5′-*O*-Tritylinosine and Analogues as Allosteric Inhibitors of Human Thymidine Phosphorylase

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On the basis of our previous findings that 5′-*O*-tritylinosine (KIN59) behaves as an allosteric inhibitor of the angiogenic enzyme thymidine phosphorylase (TPase), we have undertaken the synthesis and enzymatic evaluation of a novel series of nucleoside analogues modified at positions 1, 2, or 6 of the purine ring and at the 5′-position of the ribose moiety of the lead compound KIN59. SAR studies indicate that quite large structural variations can be performed on KIN59 without compromising TPase inhibition. Thus, incorporation of a cyclopropylmethyl or a cyclohexylmethyl group at position N^1 of 5′-*O*-tritylinosine increases the inhibitory activity against TPase 10-fold compared to KIN59. Moreover, the trityl group at the 5′-position of the ribose seems to be crucial for TPase inhibition. The here reported results further substantiate that 5′-*O*-trityl nucleosides represent a new class of TPase inhibitors that should be further explored in those biological systems where TPase plays an instrumental role (i.e. angiogenesis).

Introduction

Nucleoside analogues constitute an important contribution to the chemotherapy of cancer, viral diseases, and immunomodulating therapies. Despite their long tradition as chemotherapeutic agents, novel applications are still being explored and new nucleoside structures reach the clinic. Some very recent examples of nucleosides that have been approved for anticancer or antiviral applications include nelarabine,[1] entecavir,[2] clofarabine,[3] or azacitidine.[4] In most cases, nucleosides behave as prodrugs or bioprecursors that need to be activated to their pharmacologically active metabolite by cellular and/or viral enzymes. Still, there are an increasing number of compounds with a nucleoside structure that do not require this activation to become pharmacologically active. In some cases, these nucleoside analogues bind at the substrate-binding site of the target enzyme but are not recognized as substrates, and therefore, they exert mostly a competitive inhibition with the natural substrate. In a few other cases, the nucleoside analogues interact with the target enzyme at a site different from the substrate-binding site, and therefore, they behave as noncompetitive inhibitors with respect to the natural substrate. This binding site, which is topographically distinct, and quite often, distant, from the substrate-binding site, can be designated as an allosteric binding site.[5] Allosteric inhibitors of enzymes may offer some advantages when compared to compounds interacting at the substrate-binding site. Since these allosteric ligands do not need to be structurally related to the enzyme substrates, they may be safer and more efficacious by overcoming selectivity issues and substrate competition.[5] Therefore, allosteric inhibitors are considered as novel pharmacologically active compounds that may increase our understanding of basic enzymatic or biological processes involving the target enzymes.[5,6] A typical example of such allosteric inhibitors is represented by the non-nucleoside reverse transcriptase inhibitors of the human immunodeficiency virus (HIV), where a variety of apparently different structures tightly interact with an allosteric site on the enzyme that is ∼15 Å distanct from the substrate active site.[7,8]

Since 1998, our research groups have been involved in the discovery of novel inhibitors of the human enzyme thymidine phosphorylase (TPase). TPase catalyses the reversible phosphorolysis of pyrimidine 2′-deoxynucleosides to 2-deoxyribose-1-phosphate and their respective pyrimidine bases, including the phosphorolysis of nucleoside analogues with important antiviral or anticancer properties. Moreover, TPase, identified also as the angiogenic platelet-derived endothelial cell growth factor (PD-ECGF), stimulates endothelial cell migration in vitro and angiogenesis in vivo and plays an important role in tumor progression and metastasis.[9−11] We have identified several series of TPase inhibitors, including purine bases such as 7-deazaxanthine (7-DX)[12] or multisubstrate inhibitors such as TP65[13−15] (Chart 1). More recently, we reported the inhibitory activity of the purine riboside derivative KIN59 (5′-*O*-tritylinosine) (**1**) against human and *Escherichia coli* recombinant TPase.[16] In contrast to previously described TPase inhibitors, KIN59 competes neither with the pyrimidine nucleoside nor with the phosphate-binding site of the enzyme but noncompetitively inhibits TPase when thymidine or phosphate is used as the variable substrate.[16] In addition, KIN59 is far more active than our previously identified TPase inhibitors against TPase-induced angiogenesis in an established angiogenesis assay (chicken chorioallantoic membrane assay, CAM assay).[16] It was demonstrated that inosine, the parent nucleoside of KIN59, neither inhibits the enzyme nor the angiogenic activity of TPase in the CAM assay.[16] This indicates that the antiangiogenic activity of KIN59 requires the intact trityl-containing nucleoside. Therefore, KIN59 is the prototype of a new family of nucleosides that behave as allosteric inhibitors of TPase. Since the TPase inhibitor KIN59 has such a marked effect on angiogenesis, we speculated that KIN59 and analogues could help to explore a novel allosteric binding site at TPase, different from the thymidine and phosphate-binding sites that could play an important role in TPase-triggered angiogenesis stimulation. In this study, we performed modifications on the lead compound

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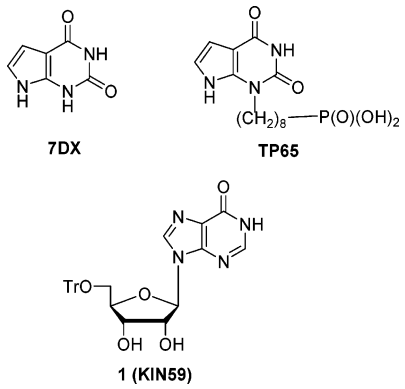
Chart 1. Structural Formulas of the TPase Inhibitors 7-Deazaxanthine (7DX), TP65, and KIN59



KIN59 to determine the structural requirements among this series of purine nucleosides for TPase inhibition.

Chemistry

When considering structural modifications on our lead compound **1**, we have focused on positions 1 and 6 of the purine base and the 5′-OH of the ribose moiety. Moreover, we have cleaved the pyrimidine ring of the purine base by synthesizing the corresponding AICAR (5-amino-(1-β-D-ribofuranosyl)imidazole-4-carboxamide) derivatives, and we have also opened the ribose ring of compound **1** by synthesizing the corresponding 2′,3′-seconucleoside.

On the basis of the known reactivity of position 1 of inosine, the first series of targeted compounds were N^1 substituted 5′-*O*-tritylinosines that could be synthesized by the introduction of different alkyl, allyl, or benzyl substituents at position N^1 of 5′-*O*-tritylinosine (**1**). Thus, treatment of **1**[16] with the corresponding halide in dimethylacetamide (DMAC) and in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[17] afforded the N^1-substituted 5′-*O*-tritylinosines (**2a**−**f**) in good yields (56−89%) (Scheme 1); only the (*E*)-3-methoxycarbonyl-2-propenyl derivative **2g** was obtained in a lower yield (32%). As previously shown in different series of compounds, substitution occurs regioselectively at N^1, while the O^6-substituted byproducts[18] were, in our case, not detected.

It is well-known that N^1-alkylinosines can be cleaved at the pyrimidine ring by treatment with aqueous alkali to yield 5-amino-4-(*N*-alkylcarbamoyl)imidazole ribosides.[19−21] Several compounds from the above series were subjected to this pyrimidine-opening reaction. Thus, reaction of **2a**−**d** with 5 N NaOH in refluxing EtOH afforded the 4-*N*-methyl, 4-*N*-propyl, 4-*N*-allyl, and 4-*N*-cyclopropylmethyl carbamoyl derivatives **3a**−**d** in 46−53% yield (Scheme 1). In every case, the opening of the pyrimidine ring is clear by the appearance in the ^{1}H NMR spectra of a broad singlet, corresponding to the 5-NH_2 signal, around 5.7 ppm and a triplet around 7.5 ppm that corresponds to the 4-CONH, when the spectra were recorded in DMSO-d_6. It is also worth mentioning the upfield movement of the signal corresponding to the anomeric proton H-1′ ($\Delta\delta = -0.4$) when comparing the open (**3a**−**d**) to the close analogues (**2a**−**d**).

We also considered of interest the synthesis of the free 4-$CONH_2$ derivative that should correspond to the 5′-*O*-trityl-AICAR (**8**). The synthesis of AICAR derivatives from inosine by using *p*-toluenesulfonyl or methoxymethyl groups as N^1-substituents was described by Shaw.[22] According to this report, treatment of these N^1-substituted inosines by aqueous alkali in refluxing ethanol could simultaneously open the pyrimidine ring and remove the N^1-substituent to afford the AICAR compounds. A more recent paper makes use of a (2-methoxyethoxy)methyl (MEM) as the substituent of choice to perform this transformation from inosine to AICAR.[23] Due to the high reactivity of the MEMCl with alcohols, the 2′- and 3′-OH of 5′-*O*-tritylinosine (**1**) were acetylated prior to the treatment with MEMCl. Thus, reaction of **4**[24] with MEMCl in dry CH_2Cl_2 in the presence of *i*-Pr_2NEt afforded the N^1-MEM inosine derivative **5** in 72% yield (Scheme 2). Treatment of **5** with refluxing 0.2 N NaOH for 24 h, as described,[23] afforded **6** as the major compound (42%) together with a smaller amount of the open analogue **7** (18% yield) that still keeps the MEM moiety on the N^4-carboxamide. Only after longer reaction times was the 5′-*O*-trityl AICAR derivative (**8**) detected. These results indicate that the opening reaction of the pyrimidine ring is prior to the removal of the MEM moiety, results that agree with the proposal of Shaw.[22] Alternatively, treatment of **5** with refluxing 5 N NaOH for 6 h afforded the 5′-*O*-trityl AICAR derivative **8** in 20% yield.

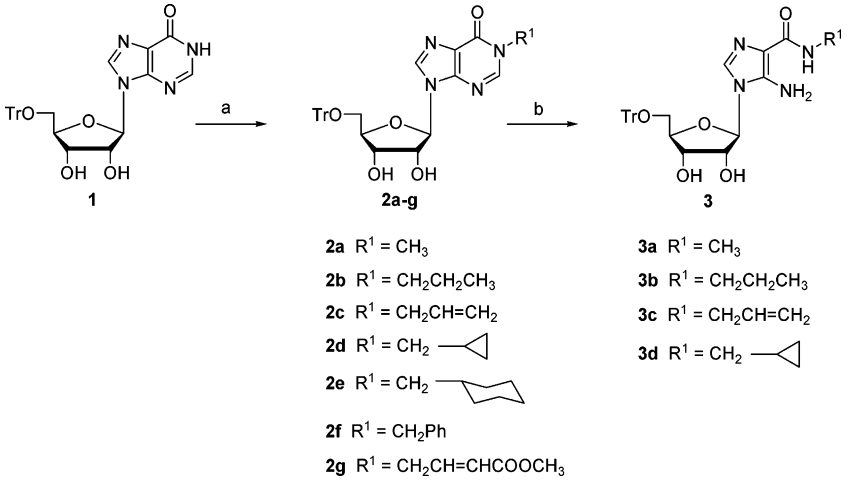
Next, we decided to replace the hypoxanthine moiety of the lead compound **1** by other purines. In particular, we chose the ribofuranosyl derivatives of 9*H*-purine (**9**) and 6-chloro-9*H*-purine (**10**) (Scheme 3). Treatment of **9** with trityl chloride in pyridine with a small amount of DMAP at 80 °C afforded the 5′-*O*-trityl nucleoside **11** in 35% yield. However, this procedure failed when applied to the 6-chloropurin-9-yl riboside (**10**). Alternatively, the 6-chloro-9-(5-*O*-trityl-β-D-ribofuranosyl)purine (**12**) was synthesized by reaction of **10** with TrCl in DMF at 40 °C and in the presence of *i*-Pr_2NEt, as described.[25] Further reaction of **12** with $MeNH_2$ in MeOH afforded the 6-NHMe purin-9-yl derivative (**13**) in 42% yield.

We also wanted to evaluate the impact of substituents introduced on the trityl moiety on TPase inhibition (Scheme 4). For this purpose, the 5′-*O*-(4,4′-dimethoxytrityl)inosine (**16**) was synthesized following described procedures.[26] We also prepared the 4-chlorotrityl derivative (**17**) by reaction of inosine (**14**) with 4-chlorotrityl chloride. This reagent was synthesized by treatment of the 4-chlorobenzophenone with phenylmagnesium bromide and further transformed into the desired chloride by reaction of the resulting carbinol with acetyl chloride.[27] Moreover, reaction of 2′-deoxyinosine (**15**) with trityl chloride in pyridine, in the presence of DMAP, at 60 °C, afforded 5′-trityl-2′deoxyinosine (**18**). It is worth mentioning that when the reaction between inosine (**14**) and trityl chloride was performed in DMF at 40 °C and in the presence of *i*-Pr_2NEt, conditions employed for the 5′-*O*-tritylation of 6-chloro-(1-β-D-ribofuranosyl)purine,[25] the reaction on inosine did not occur at the 5′-OH, as expected, but at position 1, yielding the N^1-tritylinosine derivative (**19**) as the major product (30% yield). To the best of our knowledge there is only one previous report on the introduction of a trityl moiety at N^1 of inosine, although in this report the 5′- and 3′-positions of 2′-deoxyinosine were protected with acetyl groups prior to the introduction of the trityl moiety at N1.[28] The selective introduction of the trityl moiety at N1 under the experimental conditions above-described (in the presence of the free OH groups of the ribose) has no precedent and could be of value as a protective strategy.

To highlight the importance of the presence of the furanose moiety in our lead structure **1**, we have performed the synthesis of the corresponding 2′,3′-seconucleoside (**20**). Thus, reaction of **1** with sodium periodate followed by "in situ" reduction of the resulting dialdehyde with sodium borohydride[26,29] afforded the target compound **20** in 60% yield (Scheme 5).

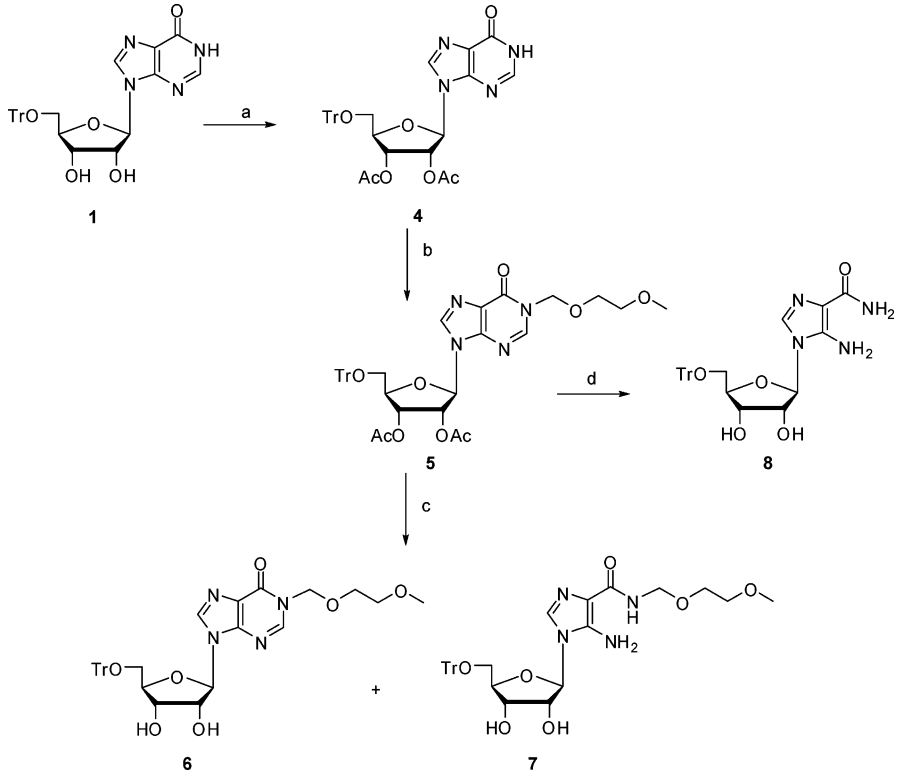
Different attempts have been made in our laboratory to synthesize 5′-*O*-benzylinosine. The only reported procedure involves reaction of 2′,3′-*O*-isopropylideneinosine with benzyl

Scheme 1. Synthesis of N^1 Substituted 5′-*O*-Tritylinosines and Their Open Pyrimidine-Ring Analogues[a]



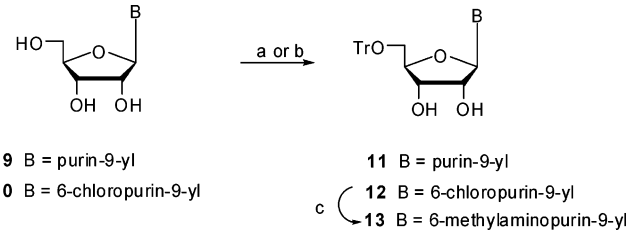
[a] (a) R1−X, DBU, DMAC, Ar, rt; (b) 5 N NaOH, EtOH, Δ.

Scheme 2. Synthesis of 5′-*O*-Trityl AICAR[a]



[a] (a) Ac_2O, Py, rt; (b) MEMCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C; (c) 0.2 N NaOH, Δ; (d) 5 N NaOH, Δ.

Scheme 3. Synthesis of 9-(5-*O*-Trityl-β-D-ribofuranosyl)purines **11**, **12** and **13**[a]



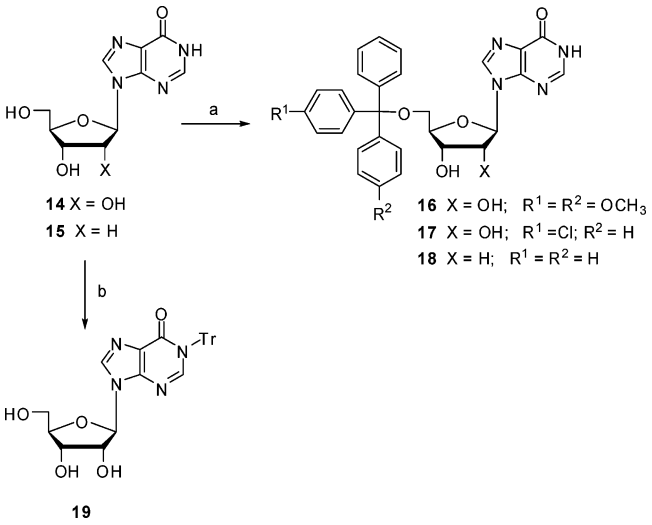
[a] (a) TrCl, DMAP, Py, 80 °C; (b) TrCl, *i*-Pr_2NEt, DMF, 40 °C; (c) $MeNH_2$ in EtOH, 70 °C.

bromide and potassium *tert*-butoxide in dioxane.[30] In our hands, this reaction afforded a mixture of 1- and 1,5′-dibenzyl products. This is a reasonable result since position 1 of inosine is highly reactive and reacts with benzyl bromide under the same experimental conditions as the 5′-OH.[31] On the basis of these results, we have undertaken the synthesis of the 1,5′-dibenzylinosine (**23**). Thus, reaction of 2′,3′-*O*-isopropylideneinosine (**21**) with benzyl bromide in DMF in the presence of NaH at room temperature afforded the dibenzylated derivative (**22**) in 71% yield (Scheme 6). In our hands, and in contrast to the described method,[31] it was important to perform this reaction at room temperature to avoid undesired products. Deprotection of the isopropylidene moiety by treatment with aqueous TFA afforded **23**[31] in 95% yield.

Biological Results and Discussion

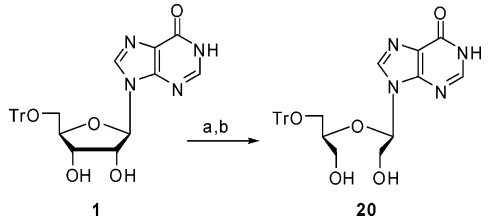
The synthesized compounds have been evaluated for their inhibitory activity against human TPase in the presence of 100 μM thymidine as the natural substrate. The 50% inhibitory

Scheme 4. Reaction of Inosine and 2′-Deoxyinosine with Trityl Chlorides[a]



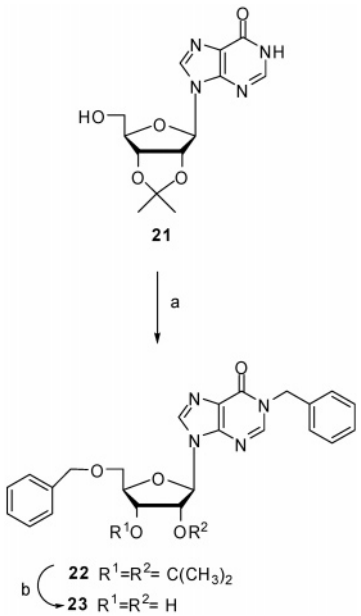
[a] (a) DMTrCl or 4-ClTrCl or TrCl, Py, DMAP, 60 or 80 °C; (b) TrCl, *i*-Pr_2NEt, DMF, 40 °C.

Scheme 5. Synthesis of 5′-*O*-Trityl-2′,3′-secoinosine[a]



[a] (a) $NaIO_4$, dioxane:H_2O, rt; (b) $NaBH_4$, dioxane:H_2O.

Scheme 6. Synthesis of 1,5′-*O*-Dibenzylinosine[a]



[a] (a) NaH, BnBr, DMF, rt; (b) 30% aqueous TFA.

concentrations (IC_{50} values), that is, the concentrations at which these compounds inhibit thymidine to thymine conversion by 50%, are shown in Table 1. For comparative purposes, we may indicate that 7-deazaxanthine (Chart 1), one of our previously described TPase inhibitors that is competitive to the thymidine substrate, shows an IC_{50} of 48 μM under similar experimental conditions. Interestingly, several of the synthesized derivatives are more inhibitory than the lead compound **1** against human TPase. In particular, incorporation of an alkyl (propyl, **2b**), alkenyl (propenyl, **2c**), or a benzyl (**2f**) group at N^1 of the lead compound **1** increases the inhibitory activity of the new compounds by 2.5−3-fold. Interestingly, those compounds that incorporate a methylcycloalkyl substituent (**2d**, **2e**) are at least 10-fold more inhibitory than **1**. Moreover, the corresponding AICAR analogues (**3b**, **3c**) also show an inhibitory potency similar to that of their parent inosine analogues (**2b**, **2c**). However, compounds with a MEM substituent at N^1 either in the inosine (**6**) or in the AICAR series (**7**) were devoid of inhibitory activity against TPase up to 75−100 μM. The 5′-*O*-trityl-AICAR **8** at the highest concentration tested (40 μM) inhibited thymidine to thymine conversion at almost 50%.

Table 1. Inhibitory Effect of the Synthesized Compounds on Human TPase Enzymatic Activity

compd	IC_{50} (μM)[a]	compd	IC_{50} (μM)[a]	compd	IC_{50} (μM)[a]
1	30 ± 15	**3a**	137	**11**	45 ± 3
2a	49 ± 4	**3b**	13 ± 3	**12**	26
2b	13 ± 2	**3c**	23 ± 5	**16**	18 ± 3
2c	11 ± 2	**3d**	19	**17**	4.5 ± 2.3
2d	2.3 ± 0.4	**5**	>20	**18**	96
2e	4.4 ± 1.8	**6**	>100	**20**	>200
2f	11 ± 1	**7**	>75	**23**	>200
2g	24 ± 41	**8**	≥40		

[a] IC_{50}: concentration at which the conversion of 100 μM thymidine to thymine is inhibited by 50%.

Replacement of the hypoxanthine base of the lead compound by other structurally related purines modified at position 6, like in compounds **11** or **12**, leads to compounds with activity similar to that of the inosine derivative **1**.

Concerning the 5′-position of the ribose moiety, the trityl group of **1** can be replaced by a 4,4′-dimethoxytrityl (such as in **16**) or a 4-chlorotrityl (such as in **17**) and the inhibitory activity against TPase is preserved or even increased. However, replacement of the 5′-*O*-trityl by a 5′-*O*-benzyl group results in an inactive compound (compare compound **23** with the trityl analogue **2f**). These data further stress the crucial importance of a trityl group at the 5′-position of these nucleoside derivatives for TPase inhibition. Interestingly, the 5′-*O*-trityl-2′deoxyinosine (**18**) also shows TPase inhibition although at higher concentrations (IC_{50}= 96 μM) than the ribose analogue **1**. On the other hand, the inactivity of the 5′-*O*-trityl-2′,3′-secoinosine derivative **20** compared to the intact nucleoside **1** indicates that not only the 5′-*O*-trityl and the hypoxanthine moieties are important for TPase inhibition but that the sugar scaffold of the nucleoside seems to be instrumental to position the two substituents (the 5′-*O*-trityl and the hypoxanthine moiety) in the right way to allow interaction with TPase. It should be mentioned, in this respect, that we ascertained that the parent compound **1** and several of its derivatives are fully stable in the presence of CEM cell extracts and bovine serum, further attesting the important role of the 5′-trityl group in the eventual biological activity of the test compounds. Indeed there are an increasing number of examples where a trityl moiety might be considered as a pharmacophoric group, both in the nucleoside[32−34] and in the peptide field.[35,36]

Some of the most potent TPase inhibitors described in this paper have also been tested against TPase-triggered angiogenesis in the CAM assay.[37] In particular, the 4-chlorotrityl derivative **17** has shown a marked inhibition of TPase-induced neovascularization at 250 nmol.[37] At a lower concentration (100 nmol), compound **17** was still able to inhibit TPase-induced angiogenesis by 75% while, at the same concentration, the parent compound **1** showed 32% inhibition.

Conclusions

We have performed the synthesis and enzymatic evaluation against human thymidine phosphorylase of a series of purine nucleoside derivatives structurally related to the TPase lead inhibitor 5′-*O*-tritylinosine. SAR studies indicate that quite large structural variations can be performed on the lead structure **1** without compromising TPase inhibition. Modification of the N^1 position of the hypoxanthine base by incorporating alkyl, alkenyl, or cyclic alkyl substituents results in maintained or increased TPase inhibition. In particular, those compounds that incorporate a cyclopropylmethyl (**2d**) or cyclohexylmethyl (**2e**) moiety are 10-fold more inhibitory against TPase than the parent compound. Moreover, several 5-amino-4-(*N*-substituted-carbamoyl)imidazole ribosides (**3b**−**d**), derived from the cleavage of the pyrimidine ring of the N^1-substituted inosines, were equally active against TPase as the parent **1**. Also other purine bases that lack a carbonyl (**11**) or have a chlorine (**12**) at position 6 retain activity. The trityl substituent at the 5′-position is crucial for activity, since the corresponding 5′-*O*-benzyl analogue was inactive. Moreover, the intact nucleoside structure in this family of compounds is important to correctly position the nucleic base and the 5′-*O*-trityl substituent for interaction with the target enzyme, since the oxidative cleavage at positions 2′,3′ of the ribose as in the 2′,3′-secoinosine derivative **20** renders an inactive compound.

Attempts are now being made to cocrystallize 5′-*O*-tritylinosine with human TPase, but so far these attempts have not been successful. Therefore, the allosteric binding site where 5′-*O*-tritylinosine binds to TPase remains elusive. Alternatively, the data reported in this paper, together with our recent report that 5′-*O*-trityl derivatives of pyrimidines, exemplified by 1-(5′-*O*-trityl-*β*-D-ribofuranosyl)thymine, also noncompetitively inhibit TPase,[37] indicate that 5′-*O*-tritylnucleosides constitute a novel class of TPase inhibitors. This family of compounds accepts significant structural modifications without impairment or even with a marked increase of their inhibitory activity against TPase, a feature that is not unusual among allosteric inhibitors. Some of the here described compounds, such as **17**, have been shown to be even more potent than the parent compound **1** in biological assays (i.e., TPase-triggered angiogenesis), supporting the interest of this class of nucleosides for further optimization.

Experimental Section

Chemical Procedures. Melting points were obtained on a Reichert-Jung Kofler apparatus and are uncorrected. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MS HP 1100). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini operating at 200 MHz (^{1}H) and 50 MHz (^{13}C), respectively, on a Varian INNOVA 300 operating at 299 MHz (^{1}H) and 75 MHz (^{13}C), respectively, and Varian INNOVA-400 operating at 399 MHz (^{1}H) and 99 MHz (^{13}C), respectively.

Analytical TLC was performed on silica gel 60 F_{254} (Merck) precoated plates (0.2 mm). Spots were detected under UV light (254 nm) and/or by charring with phosphomolybdic acid. Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron^R (Kiesegel 60 PF_{254} gipshaltig (Merck)), with layer thickness of 1 or 2 mm and flow rate of 4 or 8 mL/min, respectively. Flash column chromatography was performed with silica gel 60 (230−400 mesh) (Merck).

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Triethylamine and acetonitrile were dried by refluxing over calcium hydride. Tetrahydrofuran was dried by refluxing over sodium/benzophenone. Anhydrous *N*,*N*′-dimethylformamide was purchased from Aldrich. Anhydrous dimethylacetamide (DMAC) was purchased from Aldrich. 2′,3′-*O*-Isopropylideneinosine was purchased from Aldrich.

General Procedure for the Preparation of N^1-Substituted 5′-*O*-Tritylinosines. To a solution of 5′-*O*-tritylinosine (**1**)[16] (200 mg, 0.39 mmol) in DMAC (3 mL) were added the corresponding alkyl or benzyl halide (0.51 mmol) and DBU (0.077 mL, 0.52 mmol). The reaction mixture was stirred at room temperature under argon overnight. The reaction was quenched by addition of a mixture of ether:hexane 1:1 (8 mL) and the resulting suspension was placed at −20 °C overnight. Solvents were decanted while still chilly, and the resulting gum was evaporated under reduced pressure. The resulting residue was partitioned into EtOAc (25 mL) and a $NaHCO_3$ solution (15 mL). The organic phase was further washed with a $NaHCO_3$ solution (2 × 10 mL) and finally with brine. The organic phase was dried on anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by CCTLC in the Chromatotron or by flash column chromatography as indicated for each compound.

1-Methyl-5′-*O*-tritylinosine (2a). Following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, reaction of **1** with methyl iodide (32 μL, 0.51 mmol) in the presence of DBU afforded a residue that was purified in the Chromatotron (CH_2Cl_2:MeOH, 10:1) to afford 159 mg (77% yield) of **2a** as a white solid: mp 181−184 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 525 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.24 (m, 2H, H-5′), 3.49 (s, 3H, NCH_3), 4.06 (q, J = 5.1 Hz, 1H, H-4′), 4.20 (m, 1H, H-3′), 4.55 (m, 1H, H-2′), 5.25 (d, J = 5.9 Hz, 1H, OH), 5.57 (d, J = 5.9 Hz, 1H, OH), 5.88 (d, J = 4.4 Hz, 1H, H-1′), 7.22−7.42 (m, 15 H, Ph), 8.20 (s, 1H, H-8), 8.32 (s, 1H, H-2). Anal. ($C_{30}H_{28}N_4O_5 \cdot H_2O$) C, H, N.

1-Propyl-5′-*O*-tritylinosine (2b). Reaction of **1** with propyl iodide (50 μl, 0.51 mmol), following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, afforded a residue that was purified by flash column chromatography (CH_2Cl_2:MeOH, 15:1) to yield 193 mg (89%) of **2b** as a white solid: mp 115−118 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 553 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, J = 7.0 Hz, 3H, CH_3), 1.65 (m, 2H, CH_2CH_3), 3.20 (m, 2H, H-5′), 3.94 (t, J = 7.0 Hz, 2H, NCH_2), 4.05 (m, 1H, H-4′), 4.20 (m, 1H, H-3′), 4.55 (m, 1H, H-2′), 5.23 (d, J = 5.7 Hz, 1H, OH), 5.57 (d, J = 5.1 Hz, 1H, OH), 5.88 (d, J = 4.6 Hz, 1H, H-1′), 7.20−7.36 (m, 15 H, Ph), 8.20 (s, 1H, H-8), 8.32 (s, 1H, H-2). Anal. ($C_{32}H_{32}N_4O_5 \cdot 1.5H_2O$) C, H, N.

1-Allyl-5′-*O*-tritylinosine (2c). Reaction of **1** with allyl bromide (44 μl, 0.51 mmol), following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, afforded a residue that was purified by flash column chromatography (CH_2Cl_2:MeOH, 20:1) to yield 184 mg (85%) of **2c** as a white solid: mp 93−94 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 551 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.20 (m, 2H, H-5′), 4.07 (m, 1H, H-4′), 4.22 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 4.63 (d, J = 5.3 Hz, 2H, NCH_2), 5.04−5.19 (m, 2H, CH=CH_2), 5.23 (d, J = 5.9 Hz, 1H, OH), 5.57 (d, J = 5.7 Hz, 1H, OH), 5.89 (d, J = 4.8 Hz, 1H, H-1′), 6.98 (m, 1H, CH=CH_2), 7.20−7.43 (m, 15 H, Ph), 8.22 (s, 1H, H-8), 8.29 (s, 1H, H-2). Anal. ($C_{32}H_{30}N_4O_5 \cdot H_2O$) C, H, N.

1-(Cyclopropyl)methyl-5′-*O*-tritylinosine (2d). Reaction of **1** with (bromomethyl)cyclopropane (50 μL, 0.51 mmol), following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, afforded a residue that was purified by flash column chromatography (CH_2Cl_2:MeOH, 20:1 to 10:1) to yield 180 mg (81%) as a white solid: mp 120−123 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 565 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 0.39−0.48 (m, 4H, CH_2), 1.23 (m, 1H, CH), 3.22 (m, 2H, H-5′), 3.86 (d, J = 7.3 Hz, 2H, NCH_2), 4.11 (m, 1H, H-4′), 4.21 (m, 1H, H-3′), 4.56 (m, 1H, H-2′), 5.23 (d, J = 5.9 Hz, 1H, OH), 5.58 (d, J = 5.9 Hz, 1H, OH), 5.89 (d, J = 4.8 Hz, 1H, H-1′), 7.25−7.62 (m, 15 H, Ph), 8.21 (s, 1H, H-8), 8.35 (s, 1H, H-2). Anal. ($C_{33}H_{32}N_4O_5 \cdot 0.5H_2O$) C, H, N.

1-(Cyclohexyl)methyl-5′-*O*-tritylinosine (2e). Reaction of **1** with (bromomethyl)cyclohexane (72 μL, 0.50 mmol) during 3 days,

following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, afforded a residue that was purified in the Chromatotron (CH_2Cl_2:MeOH, 10:1) to yield 134 mg (56%) of **2e** as a white solid: mp 121−123 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 607 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 0.92−1.25 (m, 6H, C_6H_{11}), 1.55−1.70 (m, 5H, C_6H_{11}), 3.39 (m, 2H, H-5′), 3.43 (d, J = 2.9 Hz, 1H, OH), 3.82 (d, J = 7.3 Hz, 2H, NCH_2), 4.35−4.44 (m, 2H, H-3′, H-4′), 4.78 (m, 1H, H-2′), 5.35 (d, J = 4.0 Hz, 1H, OH), 5.95 (d, J = 5.9 Hz, 1H, H-1′), 7.18−7.38 (m, 15 H, Ph), 7.80 (s, 1H, H-8), 7.92 (s, 1H, H-2). Anal. ($C_{36}H_{38}N_4O_5 \cdot 1.5H_2O$) C, H, N.

1-Benzyl-5′-*O*-tritylinosine (2f). Reaction of **1** with benzyl bromide (51 μL, 0.43 mmol), following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, afforded a residue that was purified in the Chromatotron (CH_2Cl_2:MeOH, 10:1) to yield 156 mg (66%) of **2f** as a white solid: mp 120−121 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 601 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.19 (m, 2H, H-5′), 4.07 (m, 1H, H-4′), 4.22 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 5.22 (s, 2H, NCH_2), 5.26 (d, J = 5.8 Hz, 1H, OH), 5.61 (d, J = 5.5 Hz, 1H, OH), 5.89 (d, J = 4.4 Hz, 1H, H-1′), 7.20−7.37 (m, 15 H, Ph), 8.23 (s, 1H, H-8), 8.52 (s, 1H, H-2). Anal. ($C_{36}H_{32}N_4O_5 \cdot H_2O$) C, H, N.

1-[(*E*)-3-Methoxycarbonyl-2-propenyl]-5′-*O*-tritylinosine (2 g). Reaction of **1** with methyl-4-bromocrotonate (60 μL, 0.50 mmol) following the general procedure for the preparation of N^1-substituted 5′-*O*-tritylinosines, afforded a residue that was purified in the Chromatotron (CH_2Cl_2:MeOH, 10:1) to yield 78 mg (32%) of **2g** as a white solid: mp 115−116 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 609 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 3.34 (m, 2H, H-5′), 3.65 (s, 3H, OCH_3), 4.28 (m, 1H, H-4′), 4.36 (m, 1H, H-3′), 4.69−4.72 (m, 3H, H-2′, NCH_2), 5.00 (br s, 1H, OH), 5.76 (d, J = 15.7 Hz, 1H, =CHCO), 5.91 (d, J = 5.1 Hz, 1H, H-1′), 6.90 (m, 1H, NCH_2CH=), 7.09−7.32 (m, 15 H, Ph), 7.75 (s, 1H, H-8), 7.90 (s, 1H, H-2). Anal. ($C_{34}H_{32}N_4O_7 \cdot H_2O$) C, H, N.

5-Amino-1-(5-*O*-trityl-β-D-ribofuranosyl)imidazole-4-(*N*-methyl)carboxamide (3a). A solution containing **2a** (100 mg, 0.18 mmol) in EtOH (4 mL) and 5 N NaOH (2 mL) was refluxed for 3 h. Volatiles were removed, and the residue was purified by flash column chromatography (CH_2Cl_2:MeOH:NH_4OH, 10:1:0.5) to yield 49 mg (53%) of **3a** as a white solid: mp 197−200 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 515 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 2.66 (d, J = 4.8 Hz, 2H, CH_3), 3.16 (m, 2H, H-5′), 4.01 (m, 1H, H-4′), 4.09 (m, 1H, H-3′), 4.30 (m, 1H, H-2′), 5.24 (br s, 1H, OH), 5.50 (d, J = 5.4 Hz, 1H, H-1′), 5.55 (br s, 1H, OH), 5.75 (br s, 2H, NH_2), 7.25−7.37 (m, 16 H, Ph, H-2), 7.42 (m, 1H, CONH). Anal. ($C_{29}H_{30}N_4O_5 \cdot 0.5H_2O$) C, H, N.

5-Amino-1-(5-*O*-trityl-β-D-ribofuranosyl)imidazole-4-(*N*-propyl)carboxamide (3b). Following a procedure analogous to that described for the synthesis of **3a**, compound **2b** (100 mg, 0.18 mmol) reacted with 5 N NaOH to yield 53 mg (52%) of **3b** as a white solid: mp 81−84 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 543 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 0.82 (t, J = 7.6 Hz, 3H, CH_3), 1.43 (m, J = 7.6 Hz, 2H, $CH_3CH_2CH_2$), 3.10 (q, J = 7.6 Hz, 2H, $HNCH_2$), 3.14 (d, J = 4.0 Hz, 2H, H-5′), 4.00 (m, 1H, H-4′), 4.10 (dd, J = 4.8 Hz, 1H, H-3′), 4.31 (dd, J = 5.1 Hz, 1H, H-2′), 5.21 (d, J = 5.5 Hz, 1H, OH), 5.47 (d, J = 5.4 Hz, 1H, H-1′), 5.48 (d, J = 5.4 Hz, 1H, OH), 5.78 (br s, 2H, NH_2), 7.26−7.37 (m, 16H, Ph, H-2), 7.42 (t, J = 6.0 Hz, 1H, CONH). Anal. ($C_{31}H_{34}N_4O_5 \cdot 2H_2O$) C, H, N.

5-Amino-1-(5-*O*-trityl-β-D-ribofuranosyl)imidazole-4-(*N*-allyl)carboxamide (3c). Following a procedure analogous to that described for the synthesis of **3a**, compound **2c** (100 mg, 0.18 mmol) reacted with 5 N NaOH to yield **3c** (45 mg, 46%) as a solid: mp 93−94 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 541 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.17 (d, J = 3.6 Hz, 2H, H-5′), 3.78 (t, J = 5.5 Hz, 2H, $HNCH_2$), 4.01 (m, 1H, H-4′), 4.09 (m, 1H, H-3′), 4.30 (m, 1H, H-2′), 4.98−5.11 (m, 2H, CH=CH_2), 5.21 (d, J = 5.3 Hz, 1H, OH), 5.51−5.54 (m, 2H, OH, H-1′), 5.78 (br s, 2H, NH_2), 5.85 (m, 1H, CH=CH_2), 7.23−7.38 (m, 16 H, Ph, H-2), 7.54 (t, J = 6.0 Hz, 1H, CONH). Anal. ($C_{31}H_{32}N_4O_5 \cdot H_2O$) C, H, N.

5-Amino-1-(5-*O*-trityl-β-D-ribofuranosyl)imidazole-4-[(*N*-cyclopropyl)methyl]carboxamide (3d). Following a procedure analogous to that described for the synthesis of **3a**, compound **2d** (100 mg, 0.18 mmol) reacted with 5 N NaOH to yield **3d** (54 mg, 55%) as a white solid: mp 92−93 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 555 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 0.24 (m, 2H, CH_2), 0.51 (m, 2H, CH_2), 0.95 (m, 1H, CH), 3.17−3.22 (m, 3H, H-5′, OH), 3.46 (m, 2H, $HNCH_2$), 4.12 (m, 1H, H-4′), 4.43 (m, 1H, H-3′), 4.58 (m, 1H, H-2′), 5.16 (br s, 2H, NH_2), 5.31 (br s, 1H, OH), 5.42 (d, J = 5.5 Hz, 1H, H-1′), 6.79 (t, J = 5.0 Hz, 1H, NHCO), 6.90 (d, J = 1.3 Hz, 1H, H-2), 7.21−7.39 (m, 15 H, Ph). Anal. ($C_{32}H_{34}N_4O_5 \cdot H_2O$) C, H, N.

2′,3′-Di-*O*-acetyl-5′-*O*-trityl-1-[(2-methoxyethoxy)methyl]-inosine (5). To a solution of **2′,3′-bis-*O*-acetyl-5′-*O*-tritylinosine (4)**[24] (235 mg, 0.39 mmol) in dry CH_2Cl_2 (3 mL) at 0 °C were added *i*-Pr_2NEt (83 μL, 0.47 mmol) and 2-methoxyethoxymethyl chloride (MEMCl) (54 μL, 0.47 mmol). The reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched by addition of H_2O (10 mL), stirred for an additional 20 min, and diluted with CH_2Cl_2 (15 mL). The organic phase was decanted and the aqueous phase was further extracted with CH_2Cl_2 (2 × 15 mL). The combined organic phases were dried on $MgSO_4$, filtered, and evaporated. The final residue was purified by column chromatography (CH_2Cl_2:acetone, 4:1) to yield 191 mg (72%) of **5** as white solid: mp 68−69 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 683 $(M + 1)^+$; ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 3.34 (s, 3H, OCH_3), 3.46−3.52 (m, 4H, OCH_2CH_2O), 3.79 (m, 2H, H-5′), 4.34 (m, 1H, H-4′), 5.53 (m, 2H, NCH_2O), 5.68 (m, 1H, H-3′), 6.06 (t, J = 6.5 Hz, 1H, H-2′), 6.15 (d, J = 6.5 Hz, 1H, H-1′), 7.21−7.44 (m, 15 H, Ph), 7.89 (s, 1H, H-8), 7.99 (s, 1H, H-2).

1-[(2-Methoxyethoxy)methyl]-5′-*O*-tritylinosine (6). A suspension containing **5** (133 mg, 0.19 mmol) in 0.2 N NaOH (2 mL) was refluxed for 6 h. Additional 0.2 N NaOH (2 mL) was added and the reaction was refluxed overnight. Volatiles were removed, and the residue was purified by flash column chromatography (CH_2Cl_2:MeOH, 15:1). The UV-positive fractions were further purified by a SPE Silica cartridge eluting with mixtures CH_2Cl_2:MeOH, 100:1 to 60:1. The fastest moving fractions afforded 48 mg (42%) of a white solid, identified as **6**: mp 82−83 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 599 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.11 (s, 3H, OCH_3), 3.16 (m, 2H, H-5′), 3.33 (m, 2H, CH_2OCH_3), 3.56 (m, 2H, NCH_2OCH_2), 4.07 (m, 1H, H-4′), 4.21 (m, 1H, H-3′), 4.56 (m, 1H, H-2′), 5.26 (d, J = 4.5 Hz, 1H, OH), 5.43 (d, J = 6.8 Hz, 2H, NCH_2O), 5.74 (br s, 1H, OH), 5.90 (d, J = 4.6 Hz, 1H, H-1′), 5.90 (br s, 2H, NH_2), 7.21−7.37 (m, 15 H, Ph), 8.23 (s, 1H, H-8), 8.38 (s, 1H, H-2). Anal. ($C_{33}H_{34}N_4O_7 \cdot 0.5H_2O$) C, H, N.

The slowest moving fractions afforded 20 mg (18%) of a white solid identified as **5-amino-1-(5-*O*-trityl-β-D-ribofuranosyl)imidazole-4-[*N*-(2-methoxyethoxy)methyl]carboxamide (7):** mp 80−81 °C; MS (ES, positive mode) *m*/*z* 589 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.18 (d, J = 3.7 Hz, 2H, H-5′), 3.21 (s, 3H, OCH_3), 3.39 (m, 2H, CH_2OCH_3), 3.51 (m, 2H, NCH_2OCH_2), 4.02 (m, 1H, H-4′), 4.10 (m, 1H, H-3′), 4.31 (m, 1H, H-2′), 4.32 (d, J = 6.8 Hz, 2H, $HNCH_2$), 5.20 (d, J = 4.9 Hz, 1H, OH), 5.52−5.54 (m, 2H, H-1′, OH), 5.90 (br s, 2H, NH_2), 7.23−7.38 (m, 16 H, H-2, Ph), 8.24 (t, J = 6.9 Hz, 1H, NHCO). Anal. ($C_{32}H_{36}N_4O_7 \cdot 0.5H_2O$) C, H, N.

5-Amino-1-(5-*O*-trityl-β-D-ribofuranosyl)imidazole-4-carboxamide (8). A suspension containing **5** (125 mg, 0.18 mmol) in 5 N NaOH (2 mL) was refluxed for 6 h. Volatiles were removed and the residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 15:1 to 10:1) to yield 22 mg (20%) of compound **8** as a white solid: mp 114−116 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m*/*z* 501 $(M + 1)^+$; ^{1}H NMR (DMSO-d_6) δ 3.16 (m, 2H, H-5′), 3.99 (m, 1H, H-4′), 4.09 (m, 1H, H-3′), 4.30 (m, 1H, H-2′), 5.20 (d, J = 5.3 Hz, 1H, OH), 5.49−5.53 (m, 2H, H-1′, OH), 5.81 (br s, 2H, NH_2), 6.64, 6.82 (br s, 2H, $CONH_2$), 7.23−7.38 (m, 16 H, Ph, H-2). Anal. ($C_{28}H_{28}N_4O_5 \cdot 2H_2O$) C, H, N.

General Procedure for the 5′-*O*-Tritylation of Ribonucleosides. Prior to reaction, the corresponding ribonucleoside was

coevaporated twice with pyridine (5 mL). Then, to a suspension of the nucleoside (1.0 mmol) in anhydrous pyridine (5 mL) were added 4-(dimethylamino)pyridine (DMAP) (5 mg, 0.04 mmol) and trityl chloride (482 mg, 1.7 mmol). The reaction was heated at 80 °C for 15 h. After reaching room temperature, EtOAc (100 mL) and a $NaHCO_3$ solution (20 mL) were added. The organic phase was decanted and further washed with H_2O (20 mL) and brine (20 mL). Then it was dried on $MgSO_4$, filtered, and evaporated to dryness. The residue was purified as indicated for each compound.

9-(5-*O*-Trityl-*β*-D-ribofuranosyl)purine (11) was prepared by reaction of **9-(*β*-D-ribofuranosyl)purine (9)** (252 mg, 1.0 mmol) with trityl chloride, according to the general procedure. The final residue was purified by flash column chromatography (CH_2Cl_2:MeOH, 10:1) to yield 173 mg (35%) of **11** as a white solid: mp 94−97 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m/z* 495 (M + 1)$^+$; ^{1}H NMR ($CDCl_3$) *δ* 3.30 (dd, *J* = 10.7, 3.2 Hz, 1H, H-5′), 3.49 (dd, *J* = 10.7, 3.4 Hz, 1H, H-5″), 4.46 (m, 2H, H-3′, H-4′), 4.90 (m, 1H, H-2′), 5.38 (br s, 1H, OH), 6.05 (d, *J* = 5.9 Hz, 1H, H-1′), 7.20−7.28 (m, 15H, Ph), 8.38 (s, 1H, H-8), 8.97 (s, 1H, H-2), 9.22 (s, 1H, H-6). Anal. ($C_{29}H_{26}N_4O_4$·H_2O) C, H, N.

6-Chloro-9-(5-*O*-trityl-*β*-D-ribofuranosyl)purine (12). This compound was prepared by a described procedure[25] that consisted of treatment of 6-chloro-9-(*β*-D-ribofuranosyl)purine (**10**) (287 mg, 1.0 mmol) in dry DMF (4 mL) and in the presence of *i*-Pr_2NEt (0.2 mL, 1.2 mmol) with trityl chloride (558 mg, 2.0 mmol) at 40 °C for 18 h. Purification of the crude mixture by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 20:1) afforded 159 mg (30%) of **12** as a white solid: mp 94−96 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m/z* 551 (M + Na)$^+$ with a Cl isotopic pattern; ^{1}H NMR (DMSO-d_6) *δ* 3.23 (m, 2H, H-5′), 4.11 (q, *J* = 4.2 Hz, 1H, H-4′), 4.33 (m, 1H, H-3′), 4.72 (m, 1H, H-2′), 5.29 (d, *J* = 6.1 Hz, 1H, OH), 5.65 (d, *J* = 5.4 Hz, 1H, OH), 6.05 (d, *J* = 3.9 Hz, 1H, H-1′), 7.19−7.34 (m, 15 H, Ph), 8.71 (s, 1H, H-8), 8.81 (s, 1H, H-2). Anal. ($C_{29}H_{25}ClN_4O_4$) C, H, N.

6-Methylamino-9-(5-*O*-trityl-*β*-D-ribofuranosyl)purine (13). To a solution of compound **12** (100 mg, 0.19 mmol) in EtOH (2 mL) was added $MeNH_2$ in EtOH (33%) (4 mL). The mixture was heated in a sealed tube at 70 °C for 4 h. Volatiles were removed, and the residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 15:1) to afford 42 mg (42%) of **13** as a white solid: mp 218−220 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m/z* 524 (M + 1)$^+$; ^{1}H NMR (DMSO-d_6) *δ* 3.20 (m, 2H, H-5′), 3.94 (s, 3H, $NHCH_3$), 4.04 (q, *J* = 4.9 Hz, 1H, H-4′), 4.30 (m, 1H, H-3′), 4.68 (m, 1H, H-2′), 5.20 (d, *J* = 5.9 Hz, 1H, OH), 5.52 (d, *J* = 5.6 Hz, 1H, OH), 5.91 (d, *J* = 4.4 Hz, 1H, H-1′), 7.18−7.36 (m, 15 H, Ph), 7.76 (br s, 1H, NH), 8.17 (s, 1H, H-8), 8.23 (s, 1H, H-2). Anal. ($C_{30}H_{29}N_5O_4$·H_2O) C, H, N.

5′-*O*-[(4-Chlorophenyl)-1,1-(diphenyl)methyl]inosine (17). This compound was prepared by reaction of inosine **14** (268 mg, 1.0 mmol) with 4-chlorotrityl chloride[27] (313 mg, 1.0 mmol) according to the general procedure for the 5′-*O*-tritylation of ribonucleosides. Purification of the residue by flash column chromatography (CH_2Cl_2:MeOH, 20:1 to 10:1) afforded 174 mg (32%) of **17** as a white solid: mp 154−157 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m/z* 545 (M + 1)$^+$ showing Cl isotopic pattern; ^{1}H NMR (DMSO-d_6) *δ* 3.22 (m, 2H, H-5′), 4.07 (q, *J* = 5.1 Hz, 1H, H-4′), 4.23 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 5.24 (d, *J* = 6.0 Hz, 1H, OH), 5.59 (d, *J* = 5.8 Hz, 1H, OH), 5.91 (d, *J* = 4.3 Hz, 1H, H-1′), 7.24−7.38 (m, 14H, Ph), 8.00 (s, 1H, H-8), 8.22 (s, 1H, H-2), 12.40 (br s, 1H, NH-1). Anal. ($C_{29}H_{25}ClN_4O_5$) C, H, N.

5′-*O*-Trityl-2′deoxyinosine (18). 2′-Deoxyinosine (**15**) (100 mg, 0.40 mmol) was coevaporated twice with anhydrous pyridine (1 mL) prior to reaction. Then it was dissolved in pyridine (2 mL), and trityl chloride (188 mg, 0.67) and DMAP (2 mg, 0.02 mmol) were added. The reaction was warmed at 60 °C for 4 h. Methanol (1 mL) was added, and volatiles were removed. Purification of the residue by flash column chromatography (CH_2Cl_2:MeOH, 30:1 to 10:1) afforded 70 mg (35%) of **18** as a white solid: mp 219−221 °C (CH_2Cl_2:MeOH); MS (ES, positive mode) *m/z* 495 (M + 1)$^+$; ^{1}H NMR (DMSO-d_6) *δ* 2.32, 2.75 (m, 2H, H-2′), 3.15 (m, 2H, H-5′), 3.95 (m, 1H, H-4′), 4.41 (m, 1H, H-3′), 5.38 (d, *J* = 4.6 Hz, 1H, OH), 6.33 (t, *J* = 6.4 Hz, 1H, H-1′), 7.19−7.41 (m, 15 H, Ph), 7.96 (s, 1H, H-8), 8.18 (s, 1H, H-2), 12.36 (br s, 1H, NH). Anal. ($C_{29}H_{26}N_4O_4$·$0.5H_2O$) C, H, N.

1-Tritylinosine (19). To a suspension of inosine **14** (268 mg, 1.0 mmol) in DMF (4 mL) were added *i*-Pr_2NEt (0.2 mL, 1.2 mmol) and trityl chloride (558 mg, 2.0 mmol), and the mixture was stirred at 40 °C for 18 h. The workup was identical to that of the general procedure. The final residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 15:1) to afford 153 mg (30%) of **19** as a white solid: mp 123−125 °C; MS (ES, positive mode) *m/z* 511 (M + 1)$^+$; ^{1}H NMR (DMSO-d_6) *δ* 3.45−3.62 (m, 2H, H-5′), 3.91 (m, 1H, H-4′), 4.11 (m, 1H, H-3′), 4.50 (m, 1H, H-2′), 5.02 (t, *J* = 5.3 Hz, 1H, OH), 5.21 (d, *J* = 4.9 Hz, 1H, OH), 5.48 (d, *J* = 6.0 Hz, 1H, OH), 5.83 (d, *J* = 5.7 Hz, 1H, H-1′), 7.23−7.36 (m, 15 H, Ph), 8.32, 8.33 (2s, 2H, H-8, H-2). Anal. ($C_{29}H_{26}N_4O_5$·$2H_2O$) C, H, N.

5′-*O*-Trityl-2′,3′-secoinosine (20). To a solution of 5′-*O*-tritylinosine (**1**)[16] (250 mg, 0.5 mmol) in 6 mL of dioxane/H_2O (5:1) was added a solution of $NaIO_4$ (111 mg, 0.5 mmol) in H_2O (1 mL). After a few minutes, a suspension formed that was stirred for 18 h. The mixture was diluted with dioxane (5 mL), and after 10 min of stirring, the solid was filtered and further washed with dioxane (3 mL). The combined filtrates were cooled at 0 °C and treated with $NaBH_4$ (18 mg, 0.5 mmol). The resulting mixture was stirred at room temperature for 2 h and then acetone (0.1 mL) was added. After 5 min, the reaction was neutralized by addition of a few drops of 10% AcOH. Volatiles were removed, and the residue was partitioned between H_2O and *i*-BuOH. The aqueous phase was further extracted with *i*-BuOH. The combined organic phases were dried on $MgSO_4$, filtered, and evaporated. The final residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 6:1) to afford 154 mg (60%) of **20** as an amorphous solid: MS (ES, positive mode) *m/z* 513 (M + 1)$^+$; ^{1}H NMR (DMSO-d_6) *δ* 2.79 (m, 2H, H-5′), 3.42 (m, 1H, H-3′), 3.78 (m, 1H, H-4′), 3.98 (m, 1H, H-2′), 4.78 (br s, 1H, OH), 5.21 (br s, 1H, OH), 5.47 (t, *J* = 6.1 Hz, 1H, H-1′), 7.07−7.26 (m, 15H, Ph), 8.06 (s, 1H, H-8), 8.28 (s, 1H, H-2), 12.28 (br s, 1H, NH). Anal. ($C_{29}H_{28}N_4O_5$·$2H_2O$) C, H, N.

1-Benzyl-5′-*O*-benzyl-2′,3′-*O*-isopropylideneinosine (22). To a solution of 2′,3′-*O*-isopropylideninosine (**21**) (200 mg, 0.63 mmol) in dry DMF (4 mL) was added NaH (60% in mineral oil, 81 mg, 2.10 mmol). The reaction was stirred at room temperature for 1 h. Then, benzyl bromide (0.17 mL, 1.41 mmol) was added and stirring was continued for 1 h at room temperature. The reaction was quenched by addition of a few drops of AcOH till the pH was 7−8. Volatiles were removed, and the residue was purified by flash column chromatography (CH_2Cl_2:MeOH, 99:1 to 97:3) to yield 221 mg (71%) of **22** as an oil: MS (ES, positive mode) *m/z* 489 (M + 1)$^+$; ^{1}H NMR ($CDCl_3$) *δ* 1.29 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.56 (m, 2H, H-5′), 4.38 (d, *J* = 1.8 Hz, 2H, CH_2O), 4.44 (m, 1H, H-4′), 4.83 (dd, *J* = 6.0, 2.1 Hz, 1H, H-3′), 5.08 (dd, *J* = 6.0, 2.4 Hz, 1H, H-2′), 5.16 (s, 2H, CH_2N), 6.02 (d, *J* = 2.4 Hz, 1H, H-1′), 7.11−7.28 (m, 10 H, Ph), 7.89 (s, 1H, H-8), 7.93 (s, 1H, H-2).

1-Benzyl-5′-*O*-benzylinosine (23).[31] A solution containing **22** (100 mg, 0.2 mmol) in 30% aqueous TFA (2.2 mL) was stirred at 0 °C for 20 min and for 3 h at room temperature. Volatiles were removed, and the residue was purified by CCTLC in the Chromatotron (CH_2Cl_2:MeOH, 20:1) to yield 87 mg (95%) of **23** as a white solid: mp 65−68 °C (CH_2Cl_2:MeOH); *m/z* 449 (M + 1)$^+$; ^{1}H NMR ($CDCl_3$) *δ* 3.59−3.72 (m, 3H, H-5′, OH′), 4.32 (m, 1H, H-4′), 4.42 (m, 1H, H-3′), 4.46 (d, *J* = 2.7 Hz, 2H, OCH_2Ph), 4.61 (m, 1H, H-2′), 5.08 (s, 2H, N CH_2Ph), 5.28 (br s, 1H, OH), 5.95 (d, *J* = 5.4 Hz, 1H, H-1′), 7.18−7.30 (m, 10 H, Ph), 7.80 (s, 1H, H-8), 7.92 (s, 1H, H-2). Anal. ($C_{24}H_{24}N_4O_5$·H_2O) C, H, N.

TPase Enzyme Assays. The phosphorolysis of thymidine (dThd) by human TPase was measured by HPLC analysis. The incubation mixture (500 *μ*L) contained 10 mM Tris-HCl (pH 7.6), 1 mM EDTA, 2 mM potassium phosphate, 150 mM NaCl, 5% DMSO, and 100 *μ*M of dThd in the presence of 0.025 U TPase. Incubations were performed at room temperature. At different time points (i.e., 0, 20, 40, and 60 min), 100-*μ*L fractions were withdrawn, transferred to an Eppendorf tube thermoblock, and boiled at 95 °C

for 5 min. Next, the samples were rapidly cooled on ice, and dThd was separated from thymine (Thy) and quantified in the samples on a reverse phase RP-8 column (Merck, Darmstadt, Germany) by HPLC analysis. The separation of Thy and dThd was performed by a linear gradient from 98% buffer B (50 mM NaH_2PO_4 + 5 mM heptane sulfonic acid, pH 3.2) and 2% acetonitrile (ACN) to 50% buffer B and 50% ACN. Retention times of Thy and dThd were 4.2 and 8.5 min, respectively. UV-based detection of Thy and dThd was performed at 267 nm.

To evaluate the inhibitory effect of the compounds, a variety of inhibitor concentrations in DMSO were added to the reaction mixture (500 μL) containing 100 μM of dThd. The final DMSO concentration in the reaction mixture was 5% for all inhibitor concentrations. Aliquots of 100 μL were withdrawn from the reaction mixture at several time points, as described above, heated at 95 °C to inactivate the enzyme, and analyzed by HPLC.

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Supporting Information Available: Elemental analysis data of compounds **2a**−**g**, **3a**−**d**, **6**−**8**, **11**−**13**, **16**−**19**, **20**, and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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