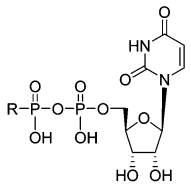
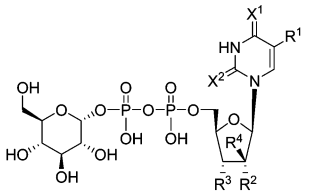
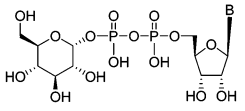
Table 1. In Vitro Pharmacological Data for UDPG, **1**, and Its Analogues in the Stimulation of PLC in COS-7 Cells Expressing Recombinant Human P2Y$_{14}$ Receptors



1 – 4, 25 – 27, 29a

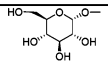
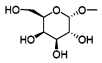
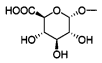
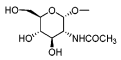
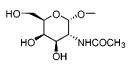
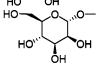
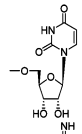
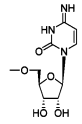


5 – 11, 13 – 18, 22 – 24

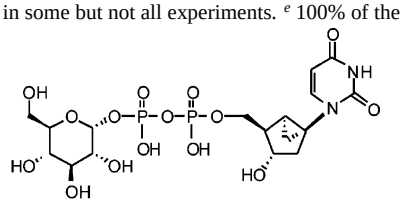


19 – 21

unless noted: R = glucose, R^1; R^4 = H; R^2, R^3 = OH; X = O; B = uracil

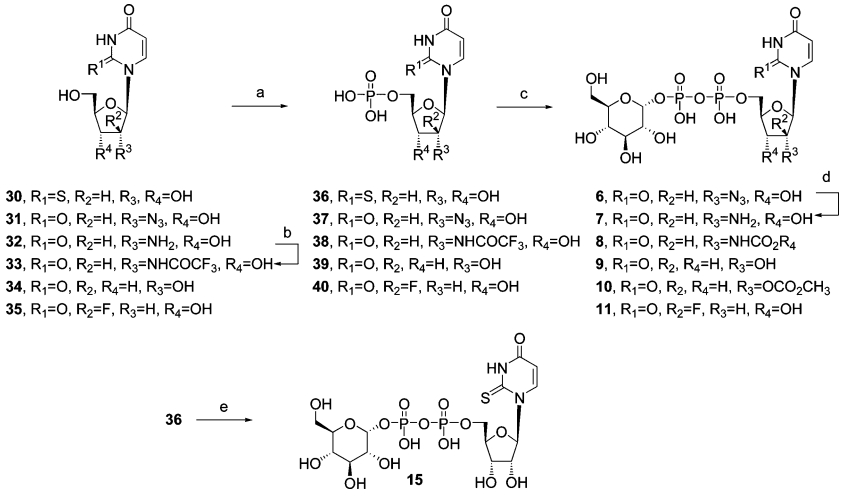
Compound	Modification	R = or other substitution	EC$_{50}$ at hP2Y$_{14}$ receptor, μM[a]
1	(= UDP-glucose)		0.35 ± 0.12
2	(= UDP-galactose)		0.67 ± 0.09[d]
3	(= UDP-glucuronic acid)		0.37 ± 0.07[d]
4a	(= UDP-*N*-acetylglucos-amine)		4.38 ± 1.05[e]
4b	(= UDP-*N*-acetylgalactos-amine)		0.81 ± 0.09[d]
Ribose-modified			
5	2′-deoxy	R^2 = H	NE
6	2′-deoxy-2′-azido	R^2 = N$_3$	NE
7	2′-deoxy-2′-amino	R^2 = NH$_2$	NE
8	cyclic-2′-deoxy-2′-aminocarbonyl-3′-O	R^2, R^3 = cyclic-NHCOO	NE
9	3′-deoxy	R^3 = H	NE
10	2′,3′-dideoxy-2′-methoxy-carbonyl	R^2 = CH$_3$OCOO, R^3 = H	NE
11	2′-fluoro-2′-deoxyara	R^2 = H, R^4 = F	NE
12	(S)-mc-2′-deoxy	f	NE
Base-modified			
13[b]	4-thio	X^1 = S	0.29 ± 0.16[e]
14	4-methylthio	X^1 = SCH$_3$	>10[c]
15[b]	2-thio	X^2 = S	0.049 ± 0.002[e]
16	5-iodo	R^1 = I	NE
17	5-azido	R^1 = N$_3$	NE
18	5-amino	R^1 = NH$_2$	NE
19	Base = C		NE
20	Base = A		NE
21	Base = G		NE
Base-and ribose modified			
22	2′-deoxy-C	R^2 = H, X^1 = NH	NE
23	2′-deoxy-T	R^1 = CH$_3$, R^2 = H	NE
24	2′-deoxy-5-F-U	R^1 = F, R^2 = H	NE
Glucose-modified			
25	UDP-inositol		1.88 ± 1.1[e]
26	UDP-fructose		0.88 ± 0.21[e]
27	UDP-mannose		0.91 ± 0.15[e]
28	2-thio-UDP-glucuronic acid		0.42 ± 0.19
Dinucleotides			
29a	Up$_2$U		NE
29b	Cp$_2$C		NE

[a] COS-7 cells were transiently transfected with expression vectors for both the human P2Y$_{14}$ receptor and an engineered Gα-q/i protein that allows coupling of Gi-coupled receptors to activation of PLC.[35] Agonist potencies were calculated using a four-parameter logistic equation and the GraphPad software package (GraphPad, San Diego, CA). EC$_{50}$ values (mean ± standard error) represent the concentration at which 50% of the maximal effect is achieved. $N = 3$, unless noted. NE = no effect at 100 μM. [b] **13**, MRS2670; **15**, MRS2690 ($n = 4$). [c] Less than or equal to 50% of the maximal effect at 10 μM. [d] Exhibited a maximal effect somewhat less than **1** in some but not all experiments. [e] 100% of the maximal effect of **1** was achieved. [f] Compound **12** =



NE - no effect at 100 μM.

Scheme 1. Synthesis of UDPG Analogues Substituted on the Ribose Moiety or the 2 Position of the Uracil Moiety[a]



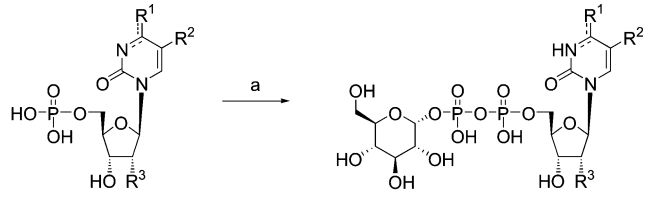
30, R_1=S, R_2=H, R_3, R_4=OH
31, R_1=O, R_2=H, R_3=N_3, R_4=OH
32, R_1=O, R_2=H, R_3=NH_2, R_4=OH
33, R_1=O, R_2=H, R_3=$NHCOCF_3$, R_4=OH
34, R_1=O, R_2, R_4=H, R_3=OH
35, R_1=O, R_2=F, R_3=H, R_4=OH

36, R_1=S, R_2=H, R_3, R_4=OH
37, R_1=O, R_2=H, R_3=N_3, R_4=OH
38, R_1=O, R_2=H, R_3=$NHCOCF_3$, R_4=OH
39, R_1=O, R_2, R_4=H, R_3=OH
40, R_1=O, R_2=F, R_3=H, R_4=OH

6, R_1=O, R_2=H, R_3=N_3, R_4=OH
7, R_1=O, R_2=H, R_3=NH_2, R_4=OH
8, R_1=O, R_2=H, R_3=$NHCO_2R_4$
9, R_1=O, R_2, R_4=H, R_3=OH
10, R_1=O, R_2, R_4=H, R_3=OCO_2CH_3
11, R_1=O, R_2=F, R_3=H, R_4=OH

[a] Reagents and conditions: (a) (i) $POCl_3$, proton sponge, $PO(OMe)_3$, 0 °C; (ii) 0.2 M triethylammonium bicarbonate, rt; (b) CF_3CO_2Et, DIEA, DMF, rt; (c) (i) 1,1′-carbonyldiimidazole, DMF, rt; (ii) Et_3N 5% in H_2O/MeOH 1/1, rt; (iii) glucose-1-monophosphate tributylammonium salt, DMF, rt; (d) H_2, Pd/C, MeOH, rt; (e) (i) morpholine, DCC, *t*-BuOH, H_2O, reflux; (ii) glucose-1-monophosphate tributylammonium salt, DMF, rt.

Scheme 2. Synthesis of UDPG Analogues Substituted on the 4 or 5 Position of the Uracil Moiety[a]

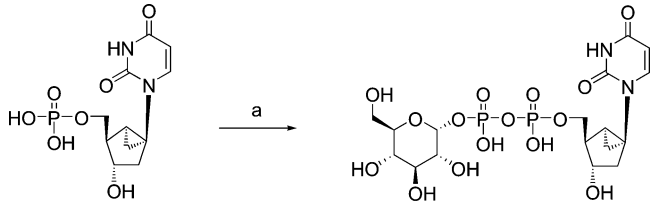


41, R_1=S, R_2=H, R_3=OH
42, R_1=SCH_3, R_2=H, R_3=OH
43, R_1=O, R_2=I, R_3=OH
44, R_1=O, R_2=F, R_3=H

13, R_1=S, R_2=H, R_3=OH
14, R_1=SCH_3, R_2=H, R_3=OH
16, R_1=O, R_2=I, R_3=OH
24, R_1=O, R_2=F, R_3=H

[a] Reagents and conditions: (a) (i) 1,1′-carbonyldiimidazole, DMF, rt; (ii) Et_3N 5% in H_2O/MeOH 1/1, rt; (iii) glucose-1-monophosphate tributylammonium salt, DMF, rt; (b) (i) 0.25 M NaOH, H_2O, MeOH, rt; (ii) iodomethane, DMF, rt.

Scheme 3. Synthesis of an (*S*)-Methanocarba Analogue of 2′-Deoxy-UDPG[a]



[a] Reagents and conditions: (a) (i) 1,1′-carbonyldiimidazole, DMF, rt; (ii) Et_3N 5% in H_2O/MeOH 1/1, rt; (iii) glucose-1-monophosphate tributylammonium salt, DMF, rt.

was modified by removal of the hydroxyl group at the 2′ position **5** and replacement of the hydroxyl at the 2′ position with various functional groups, **6**−**8**, **10**, and **11**. The hydroxyl group at the 3′ position was absent in compounds **9** and **10**. Replacement of the ribose ring with a 2′-deoxy (*S*)-methanocarba bicyclic ring[24] (**12**) also was performed. The uracil ring was modified at the 2 and 4 positions with the synthesis of 4-thiouridine-5′-diphosphoglucose (**13**) and 2-thiouridine-5′-diphosphoglucose (**15**)[25] and at the 5 position with iodide (**16**) and fluoride (**24**). Sugar-modified compounds (**25**−**27**) were synthesized with commercially available sugar monophosphates. Dinucleotides (**29**) were obtained as byproducts during the synthesis of UDPG analogues and of CDP-glucose **19**. All the nucleotide analogues were prepared in their ammonium or triethylammonium salt form according to the methods shown in Schemes 1−3 and tested in functional assays of the $P2Y_{14}$ receptor (Table 1). The nucleotide analogues were characterized using HPLC, nuclear magnetic resonance (^{1}H NMR, ^{31}P NMR), and high-resolution mass spectrometry.

The nucleoside-5′-diphosphoglucose derivatives were obtained by the following methods. 5′-Monophosphorylation by reaction of the nucleoside with phosphorus oxychloride provided the nucleoside 5′-monophosphate analogues **36**−**40** (Scheme 1).[26] 4-Methylthiouridine-5′-monophosphate (**42**) was obtained by the treatment of **41** with 0.25 M NaOH in methanol for 2 h at room temperature, followed by reacting with an excess of iodomethane at room temperature (Scheme 2).[27] All synthesized and commercially available nucleoside 5′-monophosphate analogues were passed through cation-exchange resin and neutralized with tributylamine. The salt form was changed to a tributylammonium salt to allow the subsequent reaction to proceed in an anhydrous organic solvent. The nucleoside 5′-monophosphate was activated with 1,1′-carbonyldiimidazole in DMF for 6 h at room temperature. After the reaction was quenched and the intermediate containing a 2′-cyclic carbonyl group was hydrolyzed with methanol and triethylamine, the activated intermediate was directly condensed with a glucose 1-monophosphate tributylammonium salt in DMF to afford the corresponding nucleoside-5′-diphosphoglucose derivative (**5**, **6**, **8**−**14**, **16**, **19**, **22**, **24**).[28,29] Several intermediates, including inositol-1-monophosphate, fructose-1-monophosphate, and mannose-1-monophosphate, were condensed with uridine-5′-monophosphoimidazolide to afford the compounds **25**−**27**.

The monophosphorylation of the 2′-amino-2′-deoxyuridine **32** using the above method provided only the undesired 3′-monophosphate derivative.[27] As an alternate approach to synthesize 2′-deoxy-2′-aminouridine-5′-diphosphoglucose (**7**), the 2′-trifluoroacetylamino derivative **33**, formed after protection of the amino group in **32** with a trifluoroacetyl group, was phosphorylated to give **38**. However, the protecting group of **38** was removed during the reaction to activate the phosphate with 1,1′-carbonyldiimidazole, and this reagent formed a side product containing a cyclic carbonyl group between the 2′-amino and 3′-oxygen. This intermediate was the precursor of compound

8, and it was necessary to prepare compound **7** by another route, that is, by reduction of the 2′-deoxy-2′-azidouridine-5′-diphosphoglucose (**6**) with H_2 and Pd/C.

1,1′-Carbonyldiimidazole activated not only the 5′-phosphate, but also partially activated the 2′-hydroxy group of the intermediate 3′-deoxyuridine-5′-monophosphate (**39**). The activated 2′-(imidazole-1-carbonyl) group was transformed to a methylcarbonate group when it was exposed to MeOH. After these two transient intermediates were reacted with glucose 1-monophosphate, compounds **9** and **10** were readily separated. When compound **36** was treated with 1,1′-carbonyldiimidazole, a cyclic 3′,5′-monophosphate imidazolide was produced instead of the desired 5′-monophosphate imidazolide. Alternatively, compound **15** could be prepared via the nucleoside 5′-phosphoromorpholidate using morpholine and DCC,[30] followed by the same method of condensing with glucose 1-monophosphate (Scheme 1).

Scheme 3 shows the synthesis of a sterically constrained analogue of 2′-deoxy-UDPG, the (*S*)-methanocarba analogue **12**. This analogue was prepared in an attempt to define the preferred conformation of the ribose moiety in the receptor binding site. The corresponding 2′-hydroxy analogue, which perhaps would be more definitive, was not included in this study.

To test the ability to combine the potency enhancing 2-thio modification with a substituted hexose moiety, 2-thio-UDP-glucuronic acid **28**, analogue was prepared. The method used was an adaptation of a one-step selective enzymatic oxidation starting from the corresponding glucose derivative **15**, and the product was purified using HPLC.[41]

Quantification of Pharmacological Activity. Activation of PLC was quantified in COS-7 cells transiently expressing the human P2Y$_{14}$ receptor and an engineered G protein, Gα-q/i protein (Gα$_{qi5}$) that allows coupling of Gi-coupled receptors to activation of PLC.[35] Thus, inositol lipid hydrolysis[33,34] served as a measure of agonist activity at the Gi-coupled P2Y$_{14}$ receptor.

The high potency of analogues **2** and **3** suggested a flexibility of structural modification at the glucose 4 and 6 positions. The 12-fold lower potency of **4a** in comparison to **1** suggested a limited tolerance for steric bulk at the glucose 2 position. Most of the novel UDPG analogues modified on the uracil or ribose moieties were inactive. Thus, the SAR of the P2Y$_{14}$ receptor is among the most restrictive of all of the P2Y receptors. For example, at the P2Y$_6$ receptor, most modifications of the uracil or ribose moieties reduced, but did not entirely abolish activity. At the P2Y$_2$/P2Y$_4$ receptors, some of the same ribose modifications introduced here (e.g., 2′-amino, 2′-azido) enhanced potency or selectivity.[36] Analogues with purine or pyrimidine base substitutions **19**−**21** and the dinucleotide U[5′]p$_2$[5′]U **29a** were inactive at the P2Y$_{14}$ receptor. Also, UMP, UDP, UTP, and U[5′]p$_4$[5′]U failed to activate the human P2Y$_{14}$ receptor (data not shown).

The SAR of the uracil moiety indicated that substitution of this entity was somewhat more permissive than that of the ribose moiety. Among the novel analogues prepared, a 2-thiouracil analogue **15** had a 7-fold higher potency (EC$_{50}$ 49 ± 2 nM) than that of UDPG (Figure 1). A 4-thio analogue (**13**) was equipotent to UDPG, but its *S*-methylation (**14**) was not tolerated for receptor activation. Also, three modifications at the 5 position of the uracil moiety (**16**−**18**) resulted in inactivity.

Based on the potency of compounds **2**−**4** and on the modeling results (see below) indicating as predominance of hydrophilic, H-bonding residues in the region of the hexose moiety, we proposed that the glucose moiety of UDPG should be amenable

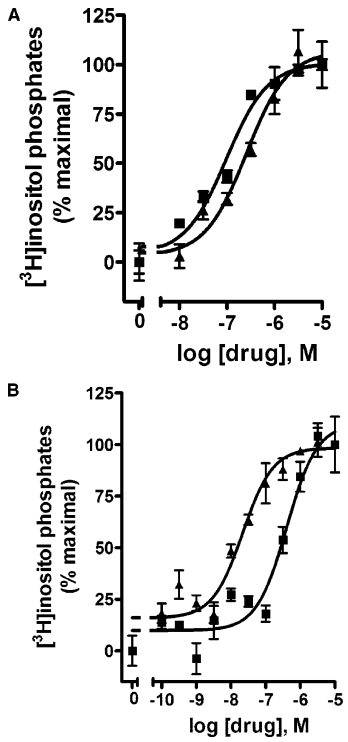


Figure 1. Activation by potent agonists **13** (A) and **15** (B) of PLC in COS-7 cells expressing both the human P2Y$_{14}$ receptor and an engineered Gα-q/i protein that allows the Gi-coupled receptor to stimulate inositol phosphate hydrolysis by PLC.

to extensive structural modification. Consequently, we synthesized UDP-fructose, UDP-mannose, and UDP-inositol and tested these derivatives as agonists of the P2Y$_{14}$ receptor. Indeed, such variation of the glucose moiety of UDPG in the form of inositol **25**, fructose **26**, and mannose **27** preserved the agonist potency at the P2Y$_{14}$ receptor.

UDP-galactose **2**, UDP-glucuronic acid **3**, and UDP-*N*-acetylglucosamine **4a** also were agonists with potencies similar to that of UDPG **1** at the P2Y$_{14}$ receptor. The maximal effect observed with **2**, **3**, and **4a** was somewhat variable. Maximal effects identical to that of UDPG were observed in some experiments, whereas the maximal effects observed with these three molecules were somewhat less than that of UDPG in other experiments. 2-Thio-UDP-glucuronic acid **28** acted as an agonist at the P2Y$_{14}$ receptor and was equipotent to compounds **1** and **3**. Thus, the favorable 2-thio modification preserved but did not enhance the potency of a hexose sugar-modified analogue.

Molecular Modeling. We recently reported a rhodopsin-based homology model of the human P2Y$_{14}$ receptor inserted into a phospholipid bilayer and refined by molecular dynamics (MD) simulation.[31] The model was utilized to study the binding mode of UDPG at the P2Y$_{14}$ receptor. Ligand docking modes were based on automatic molecular docking protocols combined with the Monte Carlo multiple minimum (MCMM) calculations. Despite being based on the ground-state of rhodopsin, the model seems to be applicable to study ligand−receptor interactions for agonists. For example, similar approaches to study the interactions of agonists with P2Y$_1$ and P2Y$_6$ receptors were recently reported.[24,37] The diphosphate moiety was found to interact with only one cationic residue in the P2Y$_{14}$ receptor, namely, Lys171 of EL2, while in other P2Y receptor subtypes three positively charged residues interact with the phosphate chain. It should be noted that in nature the phosphate groups of UDPG could be also coordinated by a metal cation. The model of the P2Y$_1$ receptor complex with UTP−Mg^{2+} was recently

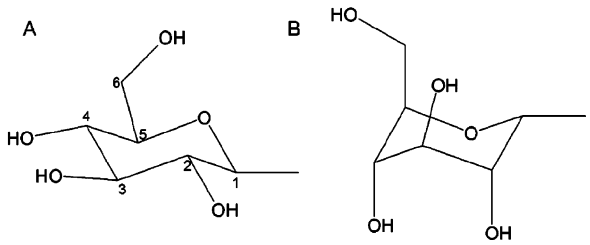


Figure 2. Possible conformations of the hexose ring of UDPG.

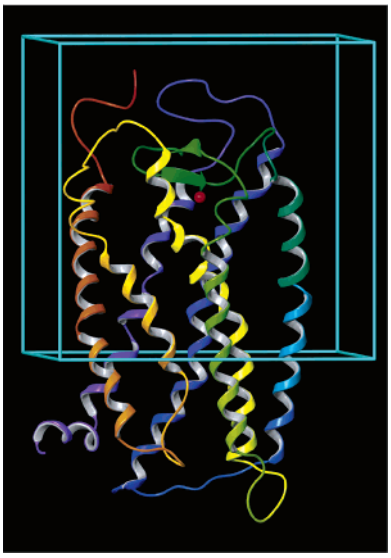


Figure 3. The molecular model of the human $P2Y_{14}$ receptor obtained after 20 ns of MD simulation. A centroid of Lys171, Arg253, and Lys277 (red sphere) was used as a center of the box for molecular docking of UDPG.

reported by Major et al.[38] The distal hexose moiety appeared to be H-bonded by other conserved cationic residues, namely, Arg253 (6.55) and Lys277 (7.35). To facilitate the comparison among receptors, throughout this paper we use the GPCR residue indexing system, as explained in detail elsewhere.[32] To refine the binding mode of UDPG, especially the binding mode of the hexose ring, the MCMM calculations were continued as described in the Experimental Section. The results of MCMM calculations suggested that two conformations of the hexose ring are possible at the receptor binding site (Figure 2). However, values of total energy of the entire ligand−receptor complex calculated for both models demonstrated that conformation **A** is more favorable. This is in a good agreement with the hexose conformation in X-ray data published for complexes of UDPG with various proteins.[39] On the other hand, conformation **B** also seems to be possible due to the interactions between Lys277 (7.35) and hydroxyl groups of the hexose ring. These interactions can lock the hexose ring in its otherwise less favorable conformation.

The UDPG binding mode **A** was proved by independently performed automatic docking of the ligand with the Glide program of MacroModel.[40] As mentioned in the Experimental Section, a box with a side of 46 Å around the centroid of three conserved cationic residues was used for the docking study covering more than half of the entire receptor (Figure 3). The binding mode of UDPG obtained after the Glide-aided molecular docking was very similar to the binding mode of UDPG obtained after MCMM calculations.

It was observed in the docking model obtained for UDPG (Figure 4) that, in the case of conformation **A**, Lys171 (EL2) can form an electrostatic interaction with the β-phosphate group

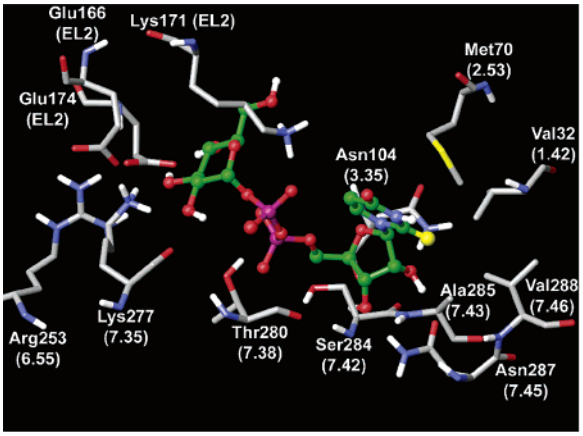


Figure 4. Molecular model of the $P2Y_{14}$−compound **15** complex, with a hexose conformation corresponding to Figure 2A. A rhodopsin-based molecular model of the human $P2Y_{14}$ receptor[11,31] was refined using 20 ns MD simulation in the phospholipid bilayer. The complex obtained after the automatic docking of **15** was used as a starting point for a MCMM conformational search analysis of the ligand inside the putative binding site of the $P2Y_{14}$ receptor.

and an additional hydrogen bond with the hydroxyl group at the 5 position of the hexose ring. Also, this hydroxyl group of UDPG can interact with the backbone oxygen atom of a conserved Cys172 (EL2). The β-phosphate group formed a H-bond with the hydroxyl group of Thr280 (7.38), while Ser284 (7.42) appeared in proximity to the α-phosphate group of UDPG. K277 (7.35) interacted with hydroxyl groups at the hexose 2 and 3 positions, while Arg253 (6.55) was found near the hydroxyl group at position 3. The glutamate residue Glu174 (EL2) can interact with hydroxyl groups at the 3 and 4 positions of the hexose ring. The second glutamate residue Glu166 (EL2) appeared near hydroxyl groups at the 2 and 3 positions and in proximity to Lys277 (7.35). Similarly, hexose binding regions in protein crystallographic structures typically contain charged amino acid side chains, which H-bond to the hydroxyl groups.[39]

The hydroxyl groups of the ribose moiety of UDPG were found to be H-bonded with two asparagine residues. In particular, it was observed that the 2′-hydroxyl group can interact with the side chain amino group of Asn104 (3.35) and the side chain C═O group of Asn287 (7.45). The 3′-hydrogen group of UDPG seemed to be H-bonded with the side chain amino group of Asn287 (7.45). These results are in a good agreement with the experimental values of EC_{50} measured for the ribose-modified UDPG derivatives, suggesting the importance of both 2′- and 3′-hydroxyl groups.

Similar to our previous model,[31] the ribose ring oxygen atom was not involved in hydrogen bonding with the receptor. This observation makes it possible to propose that replacement of this oxygen atom of UDPG by a carbon atom will not decrease the potency of a ligand. Several examples of such modifications of the ribose ring were already published for other subtypes of P2Y receptors.

As shown in Table 1, replacement of the oxygen atom at the uracil 2 position of UDPG by a sulfur atom increases agonist potency. This finding is in good agreement with the receptor docking models obtained for UDPG and 2-thio-UDPG **15** (Figure 4), in which there is a mainly hydrophobic pocket surrounding the 2 position of the uracil ring. The oxygen atom at the 2 position of UDPG was not involved in H-bonding with the receptor, implying that this oxygen is not critical for ligand recognition. The more hydrophobic sulfur atom of larger radius at the 2 position of **15** would be expected to favorably occupy

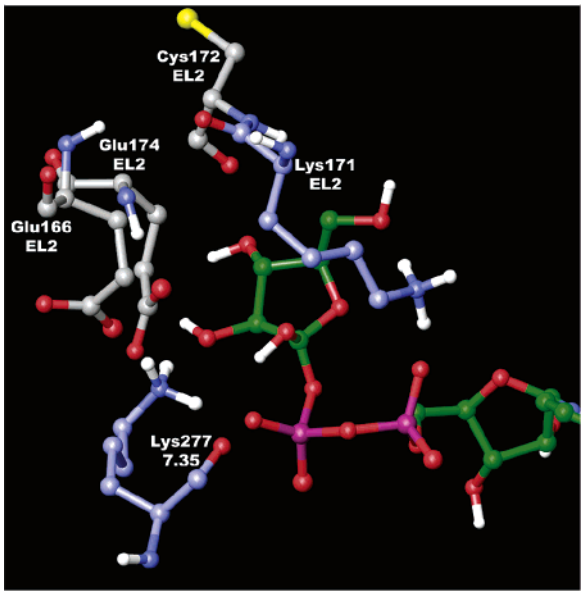


Figure 5. The fructose moiety of **26** docked within the binding site of the human P2Y$_{14}$ receptor.

more free space inside this pocket and result in improved potency of the ligand. The sulfur atom at the 2 position of the uracil ring occupied a space between several residues, namely, Val32 (1.42), Met70 (2.53), Ala285 (7.43), and Val288 (7.46).

The binding modes of several UDP derivatives with various sugar moieties were examined with molecular docking studies. In particular, binding modes of UDP-mannose **27** and UDP-inositol **25** were studied. In general, the position and conformation of these two ligands were found to be very similar to UDPG. In the case of **27**, the 2-OH group was located closer to Glu166 than to Lys277 (7.35), which formed a H-bond with the 3-OH group. Also, hydroxyl groups at the 3 and 4 positions of **27** formed H-bonds with Glu174 (EL2). The hydroxyl group at the 5 position can form a H-bond with Lys171 (EL2), while the oxygen atom of the mannose ring of **27** was not involved in H-bonding with the receptor.

The molecular model of the P2Y$_{14}$ receptor with UDP-inositol **25** obtained after MCMM calculations indicated that similar to UDPG and **27**, the ligand **25** can interact with Lys277 (7.35) and Glu174 (EL2) due to H-bonding between these residues and the hydroxyl groups at the 3 and 4 positions of the inositol moiety. In addition, the 3-OH group of the inositol moiety can interact with Arg253 (6.55). In contrast, Glu166 (EL2) appeared far from the hydroxyl groups of **25** and did not form H-bonds with the ligand. The hydroxyl group at the 6 position of the inositol ring was found near Lys171 (EL2). We propose that introduction of negatively charged groups at 2, 3, and 6 positions of the inositol ring could provide more favorable interactions with cationic residues.

Among UDP derivatives is a five-membered sugar ring derivative, UDP-fructose **26**. The docking complex of **26** in the P2Y$_{14}$ receptor indicated that the ligand can form several H-bonds with the P2Y$_{14}$ receptor (Figure 5). In particular, similar to UDPG, the oxygen atom of the hydroxyl group at the 5 position of **26** can be H-bonded to Lys171 (EL2). In addition, Lys171 can interact with the ring oxygen atom of the fructose moiety. The H-bonds between the ligand and Glu166 (EL2) were not observed in the model obtained after MCMM calculations. However, the hydroxyl group at the 4 position was H-bonded to Glu174 (EL2) and could interact with the backbone oxygen atom of Cys172 (EL2). The 3-hydroxyl group of the fructose moiety was also found to be H-bonded with Glu174 (EL2) and with Lys277.

The results of molecular docking performed for ligands containing various other sugar moieties **25**−**27** indicated that all these structures display similar binding modes at the P2Y$_{14}$ receptor. In general, the sugar moieties of these ligands were involved in similar interactions with the receptor as UDPG. For this reason, compounds **25**−**27** were proposed as potential agonists of the P2Y$_{14}$ receptor and subsequently synthesized and tested biologically (see above).

Conclusions

We have identified various modifications that deselect for this receptor subtype in relation to other uracil nucleotide-responsive receptors and one modification (i.e., 2-thio) that provides higher potency at the P2Y$_{14}$ receptor. The 4-thio analogue of UDPG was equipotent to the parent nucleotide. Most other modifications of the uracil or ribose moieties led to inactivity. Thus, the P2Y$_{14}$ receptor is among the least permissive P2Y receptors with respect to SAR. The molecular modeling studies allowed us to propose that the glucose moiety can be replaced by various six-membered or five-membered sugar rings. This prediction was tested experimentally and it was shown that variation of the glucose moiety of UDPG in the form of inositol, fructose, and mannose preserved the potency. In the models, all these sugar moieties were able to interact with various hydrophilic and H-bonding side chains located in the vicinity. Therefore, the hexose moiety provides a fertile region for chemical modification of P2Y$_{14}$ receptor agonists.

Experimental Section

Chemical Synthesis. 2-Thiouridine (**30**) was purchased from Berry and Associates, Inc. (Dexter, MI). 2′-Azido-2′-deoxyuridine (**31**) and 2′-amino-2′-deoxyuridine (**32**) were purchased from CMS Chemicals Ltd. (Oxfordshire, U.K.). 3′-Deoxyuridine (**34**) was purchased from T.R.C., Inc. (North York, Ontario, Canada). 2′-Ara-fluoro-2′-deoxyuridine (**35**) was purchased from R.I. Chemical, Inc. (Orange, CA). 5-Azidouridine diphosphoglucose (**17**) and 5-aminouridine diphosphoglucose (**18**) were purchased from A.L.T., Inc. (Lexington, KY). Compounds **1**−**4**, **20**, **21**, **23**, and all reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO). Compounds **5**, **19**, **22**, **29a**, and **29b** were synthesized as reported.[28]

^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer using D_2O as a solvent. The chemical shifts are expressed as relative ppm from HOD (4.80 ppm). ^{31}P NMR spectra were recorded at room temperature by use of Varian XL 300 spectrometer (121.42 MHz); orthophosphoric acid (85%) was used as an external standard. Purity of compounds was checked using a Hewlett−Packard 1100 HPLC equipped with a Zorbax Eclipse 5 μ XDB-C18 analytical column (250 × 4.6 mm; Agilent Technologies, Palo Alto, CA). System A: linear gradient solvent system; 5 mM TBAP (tetrabutylammonium dihydrogenphosphate)−CH_3CN from 80:20 to 40:60 in 20 min, then isocratic for 2 min; the flow rate was 1 mL/min. System B: linear gradient solvent system; 10 mM TEAA (triethylammonium acetate)−CH_3CN from 100:0 to 85:15 in 20 min, then isocratic for 2 min; the flow rate was 1 mL/min. Peaks were detected by UV absorption with a diode array detector. All derivatives tested for biological activity showed >98% purity in the HPLC systems.

High-resolution mass measurements were performed on Micromass/Waters LCT Premier electrospray time-of-flight mass spectrometer coupled with a Waters HPLC system. Purification of the nucleotide analogues for biological testing was carried out on (diethylamino)ethyl (DEAE)-A25 Sephadex columns as described below. Compounds **6**, **7**, **9**, **10**, **12**, **16**, and **25**−**27** were additionally purified by HPLC using system C. System C: gradient solvent system; 10 mM TEAA−CH_3CN from 100:0 to 90:10 in 30 min,

then isocratic for 2 min; the flow rate was 2 mL/min with a Luna 5 μ RP-C18(2) semipreparative column (250 × 10.0 mm; Phenomenex, Torrance, CA).

General Procedure for the Preparation of Nucleoside 5′-Monophosphates. Procedure A. A solution of the corresponding nucleoside (0.04−0.19 mmol) and proton sponge (1.5 equiv) in trimethyl phosphate (2 mL) was stirred for 10 min at 0 °C. Then phosphorus oxychloride (2 equiv) was added dropwise, and the reaction mixture was stirred for 2 h at 0 °C. Triethylammonium bicarbonate solution (0.2 M, 2 mL) was added to the reaction mixture, and the clear solution was stirred at room temperature for 1 h. The latter was lyophilized overnight. The residue was purified by ion-exchange column chromatography using a Sephadex-DEAE A-25 resin with a linear gradient (0.01−0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase. The portions of desired compounds were collected, frozen, and lyophilized to give the corresponding nucleoside 5′-monophosphates as the ammonium salts.

General Procedure for the Preparation of Nucleoside 5′-Diphosphoglucose Procedure B. An ammonium salt or sodium salt of the nucleoside 5′-monophosphate (0.01−0.02 mmol) was treated with ion-exchange resin (Dowex 50WX2−200 (H)), followed by neutralization with tributylamine. The mixture was evaporated and lyophilized to afford the nucleoside 5′-monophosphate tributylamine salt. To a solution of the dried corresponding nucleoside 5′-monophosphates tributylamine salt in DMF (1.5−2.0 mL) was added 1,1′-carbonyldiimidazole (3 equiv). The reaction mixture was stirred at room temperature for 6 h. Then 5% triethylamine solution in 1/1 water/methanol (2 mL) was added and stirring was continued at room temperature for an additional 2 h. After removal of the solvent, the residue was dried in high vacuum and dissolved in DMF (1 mL). Glucose-1-monophosphate tributylammonium salt (1.5 equiv, inositol-1-monophsphate for compound **25**, fructose-1-monophosphate for compound **26**, and mannose-1-monophosphate for compound **27** were used) was added to a solution of the nucleotide imidazolide in DMF. The reaction mixture was stirred at room temperature for 2 days. After removal of the solvent, the residue was purified by ion-exchange column chromatography using a Sephadex-DEAE A-25 resin with a linear gradient (0.01−0.5 M) of 0.5 M ammonium bicarbonate as the mobile phase. The portion of desired compounds were collected, frozen, and lyophilized to give the corresponding nucleoside 5′-diphosphoglucoses as the ammonium salts. Some of the products were additionally purified by HPLC using system C.

((2*R*,3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl Phosphate, Ammonium Salt (36). Procedure A. Compound **36** (8 mg, 35%) was obtained from **30** (15 mg, 0.06 mmol). ^{1}H NMR (D_2O) δ 8.21 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 2.7 Hz, 1H), 6.22 (d, J = 8.1 Hz, 1H), 4.23 (dd, J = 4.5, 3.0 Hz, 1H), 4.31 (br d, J = 3.0 Hz, 1H), 4.26 (dd, J = 12.3, 3.9 Hz, 1H), 4.11 (dd, J = 12.0, 5.0 Hz, 1H); ^{31}P NMR (D_2O) δ 0.81 (s); HRMS *m*/*z* calcd for $C_9H_{12}N_2O_8P_2S$ $(M - H^+)^-$, 339.0052; found, 339.0056.

((2*R*,3*S*,4*R*,5*R*)-4-Azido-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-hydroxytetrahydrofuran-2-yl)methyl Phosphate, Ammonium Salt (37). Procedure A. Compound **37** (7 mg, 41%) was obtained from **31** (12 mg, 0.045 mmol). ^{1}H NMR (D_2O) δ 7.91 (d, J = 8.1 Hz, 1H), 5.98 (d, J = 5.1 Hz, 1H), 5.91 (d, J = 8.1 Hz, 1H), 4.52 (t, J = 5.4 Hz, 1H) 4.32 (t, J = 5.4 Hz, 1H), 4.23 (m, 1H), 4.18 (ddd, J = 12.0, 4.5, 2.7 Hz, 1H), 4.08 (ddd, J = 12.0, 5.1, 2.7 Hz, 1H); ^{31}P NMR (D_2O) δ −3.52 (s); HRMS *m*/*z* calcd for $C_9H_{11}N_5O_8P$ $(M - H^+)^-$, 348.0345; found, 348.0366.

((2*R*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)--3-hydroxy-4-(2,2,2-trifluoroacetamido)tetrahydrofuran-2-yl)-methyl Phosphate, Ammonium Salt (38). Procedure A. Compound **38** (3.2 mg, 45%) was obtained from **33** (5.8 mg, 0.017 mmol). ^{1}H NMR (D_2O) δ 8.09 (dd, J = 8.4, 1.8 Hz, 1H), 6.05 (d, J = 8.1 Hz, 1H), 6.00 (dd, J = 7.8, 1.5 Hz, 1H), 4.41 (m, 1H), 4.31 (m, 1H), 3.96 (m, 2H), 3.80 (m, 1H); ^{31}P NMR (D_2O) δ 3.85 (s); HRMS *m*/*z* calcd for $C_{11}H_{12}N_3O_9F_3P_1$ $(M - H^+)^-$, 418.0263; found, 418.0251.

((2*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-hydroxytetrahydrofuran-2-yl)methyl Phosphate, Ammonium Salt (39). Procedure A. Compound **39** (9.2 mg, 41%) was obtained from **34** (15 mg, 0.066 mmol). ^{1}H NMR (D_2O) δ 8.09 (d, J = 8.1 Hz, 1H), 5.88 (d, J = 7.8 Hz, 1H), 5.79 (d, J = 1.2 Hz, 1H), 4.59 (m, 1H), 4.48 (m, 1H), 4.09 (ddd, J = 11.7, 4.5, 2.7 Hz, 1H), 3.86 (ddd, J = 11.7, 4.5, 4.5 Hz, 1H), 2.14 (ddd, J = 14.1, 9.6, 5.7 Hz, 1H), 2.00 (ddd, J = 13.8, 6.3, 2.7 Hz, 1H); ^{31}P NMR (D_2O) δ 2.35 (s); HRMS *m*/*z* calcd for $C_9H_{12}N_2O_8P$ $(M - H^+)^-$, 307.0331; found, 307.0309.

((2*R*,3*R*,4*S*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3-hydroxytetrahydrofuran-2-yl)methyl Phosphate, Ammonium Salt (40). Procedure A. Compound **40** (9.5 mg, 33%) was obtained from **35** (20 mg, 0.08 mmol). ^{1}H NMR (D_2O) δ 7.90 (dd, J = 8.1, 1.2 Hz, 1H), 6.31 (dd, J = 15.9, 4.2 Hz, 1H), 5.89 (dd, J = 8.1, 1.2 Hz, 1H), 5.21 (d, J = 51.5, 3.0 Hz, 1H), 4.52 (d, J = 19.2, 4.2 Hz, 1H), 4.18 (m, 3H); ^{31}P NMR (D_2O) δ 4.75 (s); HRMS *m*/*z* calcd for $C_9H_{11}N_2O_8FP$ $(M - H^+)^-$, 325.0237; found, 325.0240.

((2*R*,3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(4-(methylthio)-2-oxopyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl Phosphate, Ammonium Salt (42). To a suspension of 4-thiouridine 5′-monophosphate, **41** (sodium salt, 10 mg, 0.03 mmol) in MeOH/H_2O (1.0 mL/0.5 mL) was added 0.25 M NaOH (0.5 mL). The reaction mixture was stirred at room temperature for 2 h, and then the solvent was removed under high vacuum. The resulting residue was dissolved in dry DMF (2.0 mL), and iodomethane (0.09 mL, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, and an additional 0.09 mL of iodomethane was added. After 2 h, the solvent was removed under reduced pressure. The crude compound was purified by the same method in procedure A to give **42** (7 mg, 62%). ^{1}H NMR (D_2O) δ 8.40 (d, J = 6.9 Hz, 1H), 6.78 (d, J = 7.5 Hz, 1H), 5.97 (d, J = 2.4 Hz, 1H), 4.34−4.37 (m, 2H), 4.30 (m, 1H), 4.10−4.17 (m, 1H), 3.96−4.02 (m, 1H); ^{31}P NMR (D_2O) δ 2.09 (s); HRMS *m*/*z* calcd for $C_{10}H_{14}N_2O_8SP$ $(M - H^+)^-$, 325.0209; found, 353.0238.

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2″-Deoxy-2″-azido)uridin-5″-yl) Ester, Triethylammonium Salt (6). Procedure B. Compound **6** (2.5 mg, 63%) was obtained from **37** (ammonium salt, 2 mg, 0.005 mmol). ^{1}H NMR (D_2O) δ 7.94 (d, J = 8.1 Hz, 1H), 6.01 (d, J = 5.4 Hz, 1H), 5.96 (d, J = 7.8 Hz, 1H), 5.58 (dd, J = 7.2, 3.6 Hz, 1H), 4.80 (m, 1H), 4.36 (t, J = 5.4 Hz, 1H), 4.19−4.25 (m, 3H), 3.82−3.89 (m, 2H), 3.72−3.78 (m, 2H), 3.51 (dd, J = 9.9, 3.0 Hz, 1H), 3.44 (t, J = 9.6 Hz, 1H); ^{31}P NMR (D_2O) δ −10.89 (d, J = 20.8 Hz), −12.47 (d, J = 20.8 Hz); HRMS *m*/*z* calcd for $C_{15}H_{22}N_5O_{16}P_2$ $(M - H^+)^-$; found, 590.0531. HPLC (System A) 13.7 min (98%), (System B) 7.2 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2″-Deoxy-2″-amino)uridin-5″-yl) Ester, Triethylammonium Salt (7). H_2 at atmospheric pressure was applied to a mixture of **6** (1.5 mg, 0.002 mmol) and Pd/C 10% (0.3 mg) in MeOH (0.5 mL) for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was evaporated under the reduced pressure. The residue was purified by HPLC using system C to afford **7** (1.0 mg, 69%). ^{1}H NMR (D_2O) δ 7.96 (d, J = 7.8 Hz, 1H), 6.02 (d, J = 7.8 Hz, 1H), 5.98 (d, J = 8.1 Hz, 1H), 5.62 (dd, J = 8.1, 3.6 Hz, 1H), 4.38 (m, 1H), 4.33 (m, 1H), 4.20 (m, 2H), 3.77−3.91 (m, 4H), 3.46−3.64 (m, 3H); ^{31}P NMR (D_2O) δ −10.83 (d, J = 20.8 Hz), −12.49 (d, J = 20.8 Hz); HRMS *m*/*z* calcd for $C_{15}H_{24}N_3O_{16}P_2$ $(M - H^+)^-$,564.0632; found, 564.0600. HPLC (System A) 5.7 min (98%), (System B) 5.4 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2″-Deoxy-2″-aminocarbonyl-3″-O)uridin-5″-yl) Ester, Ammonium Salts (8). Procedure B. Compound **8** (2.0 mg, 48%) was obtained from **38** (ammonium salt, 3.0 mg, 0.007 mmol). ^{1}H NMR (D_2O) δ 7.84 (d, J = 8.4 Hz, 1H), 5.91 (m, 2H), 5.59 (dd, J = 7.5, 3.6 Hz, 1H), 5.40 (dd, J = 8.4, 3.3 Hz, 1H), 4.67−4.75 (m, 2H), 4.30 (m, 2H), 3.75−3.92 (m, 4H), 3.43−3.55 (m, 2H); ^{31}P NMR (D_2O) δ −11.25 (br s), −12.52 (br s); HRMS *m*/*z* calcd for $C_{16}H_{22}N_3O_{17}P_2$ $(M - H^+)^-$, 590.0424; found, 590.0449. HPLC (System A) 11.7 min (98%), (System B) 5.5 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((3′′-Deoxy)-uridin-5′′-yl) Ester, Triethylammonium Salt (9) and Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2′′,3′′-Dideoxy-2′′-acetyloxy)uridin-5′′-yl) Ester, Triethylammonium Salt (10). Procedure B. Compounds **9** (1.3 mg, 7%) and **10** (3.4 mg, 16%) were obtained from **39** (ammonium salts, 9 mg, 0.026 mmol). Compound **9** ^{1}H NMR (D_2O) δ 8.02 (d, J = 8.1 Hz, 1H), 5.95 (d, J = 8.1 Hz, 1H), 5.85 (d, J = 2.1 Hz, 1H), 5.62 (dd, J = 7.2, 3.3 Hz, 1H), 4.71 (m, 1H), 4.54 (m, 1H), 4.34 (m, 1H), 4.15 (m, 1H), 3.77−3.93 (m, 4H), 3.44−3.57 (m, 2H), 2.07−2.24 (m, 2H); ^{31}P NMR (D_2O) δ −10.66 (d, J = 21.5 Hz), −12.48 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{15}H_{23}N_2O_{16}P_2$ $(M - H^+)^-$, 549.0523; found, 549.0543. HPLC (System A) 12.9 min (98%), (System B) 8.5 min (98%). Compound **10** ^{1}H NMR (D_2O) δ 7.96 (d, J = 8.1 Hz, 1H), 6.02 (d, J = 1.5 Hz, 1H), 5.96 (d, J = 7.8 Hz, 1H), 5.61 (dd, J = 7.2, 3.6 Hz, 1H), 5.37 (m, 1H), 4.65 (m, 1H), 4.35 (ddd, J = 11.7, 5.1, 2.4 Hz, 1H), 4.14 (ddd, J = 11.7, 6.3, 4.2 Hz, 1H), 3.92 (m, 1H), 3.84 (s, 3H), 3.76−3.86 (m, 3H), 3.54 (dt, J = 9.9, 3.3 Hz, 1H), 3.47 (t, J = 9.3 Hz, 1H), 2.44 (m, 1H), 2.31 (ddd, J = 14.1, 6.0, 2.1 Hz, 1H); ^{31}P NMR (D_2O) δ −10.72 (d, J = 20.8 Hz), −12.47 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{17}H_{25}N_2O_{18}P_2$ $(M - H^+)^-$, 607.0578; found, 607.0568. HPLC (System A) 13.1 min (98%), (System B) 8.0 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2′-Fluoro-2′′-deoxyara)uridin-5′′-yl)ester, Ammonium Salts (11). Procedure B. Compound **11** (ammonium salt, 2.4 mg, 24%) was obtained from **40** (ammonium salt, 6 mg, 0.017 mmol). ^{1}H NMR (D_2O) δ 7.92 (dd, J = 8.1, 1.2 Hz, 1H), 6.33 (dd, J = 15.9, 3.9 Hz, 1H), 5.94 (d, J = 8.4 Hz, 1H), 5.61 (dd, J = 7.2, 3.6 Hz, 1H), 5.23 (dd, J = 51.3, 4.2 Hz, 1H), 4.57 (dd, J = 18.9, 3.3 Hz, 1H), 4.25 (m, 3H), 3.76−3.92 (m, 4H), 3.44−3.56 (m, 2H); ^{31}P NMR (D_2O) δ −10.73 (d, J = 20.8 Hz), −12.45 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{15}H_{22}N_2O_{16}FP_2$ $(M - H^+)^-$, 567.0429; found, 567.0427. HPLC (System A) 11.7 min (98%), (System B) 8.4 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-(((*S*)-Methanocarba-2′′-deoxy)uridin-5′′-yl)ester, Triethylammonium Salt (12). Procedure B. Compound **12** (1.4 mg, 59%) was obtained from **45**[24] (ammonium salt, 1.1 mg, 0.003 mmol). ^{1}H NMR (D_2O) δ 7.81 (d, J = 8.1 Hz, 1H), 5.80 (d, J = 7.8 Hz, 1H), 5.59 (dd, J = 6.9, 3.3 Hz, 1H), 4.37 (d, J = 6.6 Hz, 1H), 4.09 (m, 2H), 3.74−3.87 (m, 4H), 3.42−3.53 (m, 2H), 2.40 (m, 1H), 2.19 (m, 2H), 1.86 (m, 1H), 1.63 (m, 1H), 1.37 (m, 1H); ^{31}P NMR (D_2O) δ −10.48 (d, J = 20.8 Hz), −12.53 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{17}H_{25}N_2O_{15}P_2$ $(M - H^+)^-$, 559.0730; found, 559.0735. HPLC (System A) 11.6 min (98%), (System B) 7.9 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((4′-Thio)-uridin-5′′-yl)ester, Ammonium Salts (13). Procedure B. Compound **13** (1.9 mg, 18%) was obtained from **41** (12 mg, 0.017 mmol). ^{1}H NMR (D_2O) δ 7.86 (d, J = 7.8 Hz, 1H), 6.68 (d, J = 7.5 Hz, 1H), 5.96 (d, J = 3.6 Hz, 1H), 5.62 (dd, J = 7.5, 3.6 Hz, 1H), 4.23−4.39 (m, 5H), 3.83−3.90 (m, 2H), 3.76−3.84 (m, 2H), 3.54 (dt, J = 9.0, 3.0 Hz, 1H), 3.48 (dd, J = 9.9, 9.3 Hz, 1H); ^{31}P NMR (D_2O) δ −10.33 (d, J = 20.8 Hz), −12.46 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{15}H_{23}N_2O_{16}P_2S$ $(M - H^+)^-$, 581.0224; found, 581.0249. HPLC (System A) 13.7 min (98%), (System B) 6.6 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((4′-Methylthio)uridin-5′′-yl)ester, Ammonium Salts (14). Procedure B. Compound **14** (5.1 mg, 40%) was obtained from **42** (ammonium salt, 6.2 mg, 0.016 mmol). ^{1}H NMR (D_2O) δ 8.20 (d, J = 7.5 Hz, 1H), 6.76 (d, J = 7.2 Hz, 1H), 5.94 (d, J = 2.4 Hz, 1H), 5.59 (dd, J = 6.9, 3.0 Hz, 1H), 4.18−4.35 (m, 5H), 3.73−3.90 (m, 4H), 3.20−3.53 (m, 2H), 2.53 (s, 3H); ^{31}P NMR (D_2O) δ −10.80 (d, J = 20.8 Hz), −12.46 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{16}H_{25}N_2O_{16}P_2S$ $(M - H^+)^-$, 595.0400; found, 595.0415. HPLC (System A) 14.2 min (98%), (System B) 8.0 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2′-Thio)-uridin-5′′-yl)ester, Ammonium Salts (15). To a suspension of **36** (tributylammonium salt, 8 mg, 0.01 mmol) in *t*-BuOH/H_2O (0.5 mL/0.5 mL) were added morpholine (0.006 mL, 0.06 mmol) and DCC (14 mg, 0.06 mmol). The reaction mixture was heated to reflux for 4 h and cooled to room temperature. After removal of solvent, the reaction was diluted with H_2O and washed with diethyl ether twice. The aqueous layer was concentrated and passed through H_2O and ion-exchange resin (Dowex 50WX2−200 (H)), followed by treatment with tributylamine. After removal of all solvents, the residue was dried in high vacuum and dissolved in DMF (1 mL). Glucose-1-monophosphate tributylammonium salt was added to a solution of the nucleotide morpholidate in DMF. The reaction mixture was stirred for 2 days, followed by the same purification procedure B to give **15** (5 mg, 77%). ^{1}H NMR (D_2O) δ 8.15 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 3.0 Hz, 1H), 6.26 (d, J = 8.1 Hz, 1H), 5.61 (dd, J = 6.9, 3.3 Hz, 1H), 4.20−4.45 (m, 5H), 3.70−3.95 (m, 4H), 3.54 (dt, J = 9.0, 2.7 Hz, 1H), 3.47 (t, J = 9.3 Hz, 1H); ^{31}P NMR (D_2O) δ −10.23 (d, J = 20.8 Hz), −11.83 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{15}H_{23}N_2O_{16}P_2S$ $(M - H^+)^-$, 581.0244; found, 581.0248. HPLC (System A) 12.6 min (98%), (System B) 5.0 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((5′-Iodo)-uridin-5′′-yl)ester, Triethylammonium Salt (16). Procedure B. Compound **16** (1.4 mg, 11%) was obtained from **43** (sodium salt, 7 mg, 0.014 mmol). ^{1}H NMR (D_2O) δ 8.27 (s, 1H), 5.95 (d, J = 4.8 Hz, 1H), 5.63 (dd, J = 6.9, 4.5 Hz, 1H), 4.23−4.42 (m, 5H), 3.77−4.01 (m, 4H), 3.54 (dt, J=9.0, 3.0 Hz, 1H), 3.47 (t, J = 9.6 Hz, 1H); ^{31}P NMR (D_2O) δ −10.40 (d, J = 20.8 Hz), −11.87 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_{15}H_{22}N_2O_{17}IP_2$ $(M - H^+)^-$, 690.9439; found, 690.9444. HPLC (System A) 12.8 min (98%), (System B) 6.9 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosyl Ester 2-((2′′-Deoxy-5′-flouro)uridin-5′′-yl)ester, Ammonium Salts (24). Procedure B. Compound **24** (1.8 mg, 21%) was obtained from **44** (tributylammonium salt, 10 mg, 0.014 mmol). ^{1}H NMR (D_2O) δ 8.04 (d, J = 5.7 Hz, 1H), 6.30 (t, J = 6.9 Hz, 1H), 5.59 (dd, J = 7.5, 3.2 Hz, 1H), 4.18 (m, 2H), 3.74−3.91 (m, 4H), 3.42−3.53 (m, 2H), 3.10−3.21 (m, 2H), 2.34−2.38 (m, 2H); ^{31}P NMR (D_2O) δ −10.89 (d, J = 20.8 Hz), −12.51 (d, J = 20.8 Hz); HRMS *m/z* calcd for $C_9H_{13}N_2O_{11}P_2$ $(M - H^+)^-$, 386.9995; found, 386.9995. HPLC (System A) 14.5 min (98%), (System B) 4.8 min (98%).

UDP-Inositol, Triethylammonium Salt (25). Procedure B. Compound **25** (3.2 mg, 30%) was obtained from uridine-5′-monophosphate (tributylammonium salt, 10 mg, 0.014 mmol). ^{1}H NMR (D_2O) δ 7.97 (d, J = 7.8 Hz, 1H), 5.99 (m, 2H), 4.72 (m, 1H), 4.40 (m, 2H), 4.31 (m, 1H), 4.26 (m, 2H), 3.74 (t, J = 9.9 Hz, 2H), 3.56 (m, 2H), 3.30 (t, J = 9.3 Hz, 1H); ^{31}P NMR (D_2O) δ −9.95 (d, J = 21.4 Hz), −10.56 (d, J = 21.4 Hz); HRMS *m/z* calcd for $C_{15}H_{23}N_2O_{17}P_2$ $(M - H^+)^-$, 565.0472; found, 565.0463. HPLC (System A) 12.3 min (98%), (System B) 9.2 min (98%).

UDP-Fructose, Triethylammonium Salt (26). Compound **26** (4.0 mg, 37%) was obtained from uridine-5′-monophosphate (tributylammonium salt, 10 mg, 0.014 mmol). ^{1}H NMR (D_2O) δ 7.98 (d, J = 8.1 Hz, 1H), 6.00 (m, 2H), 4.40 (m, 2H), 4.31 (m, 1H), 4.26 (m, 2H), 4.04−4.11 (m, 2H), 3.94−4.01 (m, 2H), 3.91 (m, 1H), 3.85 (m, 1H), 3.72 (m, 1H); ^{31}P NMR (D_2O) δ −10.82 (d, J = 4.2 Hz), −10.96 (s); HRMS *m/z* calcd for $C_{15}H_{23}N_2O_{17}P_2$ $(M - H^+)^-$, 565.0472; found, 565.0468. HPLC (System A) 12.4 min (98%), (System B) 8.7 min (98%).

UDP-Mannose, Triethylammonium Salt (27). Procedure B. Compound **27** (2.8 mg, 26%) was obtained from uridine-5′-monophosphate (tributylammonium salt, 10 mg, 0.014 mmol). ^{1}H NMR (D_2O) δ 7.99 (d, J = 8.4 Hz, 1H), 6.00 (m, 2H), 5.53 (dd, J = 7.2, 2.3 Hz, 1H), 4.40 (m, 2H), 4.31 (m, 1H), 4.25 (m, 2H), 4.07 (m, 1H), 3.67−3.97 (m, 5H); ^{31}P NMR (D_2O) δ −11.17 (d, J = 20.8 Hz), −13.46 (d, J = 20.9 Hz); HRMS *m/z* calcd for $C_{15}H_{23}N_2O_{17}P_2$ $(M - H^+)^-$, 565.0472; found, 565.0471. HPLC (System A) 12.5 min (98%), (System B) 7.8 min (98%).

Diphosphoric Acid 1-α-D-Glucopyranosuronic Acid 2-((2′-Thio)uridin-5′′-yl)ester, Triethylammonium Salts (28). Compound **15** (ammonium salt, 0.7 mg, 0.001 mmol) was dissolved in 0.7 mL of stock solution (stock solution: Tris buffer (pH 7.4, 10 mL), NAD (17 mg, 0.026 mmol), sodium pyruvate (177 mg, 1.6 mmol), and lactate dehydrogenase (67 U, EC 1.1.1.27, from rabbit muscle, Sigma). The pH of this solution was adjusted to 8.7 with

0.05 NaOH). Uridine-5′-diphosphoglucose dehydrogenase (0.3 U, Sigma, from bovine liver) was added to the mixture.[41] The reaction was monitored by HPLC (System A). After 20 h at room temperature, an additional 0.1 U of UDPG dehydrogenase was added. After 5 h, the reaction mixture was lyophilized and purified by HPLC (gradient solvent system: 10 mM TEAA−CH_3CN from 100:0 to 97:3 in 40 min, then isocratic for 5 min; the flow rate was 2 mL/min with a Luna 5 μ RP-C18(2) semipreparative column (250 × 10.0 mm; Phenomenex, Torrance, CA)). The portion of desired compound was collected, frozen, and lyophilized to give **28** (0.5 mg, 0.56 μmol, 56%, starting material **15** (0.3 mg, 0.38 μmol, 38%) was recovered). ^{1}H NMR (D_2O) δ 8.17 (d, J = 8.1 Hz, 1H), 6.72 (d, J = 3.3 Hz, 1H), 6.24 (d, J = 7.5 Hz, 1H), 5.63 (dd, J = 7.2, 3.0 Hz, 1H), 4.43 (m, 1H), 4.33 (m, 2H), 4.24 (m, 1H), 4.10−4.16 (m, 2H), 3.78 (t, J = 9.6 Hz, 1H), 3.57 (m, 1H), 3.51 (t, J = 9.6 Hz, 1H); ^{31}P NMR (D_2O) δ −10.81 (br s), −12.45 (br s); HRMS *m/z* calcd for $C_{15}H_{21}N_2O_{17}P_2S$ $(M - H^+)^-$, 595.0036; found, 595.0032. HPLC (System A) 17.1 min (98%), (System B) 12.4 min (98%).

Assay of $P2Y_{14}$ Receptor-Stimulated PLC Activity. COS-7 cells were transiently transfected with the human $P2Y_{14}$ receptor and $G\alpha_{qi5}$.[35] Twenty-four hours after transfection, the inositol lipid pool of the cells was radiolabeled by incubation in 200 μL of serum-free inositol-free Dulbecco's modified Eagle's medium, containing 0.4 μCi of *myo*-[^{3}H]inositol. No changes of medium were made subsequent to the addition of [^{3}H]inositol. Forty-eight hours after transfection, cells were challenged with 50 μL of the 5-fold concentrated solution of receptor agonists in 200 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid, pH 7.3, containing 50 mM LiCl for 20 min at 37 °C. Incubations were terminated by aspiration of the drug-containing medium and addition of 450 μL of ice-cold 50 mM formic acid. After 15 min at 4 °C, samples were neutralized with 150 μL of 150 mM NH_4OH. [^{3}H]Inositol phosphates were isolated by ion exchange chromatography on Dowex AG 1-X8 columns as previously described.[34]

Data Analysis. Agonist potencies (EC_{50} values) were obtained from concentration−response curves by nonlinear regression analysis using the GraphPad software package Prism (GraphPad, San Diego, CA). All experiments were performed in triplicate assays and repeated at least three times. 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.

Molecular Modeling. Conformational Search. The recently reported complex of the $P2Y_{14}$ receptor with UDPG was utilized as a starting point for further calculations.[31] The conformational analysis of the UDP-sugars in the putative binding site of the $P2Y_{14}$ receptor was performed with MCMM implemented in MacroModel 9.0 software.[40]

Initially, an MCMM search was performed only on the glucose ring of UDPG. The atoms located within 8 Å from the ring were used as a shell. The bond between carbon atoms at positions 2 and 3 of the glucose ring was "opened" during the conformational search. The following parameters were used: MMFFs force field, water was used as an implicit solvent, a maximum of 1000 iterations of the Polak−Ribier conjugate gradient minimization method was used with a convergence threshold of 0.05 kJ·mol^{-1}·Å^{-1}, the number of conformational search steps = 1000, the energy window for saving structures = 1000 kJ·mol^{-1}. An MCMM search then was performed on the entire ligand and all residues located within 6 Å from UDPG using a shell of residues located within 2 Å. Two ligand−receptor complexes with different conformations of the hexose ring were subjected to MCMM calculations. The one hundred steps of the conformational search and an energy window for saving structures of 100 kJ·mol^{-1} were used. This protocol was applied to MCMM calculations performed for all other studied ligands.

Molecular Docking. To prove the binding mode obtained for UDPG at the $P2Y_{14}$ receptor an additional independent docking study was performed with the Glide program of MacroModel package.[40] Our initial model of the $P2Y_{14}$ receptor obtained after 20 ns of MD simulation was utilized for automatic docking of UDPG. The receptor grid generation was performed for the box with a side of 46 Å with a center in the centroid of Lys171, Arg253, and Lys277. Extra precision (XP) of docking was used. The flexibility of a ligand was allowed. The conformations of the sidechains of residues located within 6 Å from UDPG were refined by 100 steps of MCMM calculations with an energy window for saving structures of 100 kJ·mol^{-1}. The model obtained after this refinement was used for a Glide-aided automatic docking of all other ligands.

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Supporting Information Available: Graphical views of the $P2Y_{14}$−compound **15** complex (but not including those of the phospholipid bilayer and aqueous medium) are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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