Lipophilic Modifications to Dinucleoside Polyphosphates and Nucleotides that Confer Antagonist Properties at the Platelet $P2Y_{12}$ Receptor

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Platelet $P2Y_{12}$ receptors play a central role in the regulation of platelet function and inhibition of this receptor by treatment with drugs such as clopidogrel results in a reduction of atherothrombotic events. We discovered that modification of natural and synthetic dinucleoside polyphosphates and nucleotides with lipophilic substituents on the ribose and base conferred $P2Y_{12}$ receptor antagonist properties to these molecules producing potent inhibitors of ADP-mediated platelet aggregation. We describe methods for the preparation of these functionalized dinucleoside polyphosphates and nucleotides and report their associated activities. By analysis of these results and by deconstruction of the necessary structural elements through selected syntheses, we prepared a series of highly functionalized nucleotides, resulting in the selection of an adenosine monophosphate derivative (**62**) for further clinical development.

Introduction

Extracellular nucleotides and dinucleoside polyphosphates participate in the regulation of a broad range of physiological functions in numerous cell types and tissues, acting as agonists or antagonists on P2 cell surface receptors.[1–3] The P2 receptor superfamily is composed of two subfamilies designated P2X and P2Y, each having multiple receptor subtypes. The P2X subfamily, composed of ligand-gated cation channels, has seven subtypes ($P2X_1$−$P2X_7$). The P2Y subfamily, composed of G-protein-coupled receptors has eight subtypes ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$). In addition to its ubiquitous role in cellular energy pathways, adenosine 5′-triphosphate (ATP, **3**, Figure 1) is the endogenous agonist of the seven P2X receptor subtypes as well as the $P2Y_2$ and $P2Y_{11}$ receptors. Adenosine 5′-diphosphate (ADP, **2**) is the natural agonist of $P2Y_1$, $P2Y_{12}$, and $P2Y_{13}$ receptors. The pyrimidine nucleotide uridine 5′-triphosphate (UTP, **6**) is also an agonist of $P2Y_2$, with potency comparable to that of ATP. UTP also serves as the ligand for the $P2Y_4$ receptor, while its corresponding diphosphate, UDP (**5**), is the agonist of the $P2Y_6$ receptor. UDP-glucose is the agonist of the most recently described member of the P2Y subfamily, the $P2Y_{14}$ receptor.[4] Dinucleoside polyphosphates are also endogenous substances released into in the extracellular space, where they are known to stimulate several P2 receptors with activities comparable to that of their nucleotide counterparts. Natural and synthetic dinucleoside polyphosphates have agonist potencies comparable to those of ATP and UTP at $P2Y_2$ receptors and to that of UDP at the $P2Y_6$ receptor. The inherently greater stability of dinucleoside polyphosphates compared to that of their corresponding nucleotides has led us to develop two different $P2Y_2$ dinucleoside tetraphosphate agonists (**13** and **14**) for the treatment of dry eye disease and cystic fibrosis.[5,6]

Platelets are small disk-shaped anucleated cells found in circulation, which are responsible for initiating and stabilizing blood clots. While this function is vital in the maintenance of hemostasis, platelet activation under various pathological conditions can potentially lead to life-threatening thrombotic events.

Platelets express a variety of cell-surface receptors, three of which belong to the P2 superfamily ($P2X_1$, $P2Y_1$, and $P2Y_{12}$). The role of $P2X_1$ on platelet function is not completely understood; it has been demonstrated recently using $P2X_1$ receptor knockout mice that this receptor plays an important role in thrombus formation under high shear conditions, as might be found in a partially occluded vessel.[7] In contrast, the participation of $P2Y_1$ and $P2Y_{12}$ receptors in platelet activation and aggregation have been studied extensively.[8,9,10]

Activation of $P2Y_1$ by ADP results in platelet shape change and transient aggregation, whereas activation of $P2Y_{12}$ leads to degranulation and release of more endogenous nucleotides, leading to sustained aggregation. ADP is found in high concentrations in platelet granules and is released in response to stimulation with a number of platelet-aggregating agents and by the exposure of platelets to foreign surfaces or to the pro-aggregatory subendothelium following the injury of the blood vessel wall. Activated platelets also produce thromboxane A2 (TXA_2) and release fibrinogen and other active substances from storage granules. TXA_2 amplifies shape change and leads to further activation of the platelet. Platelet activation and degranulation leads to further recruitment of platelets to the site of injury. Ultimately, fibrinogen cross-links platelets via the glycoprotein IIb/IIIa receptor, leading to the formation of a stable thrombus.

ADP-promoted aggregation requires the simultaneous activation of both the $P2Y_1$ and $P2Y_{12}$ receptors, and inhibition of ADP binding at either receptor is sufficient to prevent platelet aggregation. The $P2Y_{12}$ receptor is found primarily on platelets and in the central nervous system, whereas the $P2Y_1$ receptor has a more widespread distribution.[4] The comparatively limited distribution of $P2Y_{12}$ receptors, coupled to the fact that this receptor has been validated by the thienopyridines as a clinical target, makes the $P2Y_{12}$ receptor an attractive drug discovery target for the development of medicines to treat cardiovascular diseases.[11,12]

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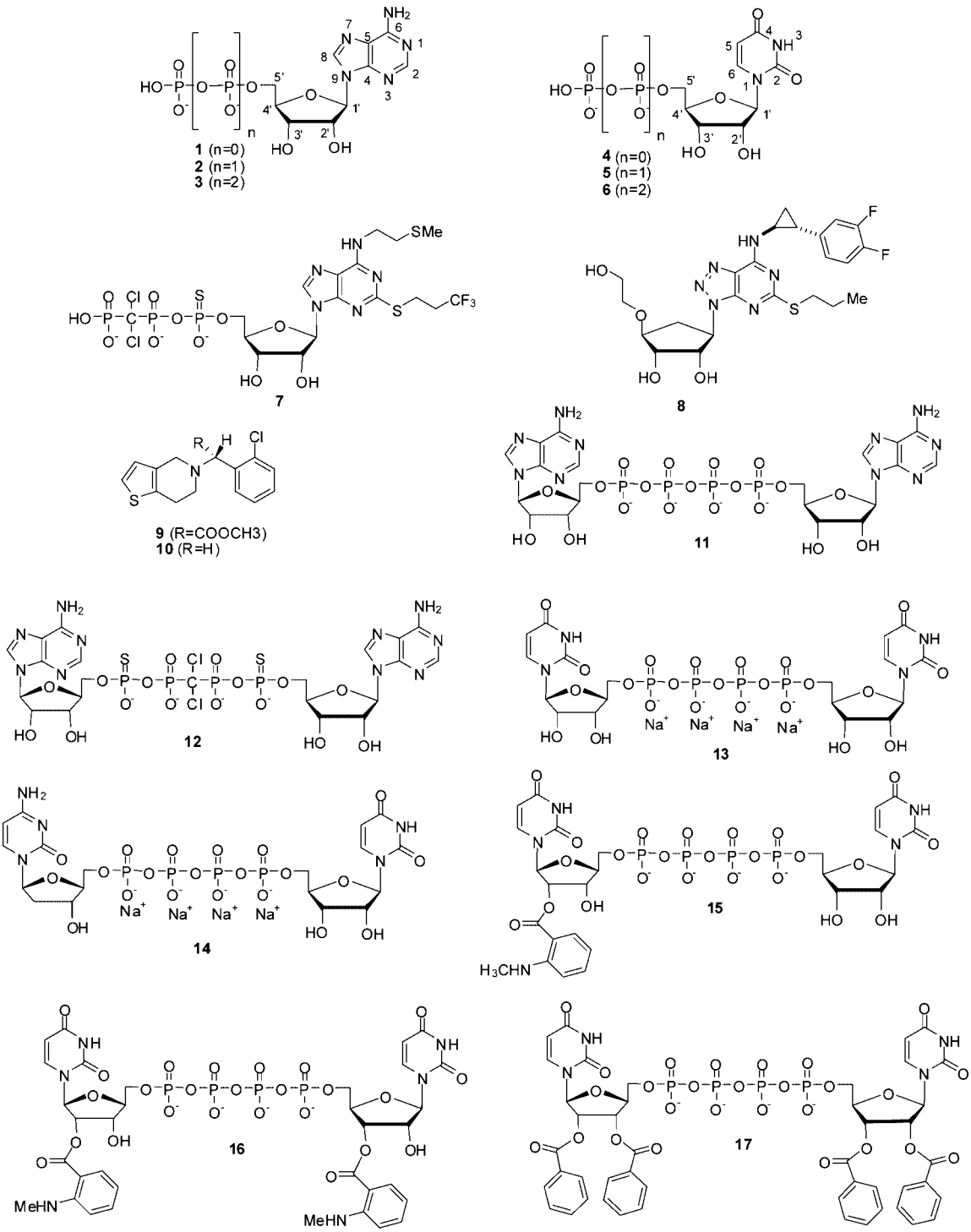


Figure 1. Structures of selected compounds.

In addition to its role as the agonist of multiple P2 receptors, ATP also antagonizes the action of ADP at the platelet $P2Y_1$ and $P2Y_{12}$ receptors.[13,14] Substitutions at the 2 and 6 positions of ATP have been shown to amplify both the potency and selectivity of this antagonist (see Figure 1 for ring numbering). The ATP analogue AR-C69931MX (cangrelor, **7**) is a highly potent $P2Y_{12}$ antagonist representative of this molecular class and is currently being evaluated in clinical trials for use during percutaneous coronary interventions.[15,16] Further work by the Astra Zeneca group led to the development of AZD6140 (**8**), a heavily modified adenosine-like molecule being evaluated as an oral drug for chronic therapy for the prevention of thrombotic events in patients with acute coronary syndrome.[17]

Several other molecular chemotypes have been shown to reversibly inhibit ADP-induced platelet aggregation. Scarborough et al.[18] reported on a series of benzothiazolo[2,3-c]thiadiazines and dioxo quinazolin sulfonyl ureas, which show high potency in platelet aggregation and $P2Y_{12}$ receptor binding assays. Recently, Wang et al.[19] described results for a quinolin/piperazin 5-oxopentanoic acid derivative, which showed an improved therapeutic index over clopidogrel in animal models.

The clinical use of the thienopyridines clopidogrel (**9**) and ticlopidine (**10**) has been shown to reduce the incidence of myocardial infarction and stroke in patients with a history of vascular disease and to provide protection from adverse events following stent implantation.[20–22] However, these drugs bind

to the P2Y$_{12}$ receptor in an irreversible manner following conversion to their respective active species by hepatic metabolism.[23] The irreversible nature of the inhibition of platelet function by clopidogrel and ticlopidine preclude their use in acute cardiovascular and cerebrovascular conditions where the strict control of platelet function is needed. Furthermore, significant interindividual variability in response to the drug has been seen in patients treated with clopidogrel.[24] Treatment with thienopyridines has also been associated with thrombotic thrombocytopenic purpura, a rare but potentially fatal side effect.[25] More recently, prasugrel, a third generation thienopyridine with apparently improved pharmacokinetic properties, has been developed.[26,27] Nevertheless, in spite of apparent progress in the thienopyridine class of irreversible inhibitors, opportunities still remain for the development of safe, rapidly reversible platelet aggregation inhibitors for the treatment of both acute and chronic clinical conditions.

Dinucleoside polyphosphates (Np$_n$N′), particularly those having adenine as the base, have been detected in chromaffin cells and platelet granules.[28,29] Physiological roles of diadenosine polyphosphates include inhibition of adenosine kinase,[30] release of nitric oxide from epithelial cells,[31] neurotransmitter release at synaptic junctions,[32] and inhibition of platelet aggregation.[33,34] In addition to being a potent P2Y$_2$ agonist, Ap$_4$A (**11**) is a modestly potent antagonist of ADP-induced platelet aggregation. Several analogues of Ap$_4$A with modifications in the phosphate chain (e.g., **12**)[35] were prepared in an effort to improve their biological stability and to examine their potency for the inhibition of platelet aggregation.

Because of our long-standing interest in the pharmacological properties and structure–activity relationships (SAR) of dinucleoside polyphosphates as agonists of the P2 superfamily of receptors, particularly P2Y$_2$, we synthesized a number of analogues bearing lipophilic modifications to the ribose and the base. During the course of this work, we found that esterification of one or more of the 2′- or 3′-ribose hydroxyls of a P2Y$_2$ receptor agonist produced compounds that antagonized the ability of ADP to induce platelet aggregation. In this article, we describe the identification and SAR of substitutions to dinucleoside polyphosphates and nucleotides that confer antagonist properties for the P2Y$_{12}$ receptor.

Chemistry

Ready access to commercially available nucleotides and an in-house collection of dinucleoside polyphosphates led us to choose these molecules as starting materials for our syntheses. To streamline the synthesis of the required analogues, the 5′-phosphate group was used as a protecting group. This strategy eliminated the long protection/deprotection procedures that have traditionally been the norm in nucleoside chemistry and facilitated the use of preparative reverse-phase HPLC to purify these water soluble but lipophilic products.

Starting with **15**, **16**, and **17** (Figure 1) as inspiration, we considered alternative modifications to the ribose hydroxyl groups. In addition to not being particularly stable, the monoesters of 2′, 3′-ribose diols demonstrate no chemoselectivity toward either the 2′ or 3′ group, providing mixtures of products upon reaction with acylating agents.[36] Furthermore, mixtures of products often result upon subsequent separation and purification because of the migration of the ester moiety between the adjacent hydroxyl groups. To achieve greater stability and access to regiochemically discreet analogues, cyclic acetals were prepared and examined. Previously, it was observed that acetal modifications to an analogue of **3** resulted in compounds that showed activity at several P2X receptors.[37]

A general method of forming cyclic acetals of acid sensitive dinucleoside polyphosphates and nucleotides was developed for the purpose of this work. Modest yielding reactions (see Table 1) were achieved with most substrates using the sodium salts of the starting dinucleoside polyphosphates or nucleotides with either 99% formic acid or anhydrous trifluoroacetic acid as both the catalyst and solvent in conjunction with 2–4 equivalents of the aldehyde or aldehyde dimethylacetal.

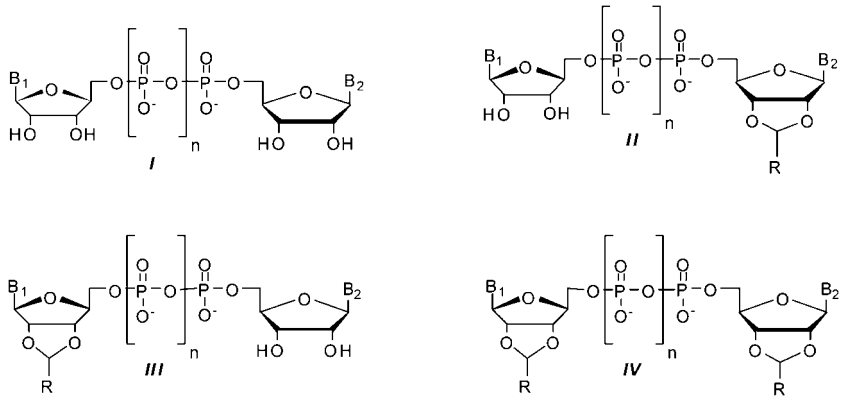
Treatment of symmetrical dinucleoside tri and tetraphosphates **11**, **13**, and **19** with a variety of aldehydes in acid gave a mixture of mono and diacetals (**37–38**, **20–31**, and **35–36**, respectively; formulas II and IV, Scheme 1). Likewise, similar treatment of the unsymmetrical dinucleoside tetraphosphate **18** gave the two possible regioisomeric monoacetals (**32** and **33**; formulas II and III) and diacetal **34** (formula IV). The structures of these compounds and their biological evaluation as antagonists of ADP-induced aggregation in a washed platelet assay are presented in Table 1.

Dinucleoside tetraphosphates **42** and **47–50** were prepared via an indirect synthetic strategy that also provided access to a variety of substituted nucleotide derivatives. This synthetic procedure gave better results than the direct action of an isocyanate on diacetals **38** and **34**.

These alternative strategies are outlined in Schemes 2 and 3. For the adenosine analogues, ADP (**2**) was converted to acetal **40** with phenylacetaldehyde dimethyl acetal in formic acid and transformed to the urea with phenylisocyanate in DMF to give **41**. Carbonyldiimidazole activation of **41** facilitated self-condensation to give **42**. To evaluate the effect of the urea modification in the platelet aggregation assay, **2** was also directly converted to the urea with phenylisocyanate to give **39**.[38] For the cytidine analogues, cytidine monophosphate (**43**) was converted to urea with *p*-fluorophenylisocyanate in DMF to give **44**. Use of **44** in the dinucleoside polyphosphate-forming reaction gave a better outcome than **43**. UTP (**6**) was activated with dicyclohexylcarbodiimide, leading to the cyclical trimeta-phosphate,[39] and was condensed with **44** to give **47**. Treatment of **47** with phenylacetaldehyde dimethylacetal in formic acid gave monoacetals **48** and **49** and diacetal **50**. For purposes of biological comparison, the same acetal and urea modifications were made to **43**, to give nucleotides **45** and **46**. Similarly, AMP (**1**) was converted to its corresponding 2′,3′-acetal (Scheme 4) providing nucleotides **52–55**. Compounds **52–55** were further modified to urea moieties at the 6-position of the purine by reaction of the amino group with an appropriate isocyanate in DMF. For these substrates, the isocyanate also acted as a weak condensing agent for the phosphate group, leading to variable amounts of the corresponding dinucleoside diphosphates as a byproduct (Scheme 4). These byproducts were easily separated from the desired nucleotides (**56–66**) during purification by HPLC. Biological results for these analogues are presented in Table 2. The biological results for compounds derived from **1** are presented in Table 3.

Acetal-forming reactions using benzaldehyde, cinnamaldehyde, and phenylpropargyl aldehyde produced mixtures of

Table 1. Inhibition of Platelet Aggregation and Selectivity for Dinucleoside Tri and Tetraphosphate Acetal Analogues[a]



compd	formula	B_1	B_2	R	n	conversion (%)	isolated yield[d] (%)	platelet aggregation IC_{50} (μM) mean ± SEM	$P2Y_1$ (EC_{50})	$P2Y_2$ (EC_{50})	$P2Y_6$ (EC_{50})
11	*I*	A	A	NA	3	ND	ND	63% inhibition at 100 μM	0.426 ± 0.236	0.072 ± 0.022	NR
13	*I*	U	U	NA	3	ND	ND	NR	NR	0.034 ± 0.011	18.64 ± 6.11
18	*I*	U	C	NA	3	ND	ND	NR	SR	0.044 ± 0.004	SR
19	*I*	U	U	NA	2	ND	ND	NR	NR	13.54 ± 5.71	0.40 ± 0.258
15	NA	U	U	NA	3	ND	31	46% inhibition at 100 μM	NR	0.130 ± 0.031	SR
16	NA	U	U	NA	3	ND	33.5	7.59 ± 0.42	NR	5.47 ± 1.16	SR
17	NA	U	U	NA	3	ND	31	52% inhibition at 100 μM	NR	NR	NR
20	*II*	U	U	benzyl	3	41	30	27.1 ± 6.64	NR	0.094 ± 0.033	SR
21	*IV*	U	U	benzyl	3	21	18	1.82 ± 0.717	NR	14.6 ± 0.94	SR
22	*II*	U	U	phenyl[b]	3	52	42	16.7 ± 3.45	NR	0.192 ± 0.052	SR
23	*IV*	U	U	phenyl[c]	3	18	6.7	8.29 ± 1.7	NR	3.07 ± 2.15	NR
24	*II*	U	U	styryl[b]	3	42	38	8.8 ± 3.96	NR	0.155 ± 0.116	NA
25	*IV*	U	U	styryl[c]	3	24	9.7	5.51 ± 2.12	NR	NR	SR
26	*II*	U	U	phenylethynyl[b]	3	37	32	8.68 ± 2.01	NR	0.068 ± 0.019	NA
27	*IV*	U	U	phenylethynyl[c]	3	9	3.5	3.3 ± 1.98	NR	0.35 ± 0.134	NR
28	*II*	U	U	propyl	3	40	23	44% inhibition at 100 μM	NR	0.098 ± 0.037	30
29	*IV*	U	U	propyl	3	50	30	60% inhibition at 100 μM	NR	SR	SR
30	*II*	U	U	heptyl	3	31	25	16.88 ± 5.19	NR	0.147 ± 0.075	SR
31	*IV*	U	U	heptyl	3	63	32	10.69 ± 3.91	NR	NA	NR
32	*II*	U	C	benzyl	3	22	20	46% inhibition at 100 μM	NR	0.545 ± 0.189	NR
33	*III*	U	C	benzyl	3	30	25	46% inhibition at 100 μM	NR	NR	NR
34	*IV*	U	C	benzyl	3	20	19	3.06 ± 0.62	NR	NR	NR
35	*II*	U	U	benzyl	2	52	38	34.73 ± 2.7	NR	NR	0.154 ± 0.041
36	*IV*	U	U	benzyl	2	28	20	4.63 ± 0.239	NR	NR	NR
37	*II*	A	A	benzyl	3	42	27.6	6.54 ± 0.63	NR	0.052 ± 0.006	NR
38	*IV*	A	A	benzyl	3	11	6.1	11.71 ± 5.17	NR	SR	NR

[a] Formula, B_1, B_2, R, and n are as defined in Scheme 1. Conversion % was determined by HPLC. Inhibition of platelet aggregation and selectivity (activity at $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors) are expressed in μM. SR = slight response (5 to 20% of the maximal activity at highest concentration tested, usually 100 μM); NR = no response at highest concentration tested (usually 100 μM); ND = not determined; NA = not applicable. [b] Mixture of 2 diastereomers. [c] Mixture of 4 diastereomers. [d] Low isolated yields relative to % conversions are a reflection of the degree of difficulty of the purifications.

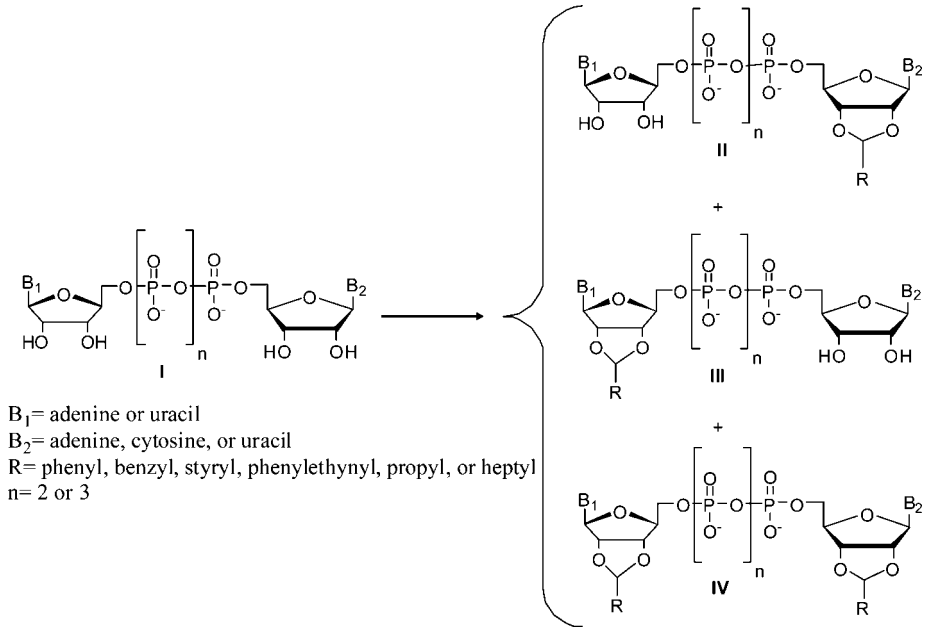
diastereomers because of the nonselective establishment of the stereogenic acetal carbon (see Figure 2).[40,41] As a result, the dinucleoside tri and tetraphosphate monoacetals derived from these aldehydes comprise two inseparable diastereomers, while the diacetals comprise four inseparable diastereomers. This pattern of selectivity was not observed for the acetals derived from aldehydes with an sp^3 carbon adjacent to the carbonyl (phenylacetaldehyde, butanal, and octanal).[42,43]

For the dinucleoside polyphosphates, the acetal configuration of phenylacetaldehyde diacetal **21**, octanal diacetal **31**, and phenylacetaldehyde diacetal **38** was established to be cis via 2D NMR experiments (NOESY).[42] The data indicated that the nature of the base (adenine in **11** vs uracil in **13**) and the number of acetals being formed had no effect on the outcome of the acetal-forming reaction. On this basis, assignment of the *cis*-acetal configuration was inferred for dinucleoside polyphosphates **20**, **28–38**, and **42**. The configuration of the acetal in cytidine analogue **45** was shown to be cis via NOE experiments.[42] On the basis of this result, the configuration of the acetal carbon in dinucleoside tetraphosphates **46** and **48−50** was inferred to be cis.

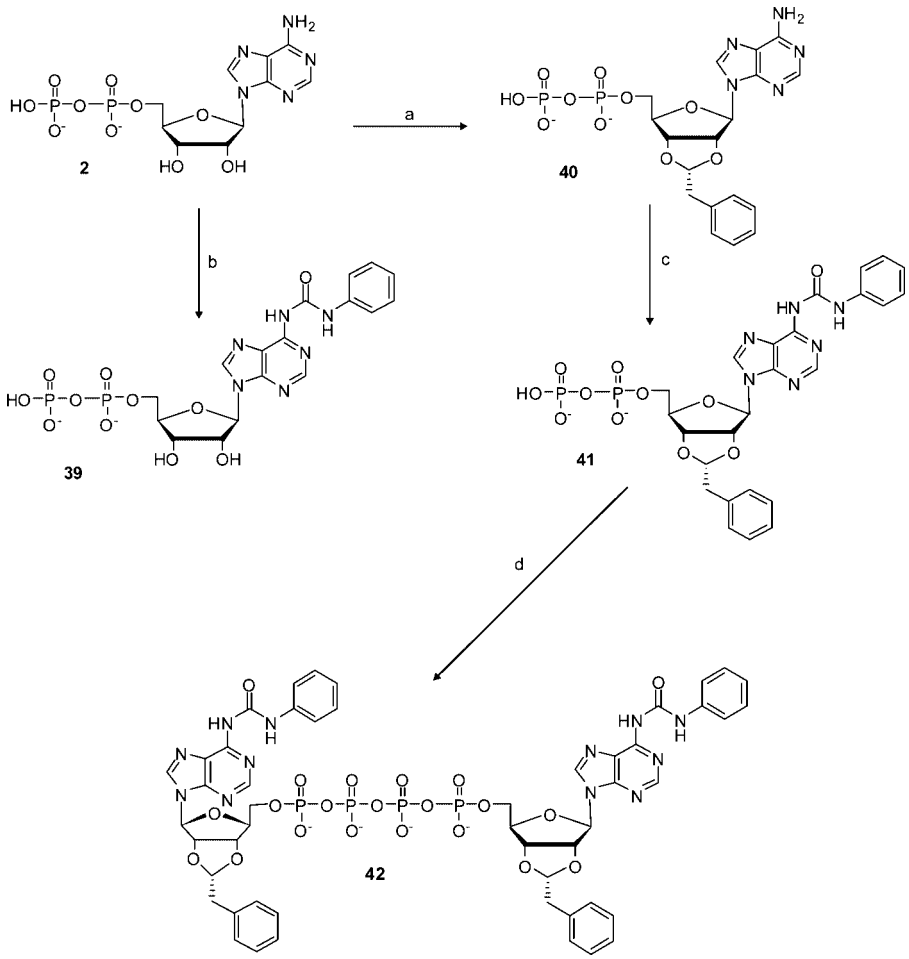
For compounds derived from monophosphate **1**, acetal-forming reactions provided either a single acetal diastereomer, such as **52**, or a mixture of two possible diastereomers such as nucleotides **53–55** (Table 3). The level of diastereoselectivity in these analogues could be quantified by HPLC and/or ^{1}H NMR (see Experimental Details). The *cis*-acetal configuration of **52** was established via 2D NMR experiments (NOESY) and was consistent with previous results in the cytidine nucleotide series (**45**), the adenosine nucleotide series (**58**), the dinucleoside polyphosphate series, and with the observations of others.[40,41]

As they were derived from compounds **53−55**, the urea products **60–66** were obtained as mixtures of diastereomers. Likewise, compounds **56–59** were all the single cis isomer, as they were derived from **52**. NOE experiments were performed on a representative of this latter group (compound **58**) to confirm that the configuration of the acetal carbon was

Scheme 1. General Synthesis of Dinucleoside Polyphosphate Acetals



Scheme 2. Synthesis of Diadenosine Tetraphosphate and Adenosine Analogues[a]

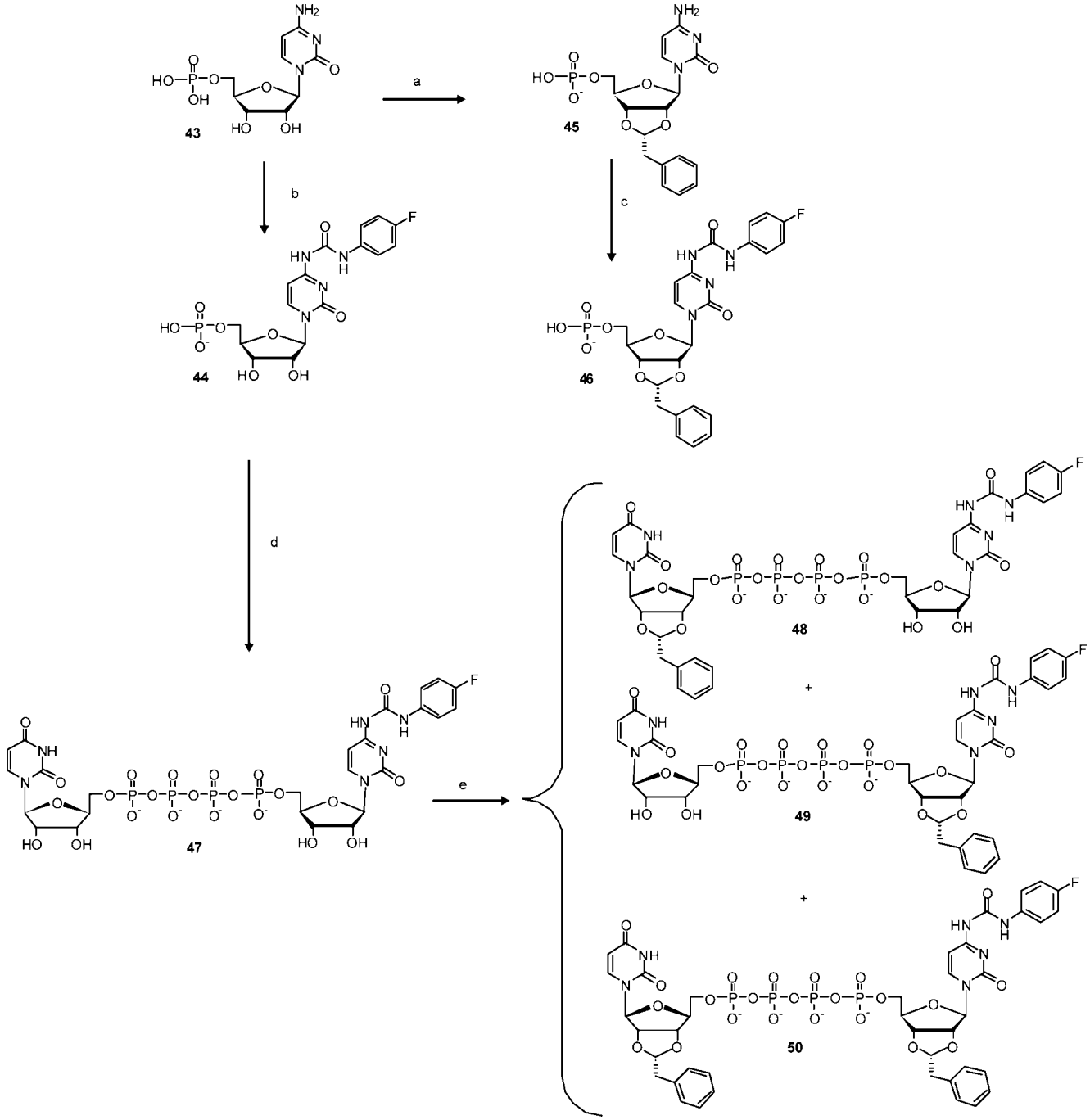


[a] Reagents and conditions: (a) Phenylacetaldehyde dimethyl acetal, HCOOH, 35°C; (b) PhNCO, DMF, 45°C; (c) PhNCO, NBu_3, DMF 30°C; (d) CDI, NBu_3, DMF, rt.

unchanged after the urea-forming reaction.[42] Assignment of the diastereomeric ratios for other analogues were made by correlation and integration of selected protons in the spectra of the mixture.

Taking advantage of the relative instability of the minor (cis) styryl acetal diastereomer in **61** toward aqueous acid, we cleaved its acetal to make the separation from the trans isomer **62** possible. NOE experiments performed on **62**

Scheme 3. Synthesis of Uridine Cytidine Tetraphosphate and Cytidine Analogues[a]



[a] Reagents and conditions: (a) Phenylacetaldehyde dimethyl acetal, HCOOH, rt; (b) *p*-F-PhNCO, DMF, 60°C, then 30°C; (c) *p*-F-PhNCO, NBu_3, DMF, rt; (d) **5**•$(NBu_3)_2$, DCC, DMF, 30°C; (e) phenylacetaldehyde dimethyl acetal, HCOOH, 30°C.

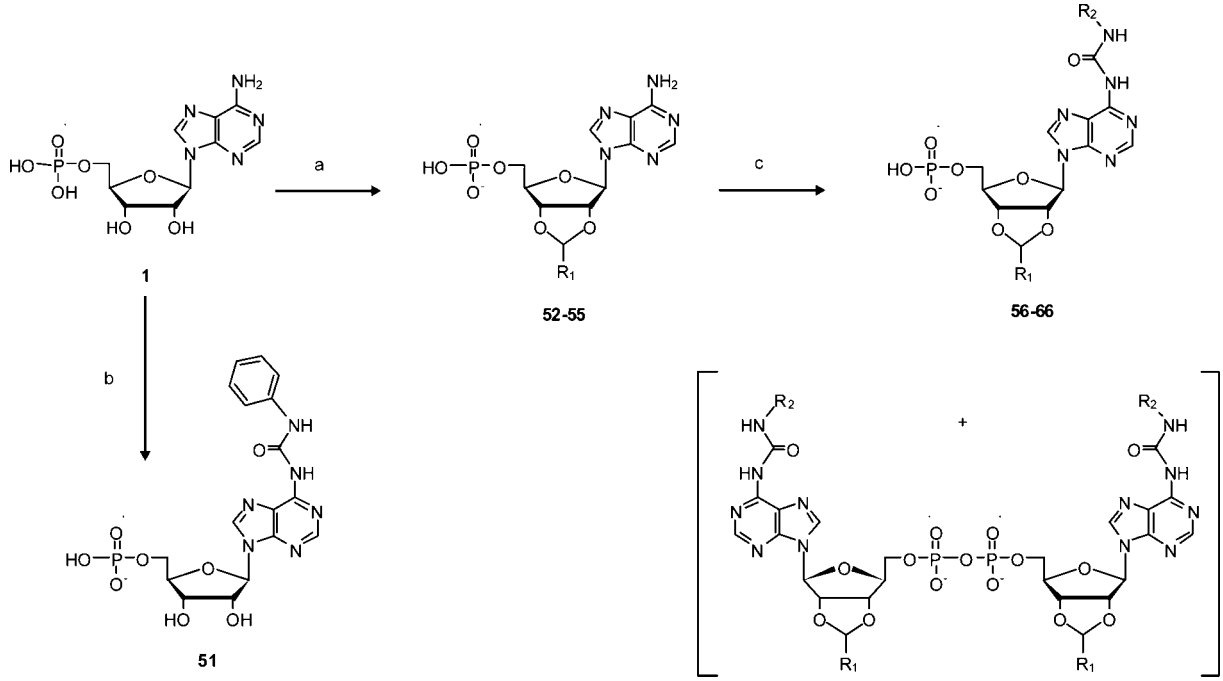
confirmed the trans orientation of the acetal moiety in this molecule (see Figure 2).[42]

Results and Discussion

Platelet Aggregation Studies. While the natural dinucleoside tetraphosphates **13** and **18** and the dinucleoside triphosphate **19** are agonists of the $P2Y_2$ receptor and the $P2Y_6$ receptor,[44] respectively, they are devoid of activity at any of the platelet nucleotide receptors. Only compound **11**, a potent $P2Y_2$ agonist (which has been found in platelet granules and is a close analogue of the natural $P2Y_{12}$ antagonist **3** has shown modest potency to inhibit platelet aggregation (63% inhibition at 100 μM in a washed platelet preparation). One of the initial results that led to this study was the observation that the *N*-methyl anthranilic acid esters (**15** and **16**, Figure 1)[45,46] or benzoic acid esters (**17**, Figure 1) of diuridine tetraphosphate **13** modestly antagonized ADP-induced platelet aggregation (46% inhibition at 100 μM, IC_{50} = 9 μM, and 52% inhibition at 100 μM, respectively). In an effort to determine whether the degree or type of substitution was important for inhibition of platelet aggregation activity, we decided to replace the ester moieties with a series of acetal substituents (propyl, heptyl, phenyl, benzyl, styryl, and ethynyl phenyl).

Review of the activities of the diuridine tetraphosphate monoacetals of **13** (compounds **20**, **22**, **24**, **26**, **28**, and **30**) revealed modest antagonism of platelet aggregation (Table

Scheme 4. Synthesis of Adenosine Monophosphate Analogues[a]



[a] Reagents and conditions: (a) R_1CHO, TFA, or HCOOH, rt; (b) PhNCO, DMF, 45°C; (c) R_2NCO, DMF.

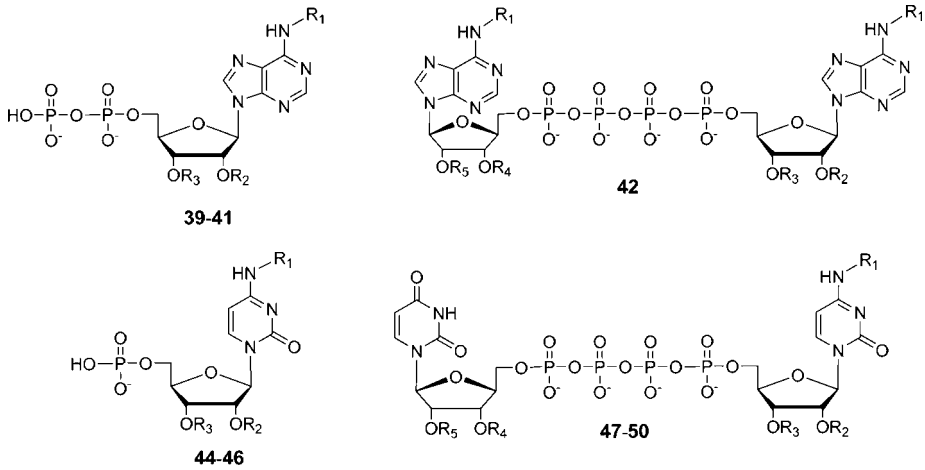
1). These diuridine tetraphosphate monoacetals demonstrated higher potency for inhibiting ADP-induced platelet aggregation than monoester **15**. There appeared to be a slight preference for the styryl (**24**) and ethynyl phenyl (**26**) derivatives. Interestingly, benzyl acetal diuridine triphosphate **35** (34.73 μM) was found to be comparable in potency to its tetraphosphate **20**, suggesting that there is some tolerance for variation in the polyphosphate chain length. Consistent with the trend observed for esters **15** and **16**, substitution of diuridine tetraphosphate **13** with two lipophilic acetals (compounds **21**, **23**, **25**, **26**, **29**, and **31**, Table 1) provided analogues with slightly improved potency when compared with their monosubstituted counterparts. Notably, the slight preference for the styryl and ethynyl phenyl derivatives remained consistent. The dibenzyl acetal diuridine triphosphate **36** also showed good potency as an inhibitor of ADP-induced platelet aggregation (IC_{50} = 4.63 μM), which was consistent with that observed for the analogous tetraphosphate **21** (IC_{50} = 1.82 μM).

Modification of diadenosine tetraphosphate **11** with either one or two benzyl acetals (**37** and **38**) provided potencies comparable to those of analogues derived from diuridine tetraphosphate **13**. Lastly, for the unsymmetrical cytidine−uridine dinucleoside tetraphosphate **18**, the two possible monobenzyl acetals (**32** and **33**) were only weakly active in the platelet aggregation assay, while the corresponding dibenzyl acetal **34** had a potency of 3.06 μM. This value was comparable to that of the most potent analogues in the diuridine tetraphosphate acetal series. These results established the importance of polysubstitution on the 2′,3′-ribose hydroxyl groups for the enhancement of potency and confirmed that more chemically stable acetal substituents could replace the *N*-methyl anthranilic ester moieties without the loss of potency.[47]

None of the tested lipophilic modifications at the ribose hydroxyl groups of **13** led to compounds with sufficient potency as platelet aggregation inhibitors to be advanced further into development. However, the benzyl acetal modifications to diadenosine tetraphosphate **11** and uridine−cytidine tetraphosphate **18** provided diacetals of comparable potency to those derived from **13**, and these molecules offered an exocyclic amino group as an additional point of modification. Accordingly, modification of **38** to a phenyl urea at the 6-amino position provided diurea **42**. Remarkably, **42** was 20-fold more potent than its unsubstituted diadenosine tetraphosphate diacetal core **38** (0.52 vs 11.71 μM). Similarly, conversion of **34** to a *p*-fluoro-phenylurea in the 4-position resulted in **50**, which was 60-fold more potent than its cytidine−uridine tetraphosphate diacetal core **34** (0.048 vs 3.06 μM). This dramatic effect led us to further examine the contributions of the benzyl acetal and the *p*-fluoro-phenylurea moieties on the potency of the cytidine−uridine tetraphosphate core. The *p*-fluoro-phenylurea of Up_4C (**47**) was found to be inactive. The monobenzyl acetal (U)/*p*-fluoro-phenylurea of Up_4C (**48**) was found to have an IC_{50} = 23.93 μM, while the monobenzyl acetal (C)/*p*-fluoro-phenylurea of Up_4C (**49**) was found to have an IC_{50} = 12.92 μM. Interestingly, it was essential for both riboses to be converted to acetals in the dinucleoside tetraphosphate core to achieve high nanomolar activity.

We next examined this dramatic substituent effect on CMP (**43**). Acylation of **43** with *p*-fluorophenylisocyanate or conversion of **43** to the corresponding *cis*-acetal with phenylacetaldehyde produced **44** and **45**, respectively, which were inactive. However, the combination of both modifications converted **43** into a micromolar antagonist (**46**, IC_{50} = 11.17 μM, Table 2). Similar modifications of ADP (**2**), a natural agonist of $P2Y_{12}$ receptor, gave a comparable SAR pattern, albeit with a more favorable outcome. The *cis*-benzyl acetal (**40**) was found to be a modestly active inhibitor. Likewise, modification of **2** with a phenylurea moiety (**39**) also converted the agonist into an antagonist. The incorporation of both of these lipophilic modifications led to **41**, with potency for inhibition of aggregation of 0.052 μM, comparable to the best of the dinucleoside tetraphosphate series (**50**). These data are summarized in Table 2.

Table 2. Inhibition of Platelet Aggregation and Selectivity for Dinucleoside Tetraphosphate Acetal and Urea Analogues, and Their Synthetic Intermediates[a]



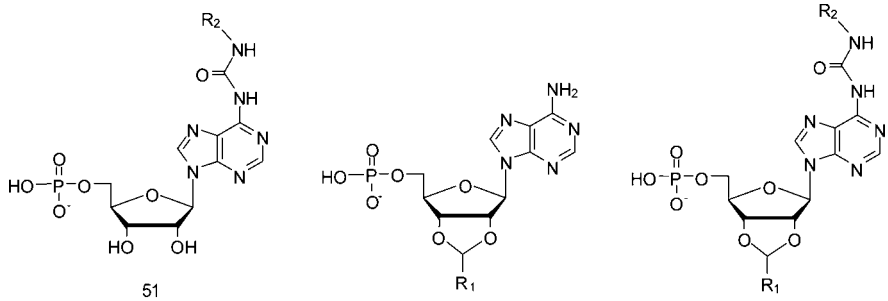
Compound	R_1	R_2/R_3	R_4/R_5	Platelet Aggregation IC_{50} (μM) mean ± SEM	$P2Y_1$ (EC_{50})	$P2Y_2$ (EC_{50})	$P2Y_6$ (EC_{50})
39		H/H	NA	10.8 ± 1.75	2.63 ± 1.25	9.4 ± 4.33	9.75 ± 4.40
40	H		NA	23 ± 2.68	4.39 ± 2.97	13.94 ± 6.22	NR
41			NA	0.052 ± 0.011	SR	13.94 ± 6.25	SR
42				0.522± 0.17	NR	NR	NT
44		H/H	NA	NR	NT	NT	NT
45	H		NA	NR	NR	NR	NR
46			NA	11.17 ± 3.62	NR	NR	NR
47		H/H	H/H	NR	SR	2.42 ± 0.23	NT
48		H/H		23.93 ± 12.58	NR	NR	NT
49			H/H	12.92 ± 8.47	NR	SR	NT
50				0.048 ± .009	NR	NR	NR

[a] Platelet aggregation, inhibition, and selectivity (agonist activity at $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors) are expressed in μM. SR = slight response (5 to 20% of the maximal activity at highest concentration tested, usually 100 μM); NR = no response at highest concentration tested (usually 100 μM); NT = not tested; NA = not applicable.

On the basis of the results for **41** and **46**, we chose to examine similar modifications for **1** (Scheme 4). Only those acetal substitutions that produced the more potent compounds of the dinucleoside polyphosphate series were prepared and tested for potency. By examining the potency of substituted adenosine diphosphate **41** and cytidine monophosphate **46**, it was clear that the purine core is an essential component for maintaining the potency for the inhibition of aggregation. Furthermore, although highly potent antagonists would likely have been found in series based on **2** (or even **3**), the chemical instability of the di and triphosphate groups was deemed a significant drug development obstacle.[48]

Much as was seen for phenylurea analogue **39**, the reaction of **1** with phenylisocyanate converted an inactive compound into a modestly active antagonist (**51**). Similarly, the formation of acetals of **1** (phenyl, benzyl, styryl, and ethynyl phenyl) also

Table 3. Inhibition of Platelet Aggregation and Selectivity for Adenosine Acetal and Urea Analogues[a]



Compound	R_1	R_2	Platelet Aggregation IC_{50} (μM) mean ± SEM	$P2Y_1$ (EC_{50})	$P2Y_2$ (EC_{50})	$P2Y_4$ (EC_{50})	$P2Y_6$ (EC_{50})
51	NA		3.11 ± 0.378	NR	NR	NR	NR
52		NA	47% inhibition at 100 μM	NR	NR	NR	NR
53		NA	75% inhibition at 100 μM	NR	NR	NR	NR
54		NA	45.07 ± 6.86	NR	NR	NR	NR
55		NA	57.07 ± 12.5	NR	NR	NR	NR
56			0.16 ± 0.027	NR	NR	NR	NR
57		n-hexyl	0.053 ± 0.016	NR	NR	NR	NR
58		ethyl	0.041 ± 0.007	NR	NR	NR	NR
59		cyclopentyl	0.0362 ± 0.0094	NR	NR	NR	NR
60		ethyl	0.013± 0.002	NR	NR	NR	NR
61		ethyl	0.011 ± 0.0012	NR	NR	NR	NR
62		ethyl	0.016 ± 0.0013	NR	NR	NR	NR
63		n-hexyl	0.237 ± 0.111	NR	NR	NR	NR
64			0.210 ± 0.06	NR	NR	NR	NR
65		n-hexyl	0.211 ± 0.08	NR	NR	NR	NR
66			0.288 ± 0.05	NR	NR	NR	NR

[a] R_1 and R_2 are as defined in Scheme 4. Inhibition of platelet aggregation is expressed in μM. SR = slight response (5 to 20% of the maximal activity at highest concentration tested, usually 100 μM); NR = no response at highest concentration tested (usually 100 μM); NA = not applicable.

provided analogues that were modestly active (**52**–**55**). Preparation of the monophosphate analogue of **41** (phenylurea and benzyl acetal, **56**) showed an approximately 3-fold loss in potency (0.052 vs 0.160 μM, respectively), which suggested that truncating the phosphate chain would not lead to an insurmountable loss of potency. Comparison of the phenyl urea moiety in the acetal series benzyl, styryl, and ethynyl phenyl (**56**, **64**, and **66**, respectively) did not reveal any obvious advantage of any of these acetals for increased potency.

A switch to aliphatic ureas led to a breakthrough in potency for the monophosphate series. However, inconsistent trends were found when comparisons were drawn across different acetal series. The ethyl urea derivatives **58** (*cis*-benzyl acetal), **60** (mixture of phenyl acetals), **61** (mixture of styryl acetals), and **62** (*trans*-styryl acetal) were 0–5-fold more potent than diphosphate **41**, making them the most potent compounds in this study (0.041, 0.013, 0.011, and 0.016 μM, respectively). Preparation of three *n*-hexyl urea derivatives **57** (*cis*-benzyl

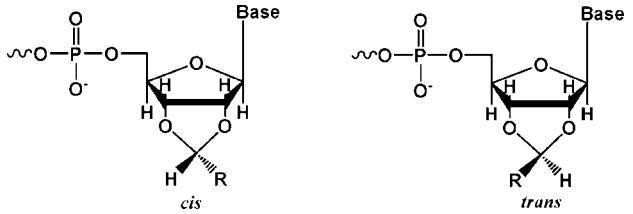


Figure 2. Acetal diastereoisomerism.

acetal), **63** (mixture of styryl acetals), and **65** (mixture of ethynyl phenyl acetals) led to derivatives with a clear preference for the benzyl acetal (0.053, 0.237, and 0.211 μM, respectively). Comparison of three alkyl ureas in the benzyl acetal series *n*-hexyl (**57**), cyclopentyl (**59**), and ethyl (**58**) revealed no preference for any of the urea alkyl groups (0.053, 0.036, and 0.041 μM, respectively). On the basis of pharmacological, physicochemical, and synthetic considerations, compound **62** was nominated as a candidate for further development as an inhibitor of platelet aggregation. A full pharmacological and biochemical characterization of this compound will be reported elsewhere.

P2Y Receptor Selectivity. Since inhibition of ADP-induced platelet aggregation could result from the blockade of either the $P2Y_1$ or the $P2Y_{12}$ receptors, we ruled out any activity of these compounds at the $P2Y_1$ receptor by conducting calcium mobilization assays in 1321N1 human astrocytoma cells expressing the recombinant human $P2Y_1$ receptor (Tables 1, 2, and 3). None of the compounds that inhibited ADP-induced platelet aggregation had any effect as an antagonist at the $P2Y_1$ receptor cell line, suggesting that the inhibition of aggregation by ADP was mediated by the antagonism of the $P2Y_{12}$ receptor. This observation was consistent with the lack of effect of these compounds on the $P2Y_1$ receptor-mediated platelet shape change induced by ADP and further confirmed in a selected number of compounds by the inhibition of the $P2Y_{12}$ receptor response in C6 rat glioma cells, which express the native $P2Y_{12}$ receptor.[49]

The diuridine tetraphosphate monoacetals **20**, **22**, **24**, **26**, **28**, and **30** were all $P2Y_2$ agonists, exhibiting potencies up to 6-fold lower than that of **13**. Consistent with this trend, the diadenosine tetraphosphate monobenzyl acetal **37** was slightly less potent as a $P2Y_2$ agonist than **11**. Similarly, the cytidine−uridine tetraphosphate monoacetal (C), **32**, was approximately 10-fold less potent at $P2Y_2$ than **18**; however, the cytidine−uridine tetraphosphate monoacetal (U), **33**, was inactive. Dinucleoside triphosphate monoacetal **35** showed slightly higher agonist potency at $P2Y_6$ than **19** (0.154 vs 0.40 μM, Table 1). The corresponding diacetal **36** was not active at this receptor. Likewise, diacetal analogues derived from the three dinucleoside tetraphosphate cores showed a marked reduction in agonist potency toward the $P2Y_2$ receptor (Table 1). For example, **27** exhibited a 10-fold loss of agonist potency (EC_{50} ($P2Y_2$) = 0.350 μM) in a calcium mobilization assay, relative to core **13**. The other diacetals in this series were even less active at $P2Y_2$ than **27** (compounds **21** and **23**) or showed little to no activity (compounds **25** and **29**). The dibenzyl acetals **34** and **38** were also inactive at $P2Y_2$. Taken together, these results suggest that increasing lipophilic modification of the hydroxyl groups of dinucleoside polyphosphates leads to both increased potency and selectivity toward the platelet $P2Y_{12}$ receptor.

In the nucleotide series, monophosphate **56** showed greater selectivity over the $P2Y_1$, $P2Y_2$, and $P2Y_6$ receptors versus its diphosphate counterpart **41**. In addition, it was not active at the $P2Y_4$ receptor. This trend held true for compounds **51**–**66** (Table 3).

On the basis of these results, the described modifications of the nucleotides and dinucleoside polyphosphates described herein confer antagonist properties to these analogues and eliminate the $P2Y_2$ and $P2Y_1$ activity, achieving selectivity for the inhibition of platelet aggregation toward the $P2Y_{12}$ receptor.

Dinucleoside polyphosphates and nucleotides modified with acetals and ureas were able to inhibit the aggregation of platelets in a concentration-dependent manner. The inhibitory effects of these compounds have been demonstrated using several platelet preparations, including whole blood, platelet rich plasma (PRP), and washed platelet preparations. The potency of a given compound in these assays varies among the different platelet preparations, mainly because of differences in the protein-binding characteristics of the compound. In general, the apparent potency observed for the compounds of this study was highest in the washed platelet preparation followed by whole blood and PRP. For example, compound **62** exhibited a potency of 0.016 μM in the washed platelet assay, while the potencies in whole blood and PRP were 0.180 μM and 0.760 μM, respectively. All data shown in this study were obtained with the washed platelet preparation.

In order to study the nature of the inhibition of platelet aggregation, we used the diuridine tetraphosphate di-2′,3′-phenylacetaldehyde acetal derivative **21**. Compound **21** produced a dose-dependent shift to the right in the platelet aggregation concentration–response curve of the ADP analogue 2-MeS-ADP (Figure 3A). The Schild analysis[50] suggested that the antagonism demonstrated by **21** on platelet aggregation is competitive (Figure 3B), with a K_B of 0.249 μM and a slope of 0.742. The reversibility of the effects of **21** on platelet aggregation was studied in a preparation of washed platelets. Platelets were treated with 50 μM **21** for 3 min at 37 °C in the absence of fibrinogen, then were either stimulated with 2-MeS-ADP and fibrinogen or were washed once by centrifugation before stimulation with 2-MeS-ADP and fibrinogen (Figure 4A and 4B). Unlike the thienopyridines **9** and **10**, the compounds reported here, as exemplified by compound **21**, behaved as competitive and reversible antagonists of platelet aggregation mediated by the $P2Y_{12}$ receptor.

Conclusions

These studies showed that acetal substitution on the 2′,3′-ribose moiety of dinucleoside polyphosphates and nucleotides was an important determinant in conferring antagonist properties toward the $P2Y_{12}$ receptor. Indeed, this effect was robust enough that dinucleoside polyphosphates such as **13** and **18**, which normally do not interact with the $P2Y_{12}$ receptor as either an agonist or antagonist, were converted via these modifications into strong antagonists. Expansion of this concept around dinucleoside polyphosphate **13** demonstrated that a variety of substituents on the acetal moiety were well tolerated, giving structurally diverse analogues on which a SAR campaign could be based. We found that the number of acetals (lipophilic substitutions) correlated directly with the potency of the molecule to inhibit aggregation, confirming the observations made with initial screening hits, **15** and **16**. Furthermore, there was a direct relationship between enhanced $P2Y_{12}$ antagonist potency with increasing lipophilic substitution and decrease in agonist potency at several other P2Y receptors, suggesting that both potent and selective compounds might be developed in this series.

We further identified the exocyclic amino group of adenine and cytidine-containing dinucleoside polyphosphates and nucleotides as a second site for lipophilic modification, where ureas

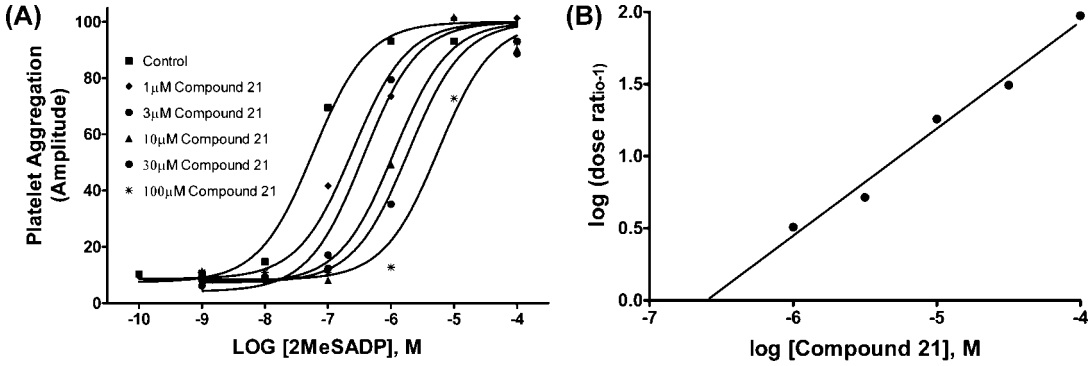


Figure 3. Effect of compound **21** on 2-MeS-ADP-promoted platelet aggregation. (A) Platelet aggregation was measured as described in General Methods. Assays were in the presence of the indicated concentrations of 2-MeS-ADP alone or in the presence of 1, 3, 10, 30, or 100 μM compound **21**. (B) Arunlakshana−Schild plot from the data presented in A. The slope of the plot is 0.7425, and K_B is 0.251 μM.

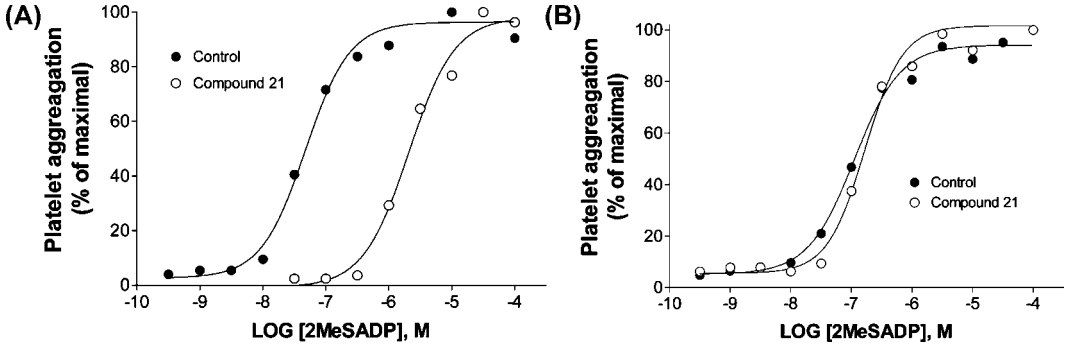


Figure 4. Reversible inhibition of platelet aggregation by compound **21**. Platelets were incubated in the absence (control) or in the presence of 50 μM of **21**. In panel A, after a three minute incubation with drug, the indicated concentrations of 2-MeS-ADP and fibrinogen were added, and platelet aggregation was monitored during the following 6–8 min. In panel B, after the three minute incubation with drug, both control and compound **21**-treated platelets were centrifuged, the supernatant was removed, and the platelets were resuspended in drug-free media. Following this, the indicated concentrations of 2-MeS-ADP and fibrinogen were added, and the aggregation was monitored as described above.

in this position greatly enhanced the antagonist potency. In most cases, modification of either a ribose with a 2′,3′-acetal moiety or a base with a urea was enough to generate at least some antagonist behavior. However, these modifications proved to be synergistic, and the disubstituted compounds were the more potent antagonists of platelet aggregation. The compounds reported here are competitive and reversible antagonists of the $P2Y_{12}$ receptor. While some of the dinucleoside polyphosphates were potent antagonists of aggregation (compounds **42** and **50**), we found that the substituted nucleotide **41**, used to prepare **42**, was more potent than its dinucleoside polyphosphate product. Optimization of **41** led to a series derived from adenosine 5′-monophosphate (**1**), of which one, **62**, was selected for in vivo preclinical studies. On the basis of positive results from animal studies, compound **62** was nominated for further development, where it was subsequently advanced to clinical trials.[51] This study provides the basis for further development of potent and selective antagonists of the $P2Y_{12}$ receptor that should prove useful for the treatment of cardiovascular diseases.

Experimental Details

General Methods. All reagents were acquired from commercial suppliers and were generally used without further purification. All reactions requiring anhydrous conditions were performed under nitrogen, using anhydrous solvents packaged in septum bottles. ^{1}H and ^{31}P NMR spectra were obtained at 300 and 121.5 MHz respectively, using Varian Gemini 2000. Generally, samples run in D_2O were pretreated with a chelating resin (Chelex 100 Na) in water, followed by lyophilization. This was done to remove paramagnetic ions in an effort to improve the NMR resolution. When resolution was still unsatisfactory, additional experiments were carried out in CD_3OD or DMSO-d_6.

Analytical HPLC chromatograms were obtained on either Waters 600S or Waters Alliance 2795 equipped with diode array detectors. The C_{18} columns used (either Waters Xterra RP_{18}, 150 × 4.6 mm, 3.5 μm, or Alltech Apollo C_{18}) were run employing a linear gradient from 0.02 M KH_2PO_4/0.005 M $NBu_4^+HSO_4$ (pH adjusted to 5.0 with KOH) to 90% ACN 10% water over 20 min (1 mL/min), a linear gradient from 0.1% TFA to ACN over 20 min (1 mL/min), or a linear gradient from 0.05 M NH_4OAc (pH 6) to ACN over 20 min (1 mL/min). The former ion pairing system allowed the analysis of unmodified parent dinucleoside polyphosphates to be carried out under reverse-phase conditions. It also was employed to detect the presence or absence of diastereomers arising from the acetal moieties and to quantify the diasteromeric ratios in compounds **53–55** and **60–66**. The NH_4OAc and TFA systems generally did not provide information on diastereomeric ratios but were used to assess the overall purity of the modified nucleotides. Purities of test compounds exceeded 95% and in most cases 98%, as measured in these systems.

Unmodified dinucleoside polyphosphates **11**, **13**, **18**, and **19** were purified using either Waters Delta 600 or Prep LC 2000 with diode array detection, in conjunction with an ion-exchange preparative column (Hamilton PRP X-100 anion exchange column, 20 μm; gradient from water to 1 M ammonium bicarbonate, 100 mL/min). Alternately, these molecules were purified using DEAE Sephadex in a medium pressure mode, utilizing a gradient from water to 1 M ammonium bicarbonate. Dinucleoside polyphosphates and nucleotides modified with lipophilic substituents were generally purified by preparative RPHPLC (Waters PrepLC system, equipped with either two or three 25 × 100 mm or two 40 × 100 mm Waters NovaPak C_{18} segments, depending on the scale of the reaction to

be purified; gradient from 0.05 M NH_4OAc to ACN, 20–70 mL/min, diode array detection) and isolated as the ammonium salts. Alternately, products from small-scale reactions were separated on a semipreparative reverse-phase column (Alltech Nucleotide/Nucleoside C_{18}, 10–250 mm, 7 μm, gradient from 0.1 M NH_4OAc to MeOH, 5 mL/min, diode array detector). Irrespective of the preparative RPHPLC purification method, dry products were obtained by partial evaporation, followed by lyophilization.

LR-LCMS were obtained using a Waters Micromass ZQ mass spectrograph (ESI+ and ESI-) coupled to a Waters Alliance 2695 HPLC. For the lipophilic dinucleoside polyphosphate and nucleotides **15**–**42** and **44**–**66**, a linear gradient from 0.05 M NH_4OAc (pH 6) to ACN over 20 min (0.5 mL/min) was used.

Platelet Aggregation Assay. Blood was collected from healthy volunteers into syringes containing 1/6 final blood volume of anticoagulant ACD (65 mM citric acid, 85 mM sodium citrate, and 110 mM dextrose). The blood was centrifuged at 180*g* for 15 min, and the supernatant (platelet rich plasma) was removed. The platelet rich plasma was centrifuged and the platelet pellet resuspended in a buffer consisting of (mM) NaCl (137), KCl (2.7), $CaCl_2$ (2) $MgCl_2$ (1), NaH_2PO_4 (3), glucose (5), HEPES (10) at pH 7.4, and 0.2% bovine serum albumin. These centrifugations and washes were repeated twice followed by resuspension in the media described above containing 0.25 U apyrase/mL. To prevent platelet activation during the procedure, all platelet resuspensions were conducted in the presence of 5 μM PGI_2, followed by incubation at 37 °C for 10 min. Platelet suspensions were further supplemented with the same concentration of PGI_2 before each centrifugation. Platelet aggregation was measured using the optical mode of a ChronoLog aggregometer (Havertown, PA). Five hundred microliters of platelet suspension containing 1 mg/mL fibrinogen was warmed to 37 °C and stirred at 1000 rpm. Several concentrations of test compound (usually between 1 nM and 100 μM) were added to the platelet suspension and incubated for two minutes, followed by the addition of a maximally effective concentration of ADP (EC_{90}; usually between 1 and 10 μM ADP). The aggregation was recorded for 8 min.

Calcium Mobilization Assay. For calcium mobilization assays, 1321N1 human astrocytoma cells were loaded with a solution of Fluo-3 AM (2.5 μM final concentration) in an assay buffer consisting of (mM) KCl (10), NaCl (118), $CaCl_2$ (2.5), $MgCl_2$ (1), HEPES (20), and glucose (10) at pH 7.4. After a 60-min incubation with Fluo-3 AM at 25 °C, cells were washed and stimulated with the indicated concentrations of P2Y receptor agonists. Intracellular calcium levels were simultaneously monitored in each well by measuring the changes in fluorescence intensity using FLIPR (Molecular Devices Corp., Sunnyvale, CA).

Data Analysis and Statistics. Data for selected compounds were expressed as the mean ± standard error of the mean (SEM). The potency of inhibitors of platelet aggregation was calculated from the inhibition of ADP-induced aggregation obtained at each concentration of test compound by fitting the data to a four-parameter logistic equation using the GraphPad software package (GraphPad Corp. San Diego, CA). When a broad range of concentrations of $P2Y_{12}$ antagonist were tested (usually from 1 nM to 100 μM), an IC_{50} value was also obtained. IC_{50} values represent the concentration of antagonist needed to inhibit by 50% the aggregation elicited by a given concentration of ADP.

General Method for the Preparation of Tributylammonium Salts of Dinucleoside Polyphosphates and Nucleotides. The nucleotides used for the procedures described herein were obtained from commercial sources as the recrystallized free acids [adenosine 5′-monophosphate (**1**) and cytidine 5′-monophosphate (**43**)] or as the sodium salts [uridine 5′-monophosphate (**4**), adenosine 5′-diphosphate (**2**), uridine 5′-triphosphate (**6**), and adenosine 5′-triphosphate (**3**)]. As a prelude to the dinucleoside polyphosphate syntheses, the nucleotide free acids were converted to their tributylammonium salts by treatment with an excess of NBu_3 in aqueous methanol. After stirring for 20 min, the solutions were evaporated to dryness and the residue coevaporated with dry DMF. Solids were obtained with overnight drying on a lyophilizer.

The nucleotides purchased as their sodium salts were first converted to their free acid forms by treatment with five weight-equivalents of Dowex 50 H^+ in water. After stirring for 10 min, the strongly acidic solutions were filtered and neutralized with an excess of NBu_3 in MeOH. Once a neutral pH or greater was obtained, the solvents were evaporated to dryness and the residues were coevaporated with dry DMF. The solids were dried to constant weight on a lyophilizer.

Tributylamine content in the solids was determined by ^{1}H NMR, and the molecular weights were adjusted accordingly. When fully dried, nucleoside monophosphates generally had 1 mol equivalent of NBu_3, 1.33–1.5 mol equivalents of diphosphates, and 2 mol equivalents of triphosphates. When easily handled solids were obtained, they were added as such to the dinucleoside polyphosphate-forming reactions described below; oily tributylammonium salts were dissolved to known concentrations in DMF.

The dinucleoside polyphosphates used as starting materials (**11**, **13**, **18**, and **19**) were synthesized from the appropriate nucleotide subunits, according to the general methods described in a previous communication.[44] Briefly, for the synthesis of **11**, nucleotide **3** in the ditributylammonium salt form was first activated with dicyclohexylcarbodiimide in DMF at room temperature, which converted the linear nucleoside triphosphate to the known cyclical trimetaphosphate.[39] Following this, **1** (as monotributylammonium salt) was added, and the reaction mixture was heated at 35 °C for 72 h. The dinucleoside polyphosphate so obtained (**11**) was isolated via ion exchange prep HPLC. Similarly, dinucleoside polyphosphates **13** and **18** were prepared via the DCC activation of **6** ($2NBu_3$ salt), followed by the reaction of the activated species with the appropriate nucleoside monophosphate in the NBu_3 salt form (**4** for dinucleoside polyphosphate **13**, and **43** for **18**). Dinucleoside triphosphate **19** was prepared from the reaction between commercially available uridine-5′-monophosphomorpholidate-4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt and the NBu_3 salt of **5** in DMF. The typical yields for these procedures ranged from 10 to 40%. Two dinucleoside polyphosphates (**11** and **47**) were converted to their free acid forms as a prelude to further synthesis. This was accomplished by treating the NBu_3 salt forms with Dowex 50 H^+ as described above for mononucleotide sodium salts, followed by direct freezing and lyophilization of the resultant dinucleoside free acids. All free acid forms were stored at –20 °C to prevent decomposition.

Synthesis. *P*^{1}-[(2′(3′)-*O*-2-(Methylamino)benzoyl)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (15) and *P*^{1}-[(2′(3′)-*O*-2-(Methylamino)benzoyl)uridine-5′-yl]-*P*^{4}-[(2′(3′)-*O*-2-(methylamino)benzoyl)uridine-5′-yl]-tetraphosphate (16). Up4U and 4Na salt (**13**, 1.0 g, 1.14 mmol) was dissolved in water (3 mL) and DMF (3 mL). To this was added *N*-methyl isatoicanhydride (303 mg, 1.71 mmol) and the reaction mixture heated at 50 °C for 5 h. The reaction mixture was diluted to 15 mL with water, and the insoluble material was removed by filtration. The products were separated via preparative HPLC. The yield of the mono MANT analogue **15** was 350 mg (31%), while the yield of the di MANT analogue **16** was 430 mg (33.5%). **15**: ^{1}H NMR (300 MHz, D_2O) δ 2.70 (d, 3H), 4.12 (m, 7H), 4.39 (s, 1H), 4.53 (t, 1H), 5.34 (m, 1H), 5.72 (m, 2H), 5.83 (dd, 1H), 6.01 (m, 1H), 6.53 (q, 1H), 6.67 (t, 1H), 7.35 (t, 1H), 7.78 (m, 3H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.84(m, 2P), −10.20 (m, 2P). MS (ES): *m*/*z* 921.9 (M − H). **16**: ^{1}H NMR (300 MHz, D_2O) δ 2.66 (t, 6H), 4.22 (m, 6H), 4.46 (q, 2H), 5.22 (t, 1H), 5.28 (d, 1H), 5.72 (d, 8.1 Hz, 1H), 5.84 (d, 1.5H), 5.93 (m, 1.5H), 6.54 (m, 4H), 7.28 (m, 2H), 7.77 (m, 4H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.76 (m, 2P), −10.16 (m, 2P). MS (ES): *m*/*z* 1055.1 (M − H).

***P*^{1}-[(2′,3′)-di-*O*-Benzoyl)uridine-5′-yl]-*P*^{4}-[(2′,3′)-di-*O*-benzoyl)uridine-5′-yl]-tetraphosphate (17).** Compound **13**, ditributylammonium salt (200 mg, 0.172 mmol), and 4-dimethylaminopyridine (5 mg, 0.04 mmol) were dissolved in pyridine (3.0 mL) and benzoic anhydride (500 mg, 2.21 mmol) added. The reaction mixture was stirred overnight at room temperature, after which the pyridine was removed. The residue was partitioned between ethyl acetate (75 mL) and 0.5 M $NaHCO_3$ (50 mL). The layers were separated and the aqueous layer containing the products concentrated to 8 mL.

The products were separated via preparative HPLC. The isolated yield of the tetra-benzoate (**17**) was 64 mg (31%). Only minimal quantities of the mono and dibenzoates and a lesser amount of the tribenzoate were produced under the chosen reaction conditions and were not isolated. **17**: ^{1}H NMR (300 MHz, D_2O) δ 4.30 (m, 4H), 5.63 (t, 2H), 5.72 (m, 2H), 5.90 (d, J = 8.1 Hz, 2H), 6.21 (d, J = 5.4 Hz, 2H), 7.27 (m, 8H), 7.41 m, 4H), 7.59 (d, 4H), 7.71 (d, 4H), 7.93 (d, J = 8.1 Hz, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.95 (m, 2P), −10.50 (m, 2P). MS (ES): *m/z* 1205.3 (M − H).

***P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (20) and *P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)-uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)uridine-5′-yl]-tetraphosphate (21).** Compound **13** and 4Na salt (290 mg, 0.332 mmol) were dissolved in 99% formic acid (3 mL) and phenylacetaldehyde dimethyl acetal (110 μL, 0.664 mmol) added. The reaction was stirred for 48 h and the formic acid removed by evaporation. The residue was partitioned between butyl acetate (15 mL) and 0.7 M $NaHCO_3$ (15 mL). The layers were separated and the products in the aqueous layer purified via preparative HPLC. The isolated yield of the monoacetal (**20**) was 88 mg (30%), and of the diacetal (**21**) 60 mg (18%). **20**: ^{1}H NMR (300 MHz, D_2O) δ 2.99 (d, 2H), 4.04 (m, 5H), 4.22 (m, 2H), 4.32 (s, 1H), 4.78 (m, 2H),5.30 (t, 1H), 5.36 (d, J = 2.4 Hz, 1H), 5.73 (d, J = 8.1 Hz, 1H), 5.81 (t, 2H), 7.20 (m, 5H), 7.63 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.00 (m, 2P), −10.78 (m, 1P), −10.18 (m, 1P). MS (ES): *m/z* 890.9 (M − H). **21**: ^{1}H NMR (300 MHz, D_2O) δ 2.98 (d, J = 3.9 Hz, 4H), 3.98 (s, 4H), 4.27 (s, 2H), 4.74 (m, 4H), 5.28 (t, J = 3.9 Hz, 2H), 5.37 (d, J = 2.7 Hz, 2H), 5.74 (d, J = 8.1 Hz, 2H), 7.21 (m, 10H), 7.61 (d, J = 8.1 Hz, 2H).4 ^{31}P NMR (121.47 MHz, D_2O) δ −21.81 (m, 2P), −10.57 (m, 2P). MS (ES): *m/z* 993.0 (M − H).

***P*^{1}-[(2′,3′)-*O*-(Phenylmethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (22) and *P*^{1}-[(2′,3′)-*O*-(Phenylmethylidene)-uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(phenylmethylidene)uridine-5′-yl]-tetraphosphate (23).** Compound **13** and 4Na salt (500 mg, 0.569 mmol) were dissolved in 99% formic acid (4 mL) and benzaldehyde dimethyl acetal (342 μL, 2.27 mmol) added. The reaction was stirred for 4 h, then the formic acid removed by evaporation. The residue was partitioned between ethyl acetate and 1 M $NaHCO_3$ and the products in the aqueous layer purified via preparative HPLC. The yield of the monoacetal (**22**, 48839) was 211 mg (42%), and of the diacetal (**23**, 48840) 37 mg (6.7%). **22**: ^{1}H NMR (300 MHz, D_2O) δ 4.07 (m, 7H), 4.43 (m, 0.4H), 4.60 (m, 0.6H), 4.97 (d, 2H), 5.73 (m, 3H), 5.86 (d, 1.6H), 6.00 (s, 0.4H), 7.36 (m, 5H), 7.64 (d, 0.4H), 7.72 (t, 1.6H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.01 (m, 2P), −10.77 (m, 0.66P), −10.32 (m, 1.33P). MS (ES): *m/z* 877.2 (M − H). **23**: ^{1}H NMR (300 MHz, D_2O) δ 4.06 (m, 4H), 4.36 (s, 0.7H), 4.54 (s, 1.3H),4.91 (m, 4H), 5.69 (t, 2H), 5.80 (d, 2H), 5.85 (s, 1.3H), 5.93 (s, 0.7H), 7.31 (m, 10H), 7.57 (d, 0.7H), 7.67 (d, 1.3H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.89 (m, 2P), −10.69 (m, 2P). MS (ES): *m/z* 965.1 (M − H).

***P*^{1}-[(2′,3′)-*O*-(Phenylethenylmethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (24) and *P*^{1}-[(2′,3′)-*O*-(Phenylethenylmethylidene)uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(phenylethenylmethylidene)uridine-5′-yl]-tetraphosphate (25).** Compound **13** and 4Na salt (1.0 g, 1.14 mmol) were dissolved in trifluoroacetic acid (3 mL) and *trans*-cinnamaldehyde (2.00 mL, 15.9 mmol) added. The reaction was allowed to proceed at ambient temperature for 1 h. The TFA was removed at <30 °C in vacuo and the residue partitioned between EtOAc and water, along with the addition of sufficient concentrated NH_4OH to render pH 7–8. The products in the aqueous layer were separated via preparative HPLC. The isolated yield of the monoacetal (**24**) was 396 mg (38%) and of the diacetal (**25**) 112 mg (9.7%). **24**: ^{1}H NMR (300 MHz, D_2O) δ 4.13 (m, 4H), 4.22 (m, 3H), 4.44 (s, 0.5H), 4.94 (d, 2H), 5.59 (d, J = 6.6 Hz, 0.4H), 5.73 (d, 6.6 Hz, 0.6H), 5.81 (m, 3H), 6.17 (ddd, 1H), 6.39 (d, 0.3H), 6.85 (dd, 1H), 7.30 (m, 3H), 7.45 (m, 2H), 7.75 (m, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.11 (m, 2P), −10.54 (m, 2P). MS (ES): *m/z* 903.1 (M − H). **25**: ^{1}H NMR (300 MHz, D_2O) δ 4.14 (m, 4H), 4.39 (m, 1H), 4.86 (m, 4H), 5.56 (d, J = 6.6 Hz, 0.8H), 5.65 (t, J = 6.9 Hz, 1.2H), 5.78 (m, 4H), 6.11 (m, 2H), 6.78 (m, 2H), 7.26 (m, 6H), 7.38 (m, 4H), 7.68 (m, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.07 (m, 2P), −10.72 (m, 2P). MS (ES): *m/z* 1017.3 (M − H).

***P*^{1}-[(2′,3′)-*O*-(Phenylethynylmethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (26) and *P*^{1}-[(2′,3′)-*O*-(Phenylethynylmethylidene)uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(phenylethynylmethylidene)uridine-5′-yl]-tetraphosphate (27).** Compound **13** and 4Na salt (250 mg, 0.28 mmol) were dissolved in 99% formic acid (2 mL) and phenylpropargyl aldehyde (105 μL, 3.42 mmol) added. The reaction was stirred for 1 h, and the formic acid was removed by evaporation. The residue was partitioned between EtOAc and 1 M $NaHCO_3$, and the products in the aqueous layer purified via preparative HPLC. The isolated yield of the monoacetal (**26**) was 82 mg (32%) and of the diacetal (**27**) 10 mg (3.5%). **26**: ^{1}H NMR (300 MHz, D_2O) δ 4.13 (m, 5H), 4.24 (m, 2H), 4.49 (s, 0.5H), 5.03 (d, 2H), 5.82 (m, 3.5H), 6.00 (s, 0.5H), 6.13 (s, 1H), 7.31 (m, 3H), 7.44 (m, 2H), 7.76 (m, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.13 (m, 2P), −10.49 (m, 2P). MS (ES): *m/z* 901.2 (M − H). **27**: ^{1}H NMR (300 MHz, D_2O) δ 4.15 (m, 4H), 4.42 (m, 2H), 5.04 (m, 4H), 5.70 (t, 1H), 5.78 (m, 2H), 5.95 (s, 0.5H), 5.98 (s, 0.5H), 6.01 (s, 0.5H), 6.08 (s, 0.8H), 6.13 (d, 0.6H), 7.29 (m, 5H), 7.40 (m, 5H), 7.67 (m, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.07 (m, 2P), −10.72 (m, 2P). MS (ES): *m/z* 1013.2 (M − H).

***P*^{1}-[(2′,3′)-*O*-(Propylmethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (28) and *P*^{1}-[(2′,3′)-*O*-(Propylmethylidene)-uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(propylmethylidene)uridine-5′-yl]-tetraphosphate (29).** Compound **13** and 4Na salt (1.0 g, 1.14 mmol) was dissolved in 99% formic acid (4 mL) and butyraldehyde (304 μL, 3.42 mmol) added. The reaction was stirred for 16 h, and the formic acid was removed by evaporation. The residue was partitioned between EtOAc and 1 M $NaHCO_3$, and the products in the aqueous layer purified via preparative HPLC. The yield of the monoacetal (**28**) was 227 mg (23%) and of the diacetal (**29**) 309 mg (30%). **28**: ^{1}H NMR (300 MHz, D_2O) δ 0.80 (t, 3H), 1.28 (m, 2H), 1.61 (m, 2H), 4.08 (m, 5H), 4.22 (m, 2H), 4.48 (s, 1H), 4.79 (m, 2H), 5.06 (t, 1H), 5.77 (m, 4H), 7.70 (d, 1H), 7.80 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.95 (m, 2P), −10.68 (m, 1P), −10.23 (m 1P). MS (ES): *m/z* 843.3 (M − H). **29**: ^{1}H NMR (300 MHz, D_2O) δ 0.80 (t, 6H), 1.29 (m, 4H), 1.62 (m, 4H), 4.06 (m, 4H), 4.47 (s, 2H), 4.79 (m, 4H), 5.06 (t, 2H), 5.76 (m, 4H), 7.70 (d, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.92 (m, 2P), −10.63 (m, 2P). MS (ES): *m/z* 897.2 (M − H).

***P*^{1}-[(2′,3′)-*O*-(Heptylmethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-tetraphosphate (30) and *P*^{1}-[(2′,3′)-*O*-(Heptylmethylidene)-uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(heptylmethylidene)uridine-5′-yl]-tetraphosphate (31).** Compound **13** and 4Na salt (1.0 g, 1.14 mmol) were dissolved in 99% formic acid (4 mL) and butyraldehyde (533 μL, 3.42 mmol) added. The reaction was stirred for 2.25 h, then the formic acid was removed by evaporation. The residue was partitioned between EtOAc and 1 M $NaHCO_3$ and the products in the aqueous layer purified via preparative HPLC. The yield of the monoacetal (**30**) was 253 mg (25%) and of the diacetal (**31**) 375 mg (32%). **30**: ^{1}H NMR (300 MHz, D_2O) δ 0.67 (t, 3H), 1.18 (m, 10H), 1.61 (m, 2H), 4.06 (m, 5H), 4.21 (m, 2H), 4.48 (s, 1H), 4.78 (m, 2H), 5.04 (t, 1H), 5.77 (m, 4H), 7.69 (d, 1H), 7.79 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.93 (m, 2P), −10.65 (m, 1P), −10.22 (m 1P). MS (ES): *m/z* 899.3 (M − H). **31**: ^{1}H NMR (300 MHz, D_2O) δ 0.69 (t, 6H), 1.20 (m, 20H), 1.61 (m, 4H), 4.05 (m, 4H), 4.47 (s, 2H), 4.78 (m, 4H), 5.04 (t, 2H), 5.76 (m, 4H), 7.71 (d, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.92 (m, 2P), −10.63 (m, 2P). MS (ES): *m/z* 1009.6 (M − H).

***P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)uridine-5′-yl]-*P*^{4}-[cytidine-5′-yl]-tetraphosphate (33), *P*^{1}-[Uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)cytidine-5′-yl]-tetraphosphate (32), and *P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)cytidine-5′-yl]-tetraphosphate (34).** Compound **18** and 4Na salt (100 mg, 0.114 mmol) were dissolved in 99% formic acid (1 mL) and phenylacetaldehyde dimethyl acetal (57 μL, 0.342 mmol) added. The reaction was stirred for 16 h, then the formic acid removed by evaporation. The residue was partitioned between EtOAc and 1 M $NaHCO_3$ and the products in the aqueous

layer purified via preparative HPLC. The yield of U-side monoacetal (**33**) was 26 mg (25%), the C-side monoacetal (**32**) was 20 mg (20%), and the diacetal (**34**) was 21 mg (19%). **33**: ^{1}H NMR (300 MHz, D_2O) δ 2.95 (d, 2H), 4.09 (m, 7H), 4.28 (m, 1H), 4.64 (m, 1H), 4.73 (m, 1H), 5.28 (t, 1H), 5.32 (d, 1H), 5.71 (m, 2H), 6.08 (d, 1H), 7.17 (m, 5H), 7.57 (d, 1H), 7.95 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.07 (m, 2P), −10.85 (m, 1P), −10.17 (m, 1P). MS (ES): *m/z* 890.3 (M − H). **32**: ^{1}H NMR (300 MHz, D_2O) δ 2.95 (d, 2H), 4.07 (m, 7H), 4.42 (m, 1H), 4.69 (m, 2H), 5.23 (d, 2H), 5.75 (m, 2H), 6.04 (d, 1H), 7.15 (m, 5H), 7.73 (dd, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.05 (m, 2P), −11.02 (m, 1P), −10.28 (m, 1P). MS (ES): *m/z* 890.3 (M − H). **34**: ^{1}H NMR (300 MHz, D_2O) δ 2.93 (d, 4H), 3.94 (m, 4H), 4.24 (s, 1H), 4.35 (s, 1H), 4.69 (m, 4H), 5.22 (m, 3H), 5.32 (d, 1H), 5.68 (d, 1H), 6.01 (d, 1H), 7.17 (m, 10H), 7.56 (d, 1H), 7.67 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −22.11 (m, 2P), −10.88 (m, 2P). MS (ES): *m/z* 992.4 (M − H).

***P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)uridine-5′-yl]-*P*^{4}-[uridine-5′-yl]-triphosphate (35) and *P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)-uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)uridine-5′-yl]-triphosphate (36).** Compound **19** and 3Na salt (100 mg, 0.129 mmol) were dissolved in 99% formic acid (1 mL) and phenylacetaldehyde dimethyl acetal (64 μL, 0.386 mmol) added. The reaction was stirred for 16 h, and the formic acid then removed by evaporation. The residue was partitioned between EtOAc and 0.5 M $NaHCO_3$ and the products in the aqueous layer purified via preparative HPLC. The yield of the monoacetal (**35**) was 40 mg (38%) and of the diacetal (**36**) 24 mg (20%). **35**: ^{1}H NMR (300 MHz, D_2O) δ 2.93 (d, 2H), 3.98 (d, 5H), 4.16 (d, 3H), 4.67 (m, 2H), 5.23 (t, 1H), 5.31 (s, 1H), 5.67 (d, 1H), 5.74 (m, 2H), 7.16 (m, 5H), 7.55 (d, 1H), 7.73 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.99 (m, 1P), −10.80 (m, 1P) 10.30 (m, 1P). MS (ES): *m/z* 811.6 (M − H). **36**: ^{1}H NMR (300 MHz, D_2O) δ 2.91 (d, 4H), 3.89 (s, 4H), 4.13 (s, 2H), 4.63 (m, 2H), 5.21 (t, 2H), 5.32 (d, 2H), 5.65 (d, 2H), 7.15 (m, 10H), 7.49 (d, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.99 (m, 1P), −10.62 (m, 2P). MS (ES): *m/z* 913.3 (M − H).

***P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)adenosine-5′-yl]-*P*^{4}-[adenosine-5′-yl]-tetraphosphate (37) and *P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)adenosine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)adenosine-5′-yl]-tetraphosphate (38).** The free acid of **11** (500 mg, 0.598 mmol) was dissolved in 99% formic acid (5 mL) and phenylacetaldehyde dimethyl acetal (297 μL, 1.79 mmol) added. The reaction was stirred for 21 h, then the formic acid removed by evaporation. The residue was partitioned between EtOAc and 1 M $NaHCO_3$ and the products in the aqueous layer purified via preparative HPLC. The yield of monoacetal (**37**, 48506) was 155 mg (27.6%) and of the diacetal (**38**, 48808) 38 mg (6.1%). **37**: ^{1}H NMR (300 MHz, D_2O) δ 3.02 (d, 2H), 4.05 (m, 2H), 4.14 (m, 2H), 4.22 (m, 1H), 4.40 (m, 2H), 4.55 (t, 1H), 4.92 (m, 1H), 5.04 (m, 1H), 5.34 (t, 1H), 5.68 (d, 1H), 5.87 (d, 1H), 7.24 (m, 5H), 8.03 (d, 1H), 8.19 (s, 1H), 8.31 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.74 (m, 2P), −10.35 (m, 1P), −9.98 (m, 1P). MS (ES): *m/z* 937.5 (M − H). **38**: ^{1}H NMR (300 MHz, D_2O) δ 2.95 (d, 4H), 4.01 (m, 4H), 4.30 (m, 2H), 4.87 (m, 2H), 4.96 (m, 2H), 5.23 (t, 2H), 5.61 (d, 2H), 7.15 (m, 10H), 7.92 (s, 2H), 8.18 (s, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.84 (m, 2P), −10.40 (m, 2P). MS (ES): *m/z* 1039.8 (M − H).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-2-benzyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-trihydrogen Diphosphate (40). Compound **2** and 1Na salt (800 mg, 1.78 mmol) were dissolved in 99% formic acid (5 mL), and phenylacetaldehyde dimethyl acetal (589 μL, 3.56 mmol) was added. The mixture was stirred overnight at 35 °C and evaporated to dryness. The residue was partitioned between EtOAc and 1 M $NaHCO_3$. The resultant partial emulsion was centrifuged (4000 rpm) and the layers separated. The aqueous layer containing the product was concentrated to 20 mL and the product purified by reverse-phase preparative HPLC. The yield of the title compound (**40**) was 252 mg (27%). **40**: ^{1}H NMR (300 MHz, D_2O) δ 3.03 (d, 2H), 3.94 (d, 2H), 4.40 (s, 1H), 4.92 (d, 1H), 5.14 (m, 1H), 5.33 (t, 1H), 5.73 (d, 1H), 7.20 (m, 5H), 8.08 (s, 1H), 8.20 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −10.31 (d, 1P), −9.52 (d, 1P). MS (ES): *m/z* 528.1 (M − H).

((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(6-(3-phenylureido)-9*H*-purin-9-yl)tetrahydrofuran-2-yl)methyl-trihydrogen Diphosphate (39). Compound **2** and 1NBu_3 salt (**2**, 1.0g, 1.63 mmol) were dissolved in dry DMF (15 mL) and phenylisocyanate (540 μL, 4.9 mmol) added. The reaction was heated for 1 h at 45 °C. The solvent was removed and the residue partitioned between EtOAc and 0.25 M $NaHCO_3$ (5 mL). The layers were separated and the product in the aqueous layer isolated by reverse-phase preparative HPLC. The yield of **39** was 431 mg (48%). **39**: ^{1}H NMR (300 MHz, D_2O) δ 4.09 (m, 2H), 4.21 (m, 1H), 4.36 (t, 1H), 4.48 (t, 1H), 5.78 (d, 1H), 6.54 (t, 1H), 6.77 (m, 4H), 8.18 (s, 1H), 8.25 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −10.08 (d, 1P), −8.76 (d, 1P). MS (ES): *m/z* 545.1 (M − H).

((3a*S*,4*R*,6*R*,6a*S*)-2-Benzyl-6-(6-(3-phenylureido)-9*H*-purin-9-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-trihydrogen Diphosphate (41). Compound **40**, 1NH_4 salt (200 mg, 0.355 mmol), and tributylamine (200 μL, 0.841 mmol) were dissolved in dry DMF (3 mL). Phenylisocyanate (194 μL, 1.78 mmol) was added and the reaction mixture heated for 60 h at 30 °C. The solvent was removed and the residue partitioned between EtOAc and 0.2 M $NaHCO_3$ (3 mL). The product in the aqueous layer was isolated by reverse-phase preparative HPLC. The yield of the title compound (**41**) was 31 mg (13.5%). Compound **41** proved to be somewhat unstable, slowly degrading to the corresponding monophosphate (**56**). Consequently, heating was avoided during the isolation of this molecule, and it was stored at −80 °C immediately after the lyophilization step. When handled appropriately, the typical content of compound **56** was 3–5%. **41**: ^{1}H NMR (300 MHz, D_2O) δ 3.06 (d, 2H), 3.94 (m, 2H), 4.44 (s, 1H), 4.97 (d,1H), 5.13 (m, 1H), 5.35 (t, 1H), 5.71 (d, J = 3.3 Hz, 1H), 6.89 (t, 1H), 7.10 (t, 2H), 7.21 (m, 7H), 8.31 (s, 1H), 8.44 (s, 1H).). ^{1}H NMR (300 MHz, MeOD + D_2O) δ 3.14 (d, 2H), 4.16 (m, 2H), 4.54 (s, 1H), 5.15 (d, 1H), 5.33 (m, 2H), 6.17 (d, J = 3.3 Hz, 1H), 7.12 (t, 1H), 7.31 (m, 7H), 7.61 (d, 2H), 8.70 (s, 1H), 8.73 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −10.44 (d, 1P), −9.52 (d, 1P). MS (ES): *m/z* 648.8 (M − H).

***P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)-6-(3-phenylureido)adenosine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)-6-(3-phenylureido)adenosine-5′-yl]-tetraphosphate (42).** Compound **41** (60 mg, 0.092 mmol) was dissolved in dry DMF (1.0 mL) and NBu_3 (100 μL, 0.42 mmol). 1,1′-Carbonyldiimidazole (20 mg, 0.123 mmol) was added and the reaction mixture allowed to stir overnight at ambient temperature. The reaction was quenched with water (1 mL) and 1 M $NaHCO_3$ (1 mL). The desired product was isolated by reverse-phase preparative HPLC. The yield of the title compound (**42**) was 9 mg (16%). **42**: ^{1}H NMR (300 MHz, D_2O + acetonitrile d3) δ 3.04 (t, 4H), 4.01 (s, 4H), 4.41 (s, 2H), 4.87 (d, 2H), 4.99 (t, 2H), 5.22 (t, 2H), 5.95 (d, 2H), 7.08 (t, 2H), 7.26 (m, 14H), 7.51 (d, 4H), 8.39 (s, 2H), 8.44 (s, 2H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.31 (m, 2P), −10.13 (m, 2P). MS (ES): *m/z* 1279.9 (M − H).

((2*R*,3*R*,4*S*,5*R*)-5-(4-(3-(4-Fluorophenyl)ureido)-2-oxopyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl-dihydrogen Phosphate (44). Compound **43** and 1 NBu_3 salt (51 mg, 0.1 mmol) were dissolved in dry DMF (0.5 mL) and *p*-fluorophenylisocyanate (34 μL, 0.3 mmol) added. The reaction was heated at 60 °C for 5 min, then at 30 °C for 1 h. The solvent was removed by evaporation under vacuum and the residue partitioned between EtOAc (10 mL) and 0.5 M $NaHCO_3$. The product in the aqueous layer was isolated by reverse-phase preparative HPLC. The yield of the title compound (**44**) was 51 mg (80%). **44**: ^{1}H NMR (300 MHz, D_2O) δ 4.05 (m, 5H), 5.78 (d, 1H), 6.24 (d, 1H), 6.97 (t, 2H), 7.28 (m, 2H), 8.14 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.39 (s). MS (ES): *m/z* 461.4 (M − H).

((3a*S*,4*R*,6*R*,6a*S*)-6-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-2-benzyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (45). Compound **43** (1.0g, 3.09 mmol), in the free acid form, was dissolved in 99% formic acid (7.5 mL) and phenylacetaldehyde dimethyl acetal (1.03 mL, 6.2 mmol) added.

The reaction was stirred overnight at ambient temperature, after which the volatiles were removed under vacuum. The residue was partitioned between EtOAc and 1 M $NaHCO_3$, and the product in the aqueous layer was isolated by reverse-phase preparative HPLC. The yield of the title compound (**45**) was 650 mg (49%). **45**: ^{1}H NMR (300 MHz, D_2O) δ 2.98 (d, 2H), 3.77 (m, 2H, 4.17 (s, 1H), 4.71 (m, 2H), 5.28 (t, 1H), 5.52 (d, 1H), 5.86 (d, 1H), 7.20 (m, 5H), 7.63 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 2.87 (s). MS (ES): *m/z* 424.2 (M − H).

((3a*S*,4*R*,6*R*,6a*S*)-2-Benzyl-6-(4-(3-(4-fluorophenyl)ureido)-2-oxopyrimidin-1(2*H*)-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-methyl-hydrogen Phosphate (46). Compound **45** (100 mg, 0.218 mmol) and NBu_3 (104 μL, 0.436 mmol) were dissolved in dry DMF (2 mL). 4-Fluorophenylisocyanate (99 μL, 0.871 mmol) was added and the reaction stirred at room temperature. At 1 h, an additional portion of 4-fluorophenylisocyanate (99 μL, 0.871 mmol) was added. After 3 h, the solvent was removed and the residue partitioned between EtOAc and 1 M $NaHCO_3$. The product in the aqueous layer was isolated by reverse-phase preparative HPLC. The yield of the title compound (**46**) was 102 mg (83%). **46**: ^{1}H NMR (300 MHz, D_2O) δ 3.00 (d, 2H), 3.81 (m, 2H), 4.34 (m, 1H), 4.72 (m, 2H), 5.28 (t, 1H), 5.44 (d, 1H), 6.20 (d, 1H), 6.99 (t, 2H), 7.27 (m, 2H), 7.92 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 2.39 (s). MS (ES): *m/z* 563.2 (M − H).

***P*^{1}-[Uridine-5′-yl]-*P*^{4}-[4-(3-(4-fluorophenyl)ureido)cytidine-5′-yl]-tetraphosphate (47).** Compound **6** and 2NBu_3 salt (8.93 g, 10.5 mmol) were dissolved in dry DMF (total solution volume 50 mL) and DCC (2.04 g, 9.9 mmol) added. The reaction was stirred for 1.25 h at 30 °C, during which time it became heterogeneous because of precipitated dicyclohexylurea. ^{31}P NMR showed complete conversion to the cyclic trimeta phosphate. A solution of CMP 6-(*p*-fluoro)phenylurea and 1NBu_3 salt (**44**, 3.38 g, 5.25 mmol) in dry DMF (50 mL) was added, and the solution was kept at 30 °C for 3 days. Water (10 mL) was added, and the reaction mixture was stirred for 30 min, after which the solvent was removed by evaporation. The residue was dissolved in water (100 mL), and the precipitated dicyclohexylurea was removed by filtration. The filtrate was concentrated and the product isolated by reverse-phase preparative HPLC. The yield of the title compound (**47**) was 2.12 g (41%, based on **44**). **47**: ^{1}H NMR (300 MHz, D_2O) δ 4.11 (m, 10H), 5.74 (m, 3H), 6.30 (d, 1H), 6.97 (t, 2H), 7.29 (m, 2H), 7.71 (d, 1H), 8.15 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.67 (m, 2P), −10.32 (m, 2P). MS (ES): *m/z* 925.4 (M − H).

***P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)uridine-5′-yl]-*P*^{4}-[4-(3-(4-fluorophenyl)ureido)cytidine-5′-yl]-tetraphosphate (48), *P*^{1}-[Uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)-4-(3-(4-fluorophenyl)ureido)cytidine-5′-yl]-tetraphosphate (49), and *P*^{1}-[(2′,3′)-*O*-(2-Phenylethylidene)uridine-5′-yl]-*P*^{4}-[(2′,3′)-*O*-(2-phenylethylidene)-4-(3-(4-fluorophenyl)ureido)cytidine-5′-yl]-tetraphosphate (50).** Compound **47** (100 mg, 0.108 mmol), in the free acid form, was dissolved in 99% formic acid (2 mL) and phenylacetaldehyde dimethyl acetal (107 μL, 0.65 mmol) added. The reaction was heated at 30 °C for 45 min, after which the formic acid was removed at ambient temperature. The residue was partitioned between EtOAc and 0.8 M $NaHCO_3$. The products in the aqueous layer were purified by reverse-phase preparative HPLC. In addition to the recovered starting material (**47**, 38 mg), the yields were 39 mg of the uridine-side monoacetal (**48**, 32%), 14 mg of the cytidine-side monoacetal (**49**, 12%), and 15 mg of the diacetal (**50**, 12%). **48**: ^{1}H NMR (300 MHz, D_2O) δ 2.86 (d, 2H), 4.10 (m, 8H), 4.66 (m, 2H), 5.19 (t, 1H), 5.31 (d, 1H), 5.69 (d, 1H), 5.78 (d, 1H), 6.30 (d, 1H), 6.97 (t, 2H), 7.13 (m, 5H), 7.30 (m, 2H), 7.54 (d, 1H), 8.16 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.71 (m, 2P), −10.62 (m, 1P), −9.92 (m, 1P). MS (ES): *m/z* 1027.2 (M − H). **49**: ^{1}H NMR (300 MHz, D_2O) δ 2.98 (d, 2H), 4.19 (m, 7H), 4.42 (m, 1H), 4.68 (m, 2H), 5.26 (t, 1H), 5.37 (d, 1H), 5.66 (d, 1H), 5.75 (d, 1H), 6.24 (d, 1H), 6.96 (t, 2H), 7.21 (m, 5H), 7.30 (d, 2H), 7.61 (d, 1H), 7.88 (d, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ −21.62 (m, 2P), −10.60 (m, 1P), −10.19 (m, 1P). MS (ES): *m/z* 1027.2 (M − H). **50**: ^{1}H NMR (300 MHz, D_2O) δ 2.88 (d, 2H), 3.00 (d, 2H), 4.00 (m, 4H), 4.18 (m, 1H), 4.36 (m, 1H), 4.68 (m, 4H), 5.17 (t, 1H), 5.28 (t, 1H), 5.35 (d, 1H), 5.41 (d, 1H), 5.68 (d, 1H), 6.24 (d, 1H), 6.98 (t, 2H), 7.18 (m, 10H), 7.31 (m, 2H), 7.53 (d, 1H), 7.90 (d, 1H).). ^{31}P NMR (121.47 MHz, D_2O) δ −21.57 (m, 2P), −10.62 (m, 2P). MS (ES): *m/z* 1129.7 (M − H).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-2-benzyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (52). The free acid of **1** (10.0g, 28.8 mmol) was dissolved in trifluoroacetic acid (50 mL) and cooled to 0–5 °C followed by the addition of phenylacetaldehyde dimethyl acetal (13.5 mL, 88.5 mmol). Three additional portions of phenylacetaldehyde dimethyl acetal (2.5 mL, 15mmol) were added at 2 h, 2.75 h, and 3.5 h. The reaction was stirred for a total of 7 h, and the volatiles were removed in vacuo at <40 °C. The residue was partitioned between EtOAc and 0.75 M $NaHCO_3$. The aqueous layer was back extracted with EtOAc, and the product in the aqueous layer was isolated by reverse-phase preparative HPLC. The yield of the title compound (**52**) was 7.5g (59%). **52**: ^{1}H NMR (300 MHz, D_2O) δ 2.99 (d, 2H), 3.80(m, 2H), 4.32 (s, 1H), 4.82 (d, 1H), 5.07 (m, 1H), 5.28 (t, 1H), 5.72 (d, 1H), 7.17 (m, 5H), 7.99 (s, 1H), 8.09 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.45 (s). MS (ES): *m/z* 448.3 (M − H). HPLC retention time (C_{18}, ion pairing): 10.64 min (100%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-2-phenyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (53). Compound **53** was prepared from **1** (free acid) according to the method for the synthesis of **52**, substituting benzaldehyde (3 equiv) for phenylacetaldehyde dimethyacetal. The yield of the title compound (**53**) was 92%, following reverse-phase preparative HPLC purification. The product consisted of a 50:50 ratio of acetal diastereomers, as determined by HPLC and NMR (MeOD). **53**: ^{1}H NMR (300 MHz, MeOD) δ 4.07 (m, 2H), 4.56 (m, 0.5H), 4.66 (m, 0.5H), 5.24 (m, 1H), 5.46 (m, 1H),6.00 (s, 0.5H), 6.24 (s, 0.5H), 6.36 (d, J = 3.0 Hz, 0.5H), 6.39 (d, J = 3.3 Hz, 0.5H), 7.39 (m, 3H), 7.52 (m, 1H), 7.58 (m, 1H), 8.20 (d, 1H), 8.49 (s, 0.5H), 8.56 (s, 0.5H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.58 (s 0.5P), 1.70 (s, 0.5P). MS (ES): *m/z* 434.5 (M − H). HPLC retention times (C_{18}, ion pairing): 9.99 min (50%), 10.17 (50%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-Amino-9H-purin-9-yl)-2-styryltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (54). Compound **54** was prepared from **1** (free acid) according to the method for the synthesis of **52**, substituting *trans*-cinnamaldehyde (2 equiv) for phenylacetaldehyde dimethyl acetal. Following overnight stirring at 0–5 °C, further portions of *trans*-cinnamaldehyde (4 × 0.9 equiv) were added over a 2 h period, to enhance the conversion to product. The yield of the title compound (**54**) was 51%, following preparative HPLC purification. The product consisted of a 66:33 ratio of acetal diastereomers, as determined by HPLC and NMR (MeOD). **54**: ^{1}H NMR (300 MHz, MeOD) δ 4.08 (m, 2H), 4.50 (m, 0.66H), 4.62 (m, 0.33H), 5.11 (dd, 0.33H), 5.18 (dd, 0.66H), 5.37 (m, 1H), 5.65 (d, J = 6.9 Hz, 0.33H), 5.89 (d, J = 6.3 Hz, 0.66H), 6.29 (m, 2H), 6.87 (d, J = 15.9 Hz, 1H), 7.33 (d, 3H), 7.47 (m, 2H), 8.22 (s, 1H), 8.49 (s, 0.66H), 8.56 (s, 0.33H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.17 (s 0.33P), 1.28 (s, 0.66P). MS (ES): *m/z* 460.6 (M − H) HPLC retention times (C_{18}, ion pairing): 10.96 min (66%), 11.11 (33%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-Amino-9*H*-purin-9-yl)-2-(phenylethynyl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (55). Compound **55** was prepared from **1** (free acid) according to the method for the synthesis of **52**, substituting phenylpropargyl aldehyde (3 equiv) for phenylacetaldehyde dimethyl acetal. The reaction was stirred for three days at RT, giving a 36% yield of the title compound (**55**) following preparative HPLC purification. The product consisted of a 35:65 ratio of acetal diastereomers, as determined by HPLC and NMR (MeOD). **55**: ^{1}H NMR (300 MHz, MeOD) δ 4.09 (m, 2H), 4.56 (m, 0.33H), 4.76 (m, 0.66H), 5.19 (dd, 0.66H), 5.30 (dd, 0.33H), 5.42 (dd, 0.65H), 5.51 (dd, 0.35H), 6.08 (s, 0.65H), 6.29 (d, J = 3.3 Hz, 0.33H), 6.31 (s, 0.35H), 6.55 (d, J = 3.0 Hz, 0.66H), 7.38 (m, 4H), 7.54 (m, 1H), 8.22 (d, 1H), 8.51 (d, 1H) ^{31}P NMR (121.47 MHz, D_2O) δ 1.35 (s). (MS (ES): *m/z* 458.4 (M − H) HPLC retention times (C_{18}, ion pairing): 10.89 min (35%), 11.09 (65%).

((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(6-(3-phenylureido)-9*H*-purin-9-yl)tetrahydrofuran-2-yl)methyl-dihydrogen Phosphate (51). Compound **51** was synthesized from **1** (NBu_3 salt) according to the method for the synthesis of **39**, using phenyl isocyanate (5 equiv). The yield of the title compound was 17%. The low yield of **51** was attributable to byproducts arising from the competitive acylation of the hydroxyl groups. **51**: ^{1}H NMR (300 MHz, D_2O) δ 3.99 (, 3H), 4.23 (s, 1H), 4.34 (t, 1H), 4.58 (t, 1H), 5.97 (d, J = 5.4 Hz, 1H), 6.90 (t, 1H), 7.10 (t, 2H), 7.18 (d, 2H), 8.40 (s, 1H), 8.42 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.65 (s).

((3a*S*,4*R*,6*R*,6a*S*)-2-Benzyl-6-(6-(3-phenylureido)-9*H*-purin-9-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (56). Compound **52** and 1 NH_4 salt (5.0g, 10.7 mmol) were dissolved in DMF (20 mL) and tributylamine (5.11 mL, 21.4 mmol) added, stirred for 5 min, and then evaporated to an oil. This oil was dissolved in DMF (35 mL), and phenyl isocyanate (3.51 mL. 32.2 mmol) was added and the reaction heated to 30–35 °C overnight. The volatiles were evaporated, and the residue was partitioned between BuOAc (100 mL), toluene (200 mL), and water (300 mL). The aqueous layer was separated, concentrated, and the product purified by reverse-phase preparative HPLC. The purified yield of the title compound **56** following lyophilization was 3.3g (54%). **56**: ^{1}H NMR (300 MHz, MeOD) δ 3.14 (d, J = 4.5 Hz, 2H), 4.01 (m, 2H), 4.53 (s, 1H), 5.07 (d, 1H), 5.31 (m, 2H), 6.19 (d, J = 3.3 Hz, 1H), 7.11 (t, 1H), 7.29 (m, 7H), 7.61 (d, 2H), 8.69 (s, 1H), 8.81 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 3.15 (s). MS (ES): m/z 567.2 (M − H). HPLC retention time (C_{18}, ion pairing): 13.48 min (100%).

((3a*S*,4*R*,6*R*,6a*S*)-2-Benzyl-6-(6-(3-hexylureido)-9*H*-purin-9-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (57). Compound **52** and 1 NH_4 salt (8.0g, 17.2 mmol) were dissolved in DMF (50 mL) and tributylamine (8.17 mL, 34.3 mmol) and the solution was concentrated to approximately one-half volume under vacuum to remove ammonia. Further DMF (25 mL) was added, after which *n*-hexylisocyanate (7.0 mL, 48.3 mmol) was added over 2 h, with vigorous stirring and at such a rate as to avoid solidification of the reaction mixture. The reaction was heated for 3 days at 35 °C and was concentrated to an oil. This residue was reconstituted in 1 M $NaHCO_3$ (20 mL) and water (300 mL), and the resulting suspension heated for 10 min at 50 °C in order to cleave any acylphosphate intermediates. The mixture was cooled, water (100 mL) and ether (200 mL) were added, and the layers were separated. The product containing the aqueous layer was concentrated to around 100 mL and an equal volume of acetonitrile added as an aid to solubility. The product was isolated by reverse-phase preparative HPLC. The yield of **57** was 5.2 g (52%). **57**: ^{1}H NMR (300 MHz, D_2O) δ 0.76 (t, 3H), 1.17 (s, 6H), 1.38 (m, 2H), 2.94 (d, 2H), 3.09 (t, 2H), 3.80 (m, 2H), 4.38 (s, 1H), 4.85 (d, 1H), 5.07 (t, 1H), 5.25 (t, 1H), 5.82 (d, 1H), 7.12 (m, 5H), 8.26 (s, 1H), 8.44 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 5.12 (s). MS (ES): m/z 575.3 (M − H). HPLC retention time (C_{18}, ion pairing): 14.90 min (100%).

((3a*S*,4*R*,6*R*,6a*S*)-2-Benzyl-6-(6-(3-ethylureido)-9*H*-purin-9-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (58). Compound **58** was prepared from **52** according to the method for the synthesis of **57**, substituting ethylisocyanate (6.5 equiv) for *n*-hexylisocyanate. The reaction was heated for a total of 3 days at 50 °C, then concentrated to an oil. Following alkaline treatment to cleave the transient acylphosphate species, the residue was partitioned between aqueous $NaHCO_3$ and ether. The products in the aqueous layer were separated by reverse-phase preparative HPLC, giving **58** in 47% yield. **58**: ^{1}H NMR (300 MHz, D_2O) δ 1.06 (t, 3H), 2.98 (d, 2H), 3.20 (q, 2H), 3.83 (d, 2H), 4.36 (s, 1H), 4.84 (d, 1H), 5.10 (s, 1H), 5.28 (t, 1H), 5.74 (s, 1H), 7.17 (s, 5H), 8.19 (s, 1H), 8.28 (s, 1H).^{31}P NMR (121.47 MHz, D_2O) δ 1.21 (s). MS (ES): m/z 519.2 (M − H). HPLC retention time (C_{18}, ion pairing): 12.00 min (100%).

((3a*S*,4*R*,6*R*,6a*S*)-2-Benzyl-6-(6-(3-cyclopentylureido)-9*H*-purin-9-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (59). Compound **59** was prepared from **52** according of the method for the synthesis of **57**, substituting cyclopentylisocyanate (12 equiv) for *n*-hexylisocyanate. The isocyanate was added in 4 equal portions, with heating at 50 °C over 6 days. The reaction was concentrated to an oil and treated with 1 M $NaHCO_3$ to cleave the transient acylphosphate species. This solution was partitioned between aqueous $NaHCO_3$ and ether, and the products in the aqueous layer were separated by reverse-phase preparative HPLC. The yield of **59** was 10%. **59**: ^{1}H NMR (300 MHz, DMSO-d_6) 1.54 (m, 4H), 1.67 (m, 2H), 1.88 (m, 2H), 3.04 (d, 2H), 3.75 (m, 2H), 4.06 (m, 1H), 4.38 (s, 1H), 4.96 (d, 1H), 5.24 (m, 2H), 6.15 (d, 1H), 7.23 (m, 5H), 8.53 (s, 1H), 8.74 (s, 1H), 9.4 (d, 1H), 9.60 (broad s, 1H). δ ^{31}P NMR (121.47 MHz, D_2O) δ 1.49 (s). MS (ES): m/z 559.3 (M − H). HPLC retention time (C_{18}, ion pairing): 13.50 min (100%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-(3-Ethylureido)-9*H*-purin-9-yl)-2-phenyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (60). Compound **60** was prepared from **53** according to the method for the synthesis of **57**, substituting ethylisocyanate (6 equiv) for *n*-hexylisocyanate. The reaction was heated at 35–40 °C for 4 days, with daily additions of the isocyanate (1.5 equiv × 4) over this time. The conversion by this time was around 92%. Following alkaline treatment to cleave the transient acylphosphate species, workup (aqueous $NaHCO_3$/ether), and preparative HPLC, the yield of **60** was 86%. The product consisted of an approximately 60:40 ratio of acetal diastereomers (60:40 by NMR (MeOD); 56:44 by HPLC). **60**: ^{1}H NMR (300 MHz, MeOD) δ 1.26 (t, 3H), 3.41 (q, 2H), 4.06 (m, 2H), 4.61 (m, 0.6H), 4.70 (s, 0.4H), 5.28 (m, 1H), 5.51 (m, 1H), 6.02 (s, 0.4H), 6.27 (s, 0.6H), 6.47 (t, 1H), 7.40 (m, 3H), 7.52 (m, 1H), 7.59 (m, 1H), 8.56 (d, 1H), 8.72 (s, 0.6H), 8.81 (s, 0.4H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.39 (s, 0.5P), 1.54 (s, 0.5P). MS (ES): m/z 505.2 (M − H). HPLC retention time (C_{18}, ion pairing): 11.58 min (56%), 11.95 min 44%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-(3-Ethylureido)-9*H*-purin-9-yl)-2-styryltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (61). Compound **61** was prepared from **54** according to the method for the synthesis of **57**, substituting ethylisocyanate (7 equiv) for *n*-hexylisocyanate. The isocyanate was added in daily portions (1 equiv × 7), with heating at 30–35 °C over 7 days. The conversion by this time was around 65%. Following alkaline treatment to cleave the transient acylphosphate species, workup (aqueous $NaHCO_3$/ether), and preparative HPLC, the yield of **61** was 50%. The product consisted of an approximately 66:33 ratio of acetal diastereomers (65:35 by NMR (MeOD); 66:33 by HPLC). **61**: ^{1}H NMR (300 MHz, MeOD) δ 1.26 (t, 3H), 3.41 (q, 2H), 4.07 (m, 2H), 4.54 (m, 0.65H), 4.65 (s, 0.35H), 5.14 (dd, 0.35H), 5.21 (dd, 0.65H), 5.38 (m, 1H), 5.65 (d, J = 6.6 Hz, 0.35H), 5.90 (d, J = 6.3 Hz, 0.65H), 6.21 (dd, J = 6.3 Hz, 0.65H), 6.39 (m, 1.35H), 6.89 (dd, 1H), 7.31 (m, 3H), 7.48 (m, 2H), 8.57 (d, 1H), 8.69 (s, 0.6H), 8.77 (s, 0.4H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.90 (s, 0.33P), 1.98 (s, 0.66P). MS (ES): m/z 531.2 (M − H). HPLC retention times (C_{18}, ion pairing): 12.06 min (66%), 12.39 (33%).

((2*S*,3a*S*,4*R*,6*R*,6a*S*)-6-(6-(3-Ethylureido)-9*H*-purin-9-yl)-2-styryltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (62). Compound **61** (50 mg; a 66% trans:33% cis mixture of diastereomers) was dissolved in 25% aqueous acetic acid (2 mL) and was warmed at 35 °C for 1.5 h. Ion pairing HPLC (see General Methods) indicated that the less stable *cis*-acetal isomer was completely cleaved by this time, leaving a mixture of the trans diastereomer, the 2′,3′-diol arising from the cleavage of the cis diastereomer, and cinnamaldehyde. The products were separated and purified via preparative HPLC. Following lyophilization, the yield of title compound **62** was 23 mg (69.7%). **62**: ^{1}H NMR (300 MHz, MeOD) δ 1.26 (t, 3H), 3.41 (q, 2H), 4.03 (m, 2H), 4.56 (s, 1H), 5.24 (dd, 1H), 5.45 (dd, 1H), 5.91 (d, J = 6.0 Hz, 1H), 6.22 (dd, J = 6.0 Hz, J = 15.9 Hz, 1H), 6.40 (d, J = 3.3 Hz, 1H), 6.87 (d, J = 16.2 Hz, 1H), 7.29 (m, 3H), 7.46 (d, 2H), 8.56 (s, 1H), 8.92 (s, 1H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.29 (s). MS (ES): m/z 531.2 (M − H). HPLC retention time (C_{18}, ion pairing): 12.12 min (100%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-(3-Hexylureido)-9*H*-purin-9-yl)-2-styryltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen

Phosphate (63). Compound **63** was prepared from **54** according to the method for the synthesis of **57**. *n*-Hexylisocyanate (4.3 equiv) was added and the reaction heated at 35 °C overnight. A further portion of *n*-hexylisocyanate (2.2 equiv) was added, and the reaction heated an additional 3 days. Following alkaline treatment, workup (aqueous $NaHCO_3$/ether), and preparative HPLC, the yield of **63** was 38%. The product consisted of an approximately 75:25 ratio of acetal diastereomers (80:20 by NMR (D_2O); 75:25 by HPLC). **63**: ^{1}H NMR (300 MHz, D_2O) δ 0.69 (t, 3H), 1.19 (m, 6H), 1.44 (t, 2H), 3.18 (t, 2H), 3.85 (s, 2H), 4.46 (d, 1H), 5.05 (m, 1H), 5.25 (m, 0.2H), 5.30 (m, 0.8H), 5.60 (d, 0.2H), 5.80 (d, 0.8H), 6.07 (dd, 0.8H)), 6.22 (m, 1.2H), 6.76 (t, 1H), 7.20 (m, 3H), 7.29 (m, 2H), 8.37 (s, 1H), 8.48 (s, 0.8H), 8.57 (s, 0.2H). ^{31}P NMR (121.47 MHz, D_2O) δ 4.99(s, 0.2P), 5.05 (s, 0.8P). MS (ES): *m*/*z* 587.4 (M − H). HPLC retention time (C_{18}, ion pairing): 15.43 min (75%), 16.37 (25%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-(3-Phenylureido)-9*H*-purin-9-yl)-2-styryltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (64). Compound **64** was prepared from **54** according to the method for the synthesis of **56**. The reaction was allowed to stir at 40 °C for 2 days. Following workup and preparative HPLC, the yield of the title compound was 22%. The product consisted of an approximately 65:35 ratio of acetal diastereomers (66:33 by NMR (D_2O); 65:35 by HPLC). **64**: ^{1}H NMR (300 MHz, D_2O) δ 3.88 (m, 2H), 4.47 (d, 1H), 5.07 (m, 1H), 5.28 (m, 1H), 5.63 (d, J = 6.6 Hz, 0.33H), 5.81 (d, J =6.3 Hz, 0.66H), 6.19 (m, 2H), 6.78 (dd, 1H), 7.01 (t, 1H), 7.26 (m, 9H), 8.44 (s, 0.33H), 8.46 (s, 0.66H), 8.49 (s, 0.66H), 8.59 (s, 0.33H). ^{31}P NMR (121.47 MHz, D_2O) δ 4.86 (s, 0.35P), 4.89 (s, 0.65P). MS (ES): *m*/*z* 579.3 (M − H). HPLC retention time (C_{18}, ion pairing): 13.62 min (65%), 14.19 (35%).

((3a*S*,4*R*,6*R*,6a*S*)-6-(6-(3-Hexylureido)-9*H*-purin-9-yl)-2-(phenylethynyl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (65). Compound **65** was prepared from **55** according to the method for the synthesis of **57**. *n*-Hexylisocyanate (4 equiv) was added in a single portion and the reaction heated at 80–85 °C overnight. Following alkaline treatment, workup (aqueous $NaHCO_3$/ether), and preparative HPLC, the yield of **65** was 22%. The product consisted of an approximately 48:52 ratio of acetal diastereomers (45:55 by NMR (MeOD); 48:52 by HPLC). **65**: ^{1}H NMR (300 MHz, MeOD) δ 0.92 (t, 3H),1.37 (m, 6H), 1.64 (m, 2H), 3.38 (t, 2H), 4.07 (m, 2H), 4.60 (m, 0.45H),4.76 (m, 0.55H), 5.22 (dd, 0.55H), 5.34 (dd, 0.45H), 5.46 (dd, 0.55H), 5.56 (dd, 0.45H), 6.07 (s, 0.55H), 6.32 (s, 0.45H), 6.35 (d, J = 3.3 Hz, 0.45H), 6.65 (d, J = 3.3 Hz, 0.55H), 7.40 (m, 4H), 7.56 (m, 1H), 8.56 (d, 1H), 8.76 (s, 0.45H), 8.79 (s, 0.55H). ^{31}P NMR (121.47 MHz, D_2O) δ 1.99 (s, 0.51P), 2.05 (s, 0.49P). MS (ES): *m*/*z* 585.4 (M − H). HPLC retention times (C_{18}, ion pairing): 14.80 min (48%), 15.97 (52%).

((3a*S*,4*R*,6*R*,6a*S*)-2-(Phenylethynyl)-6-(6-(3-phenylureido)-9*H*-purin-9-yl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl-dihydrogen Phosphate (66). Compound **66** was prepared from **55** according to the method for the synthesis of **56**, using phenyl isocyanate (5 equiv). The reaction was heated overnight at 30–35 °C. Following workup and preparative HPLC, the yield of the title compound was 61%. The product consisted of a 50:50 ratio of acetal diastereomers (47:53 by NMR (MeOD); 49:51 by HPLC). **66**: ^{1}H NMR (300 MHz, MeOD) δ 4.05 (m, 2H), 4.64 (s, 0.47H), 4.76 (s, 0.53H), 5.25 (d, 0.52H), 5.39 (d, 0.48H), 5.48 (m, 0.52H), 5.58 (m, 0.48H), 6.04 (s, 0.53H), 6.33 (s, 0.47H), 6.39 (d, J = 3.3 Hz, 0.49H), 6.70 (d, J = 3.3 Hz, 0.51H), 7.11 (t, 1H), 7.40 (m, 6H), 7.59 (m, 3H), 8.67 (s, 1H), 9.02 (s, 0.47H), 9.05 (s, 0.53H). ^{31}P NMR (121.47 MHz, D_2O) δ 5.07 (0.5P), 5.08 (0.5P). MS (ES): *m*/*z* 577.3 (M − H). HPLC retention times (C_{18}, ion pairing): 13.59 min (49%), 14.42 (51%).

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Supporting Information Available: ^{1}H NMR (D_2O, 300 MHz) for compounds **11**, **13**, **18**, **21**, **53**−**56**, **59**–**62**, **65**, and **66**. NOE (D_2O, 300 MHz) correlations for compounds **21**, **31**, **38**, **45**, **58**, and **62**. This material is available free of charge via the Internet at http://pubs.acs.org.

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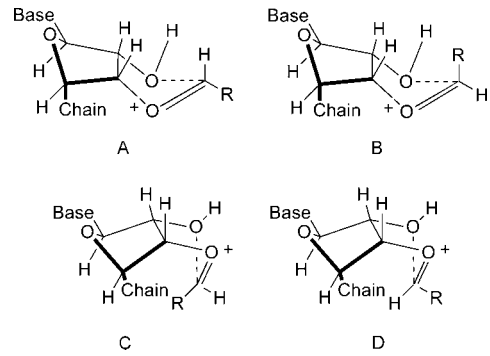
(42) Further proof of structure for compounds **21**, **31**, **38**, **45**, **58**, and **62** may be found in the Supporting Information.

(43) One plausible explanation to describe the selectivity of the acetal ring-forming reactions can be illustrated by examining the following transition states (**A**−**D**). The final ring closure could be described as a 5-endo trig cyclization of a free 2′ or 3′ hydroxyl group of the ribose ring on to the oxonium ion derived from the complementary hydroxyl group. The transition states are drawn using the 2′-hydroxyl as a nucleophile and the 3′-oxonium ion as an electrophile for illustrative purposes only. To minimize steric congestion, the conformation of the ribose ring places the base and the side chain in pseudo equatorial positions. Transition states **A** and **B**, which place the pivotal forming stereogenic center of the new ring in a pseudo exo-orientation relative to the ribose ring, should govern formation of kinetically favored products. Transition states **C** and **D**, which place the pivotal forming stereogenic center of the new ring in a pseudo endo-orientation, should be less favored. Therefore, the kinetically favored product would be derived from transition state **A**, which places the substituent in a pseudo equatorial position. Kinetic products (products derived from aldehydes that generate very unstable oxonium ions) should give predominantly the cis-product. In circumstances where the formation of products is under thermodynamic control, acetals derived from aldehydes that can generate stable oxonium ions (cinnamaldehyde, benzaldehyde, and ethynyl phenyl aldehyde), the favored product (whether derived from an open or closed oxonium ion intermediate), would result from the substituent being in the energetically more favored exo-face of the 5,5-bicyclic acetal. Thermodynamic products should give predominantly the trans-product.



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(47) In addition to the aforementioned regiochemistry problems, the ribose esters were also less stable as solids and in solution than acetals. At room temperature and a pH of greater than 7, which would be the typical physiological situation, compounds **15**–**17** showed significant decomposition after a few days. The acetal compounds were stable in solution under these conditions. Solid ester compounds typically were stored at −80 °C in their ammonium salt forms to avoid decomposition over the long term, whereas solid acetals (in their sodium or ammonium salt forms) could be stored on the benchtop for many months without ill effect. The general trend for stability of the aromatic acetals under acidic conditions was styryl < phenyl < benzyl.

(48) In general, the rank order of hydrolytic stability of the phosphates that we observed was dinucleoside polyphosphates > nucleoside monophosphates > nucleoside diphosphates > nucleoside triphosphates. Dinucleoside polyphosphates and nucleoside monophosphates

modified with the lipophilic moieties described in this article could be stored in their salt forms (sodium, ammonium, or tributylammonium) at ambient temperature for months without significant decomposition. As noted in the preparation of **41**, it was nearly impossible to avoid some degradation of the nucleoside diphosphate to the monophosphate (**56**), even with optimal handling. The corresponding triphosphate analogue to **41**and**56**(not included in this article) proved to be much more troublesome and was not pursued.

(49) These data have been partially reported in poster format at the 4th International Symposium of Nucleosides and Nucleotides, Chapel Hill, North Carolina, June, 2004. Douglass, J. G.; Patel, R. I.; Redick, C. C.; Jones, A. C.; Shave, S. R.; Boyer, J. L. (Poster 70W) Lipophilic Adenine Nucleotides as Reversible Inhibitors of P2Y$_{12}$-Mediated Platelet Aggregation. 4th International Symposium of Nucleosides and Nucleotides, Chapel Hill, North Carolina, June, 2004.

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