Synthesis, Flow Cytometric Evaluation, and Identification of Highly Potent Dipyridamole Analogues as Equilibrative Nucleoside Transporter 1 Inhibitors

Wenwei Lin and John K. Buolamwini*

Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Sciences Center, 847 Monroe Avenue, Suite 327, Memphis, Tennessee 38163

Received March 19, 2007

Dipyridamole (Persantine) is a clinically used vasodilator with equilibrative nucleoside transporters 1 and 2 (ENT1 and ENT2) inhibitory activity albeit less potent than the prototype ENT1 inhibitor nitrobenzylmercaptopurine riboside (NBMPR). Dipyridamole is a good candidate for further exploration because it is a non-nucleoside and has a proven record of safe use in humans. A series of dipyridamole analogues were synthesized with systematic modification and evaluated as ENT1 inhibitors by flow cytometry. Compounds with much higher potency were identified, the best being 2,6-bis(diethanolamino)-4,8-diheptamethyleneiminopyrimido[5,4-*d*]pyrimidine **(13)** with a K_i of 0.49 nM compared to a K_i of 308 nM for dipyridamole. Compound **13** is similar in potency to the prototype potent ENT1 inhibitor NBMPR (0.43 nM). For the first time, a dipyridamole analogue has been identified that is equipotent with NBMPR. The SAR indicated that diethanolamine substituted analogues were more active than monoethanolamine compounds. Also, free hydroxyl groups are not essential for activity.

Introduction

Nucleoside transporters are specialized integral membrane glycoproteins known to mediate the cellular influx or efflux of physiological nucleosides or nucleobases, as well as many synthetic analogues.[1,2] Currently, nucleoside transporters are classified into two families: (i) the equilibrative nucleoside transporter family (ENTs) and (ii) the concentrative nucleoside transporter family (CNTs[a]).[3,4] The equilibrative family facilitates the transport of nucleosides or nucleobases down their concentration gradients. In contrast, the concentrative family transports nucleosides against their concentration gradients by coupling with a sodium ion gradient. Nucleoside transporter inhibitors have potential therapeutic applications in ischemic heart disease and stroke,[5−10] in inflammatory disease,[11] and as biological response modifiers in antimetabolite chemotherapy.[12] A comprehensive summary of nucleoside transport inhibitors as potential therapeutic agents has been published.[13]

Equilibrative nucleoside transporters were the first to be identified because of their broad tissue distribution. They were initially subdivided into es (equilibrative sensitive) or ENT1 and into ei (equilibrative insensitive) or ENT2 according to their sensitivities to inhibition by nanomolar concentrations of 4-nitrobenzylmercaptopurine ribonucleoside (NBMPR). Four subtypes of ENTs (ENT1, ENT2, ENT3, and ENT4) have now been identified and cloned.[3] The ENT1 transporter is the most widely distributed nucleoside transporter with the highest abundance in most tissues studied.[14,15] This makes it the most relevant NT target for therapeutic exploration. Several chemical classes have been shown to inhibit ENT1.[13] Among them, three classes are most significant (Figure 1). These are purine nucleoside analogues of which NBMPR is the prototype, pyrimidopyrimidine analogues such as the antithrombotic and vasodilating agent dipyridamole, and flazine calcium channel blockers represented by lidoflazine.

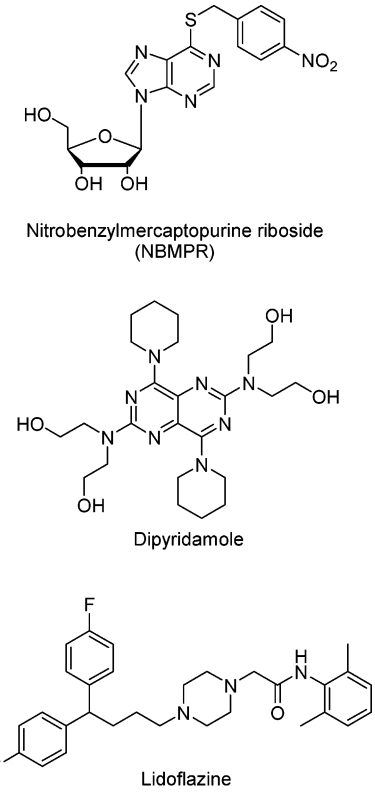


Figure 1. Representatives of the three main ENT1 inhibitory chemical classes.

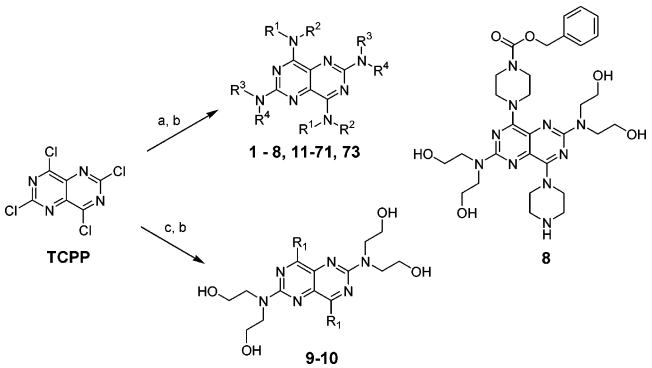
NBMPR is a more potent ENT1 inhibitor (e.g., K_i of 0.7 nM)[16] than dipyridamole (e.g., K_i of 8.8).[17] Draflazine, a lidoflazine analogue, also exhibits high ENT1 inhibitory activity

* To whom correspondence should be addressed. Phone: 901-448-7533. Fax: 901-448-6828. E-mail: Jbuolamwini@utmem.edu.

[a] Abbreviations: Cbz, carbobenzoxy; CNTs, concentrative nucleoside transporters; DMSO, dimethylsulfoxide; ENT1, equilibrative nucleoside transporter 1; ENT2, equilibrative nucleoside transporter 2; ENT3, equilibrative nucleoside transporter 3; ENT4, equilibrative nucleoside transporter 4; ESI, electrospray ionization; LC, liquid chromatography; MS, mass spectrometry; NBMPR, nitrobenzylmercaptopurine riboside; NMR, nuclear magnetic resonance; NTIs, nucleoside transporter inhibitors; SAR, structure−activity relationship; CDKs, cyclin dependent kinases; TCPP, 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine; TLC, thin-layer chromatography; TMS, tetramethylsilane.

10.1021/jm070311l CCC: $37.00
Published on Web 07/18/2007

Scheme 1[a]



[a] Reagents and conditions: (a) NHR^1R^2, anhydrous THF, 0−5 °C; (b) NHR^3R^4, DMSO, 150 °C; (c) R^1MgCl, anhydrous THF, 0−5 °C.

(IC_{50} = 0.28−10 nM).[18] However, NBMPR and the flazine compounds like draflazine are poor candidates for further exploration. NBMPR has immunosuppressive and mutagenic activities derived from its 6-mercaptopurine metabolite.[19−21] The flazines are nosnspecific, having calcium channel antagonist activity that is thought to contribute significantly to their cardioprotective effects.[22−24] As a potent ENT1 inhibitor, dipyridamole has broad pharmacological effects. It is an effective coronary vasodilator (used as an antianginal drug) through the increasing of extracellular adenosine concentration stemming from its ENT inhibitory activity.[5,25,26] Dipyridamole also has antiplatelet effects attributed to phosphodiesterase inhibition.[5] Coadministration of ENT1 inhibitors, such as dipyridamole, and antimetabolites such as 5-fluorouracil has been shown to result in synergism and might improve the therapeutic index of antimetabolites, where target cells have a higher ENT1 expression than normal cells.[27,28] Synergism results not only from inhibition of nucleoside salvage but also from the increase of the intracellular concentration of 5-fluorodeoxyuridine caused by blockade of its efflux by dipyridamole. Thus, the intracellular level of the active product, 5-fluorodeoxyuridine monophosphate, increases, resulting in higher therapeutic efficacy.[29,30]

Besides mammalian tissues, nucleoside transporters are also found in parasites such as *Plasmodium falciparum*, the malarial parasite.[31,32] Parasites rely on salvage pathways to meet their purine and purine nucleoside needs because they do not have de novo purine biosynthetic pathways.[33] Nucleoside transporters of parasites have limited homologies with the human ENT1 and have been shown to be inhibited by dipyridamole but not NBMPR or lidoflazine.[34] Some parasites like *Toxoplasma gondii* can even transport NBMPR.[35] A study of the antimalarial activity of dipyridamole showed that it was effective against all of the erythrocytic stages such as rings, trophozoites, and schizonts; it had an IC_{50} of 30 nM by itself and lowered the IC_{50} of chloroquine from 97.0 to 13.7 nM at a concentration of 0.1 nM.[36]

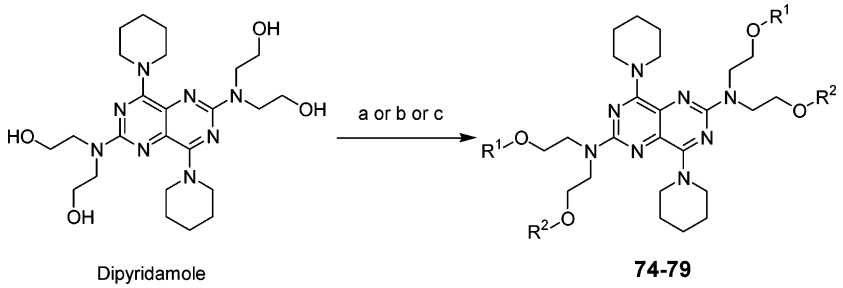
In light of these positive attributes of dipyridamole, we selected it as a candidate for further structure−activity relationship (SAR) exploration for ENT1 transporter inhibitory activity. Many dipyridamole analogues have been reported and evaluated for their effects as antiplatelet and cardioprotective agents.[37−41] Some dipyridamole analogues have also been synthesized and evaluated for their inhibitory activities against cyclin dependent kinases (CDKs), with negative results.[42] A more recent publication disclosed the synthesis and biological evaluation of a series of dipyprdamole analogues for their ENT1 inhibitory activities, and some of them showed only slightly higher activities than dipyridamole.[43] In this paper, a series of dipyridamole analogues were synthesized for a more systematic and comprehensive evaluation of ENT1 SAR. Some of the compounds showed activity comparable to that of NBMPR, which is a much more potent ENT1 inhibitor than dipyridamole.

Chemistry

For the synthesis of these dipyridamole analogues, commercially available starting materials 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (TCPP) and dipyridamole were used on the basis of the structures of individual final products. For the preparation of the major dipyridamole analogues (compounds **1**−**8**, **11**−**71**, and **73**) (Scheme 1), an excess of the appropriate amine (about 4-fold excess) was reacted with TCPP in anhydrous THF. The resulting 2,6-dichloro intermediates were individually reacted with diethanolamine, ethanolamine, or morpholine at 150 °C in DMSO as solvent to obtain the target products. For the preparation of compounds **9** and **10** (Scheme 1), the appropriate Grignard reagents were used for the first step, followed by reaction with diethanolamine in the second step.

For the preparation of compounds **74**−**79** (Scheme 2), dipyridamole was used as starting material. Dipyridamole was acylated or alkylated[44] to afford the desired products. Compound **78** was a dialkylated product, instead of the intended tetraalkylated product. It appears that the introduction of the first isopropyl group at each side of dipyridamole prevented the introduction of a second isopropyl group on the remaining hydroxyl groups under the reaction conditions. This could be possibly due to steric hindrance. In total, 79 dipyridamole analogues with diverse substituents were synthesized in this study. The core pyrimido[5,4-*d*]pyrimidine system and the symmetrical feature in dipyridamole were maintained with the exception of compound **8**, which had two different substituents at the at the 4- and 8-positions. Compound **8** was planned to be

Scheme 2[a]



[a] Reagents and conditions: (a) HCOOH, 100 °C (compound **74**); (b) CH_3COCl, DMAP, anhydrous THF, 0−5 °C (compound **75**); (c) NaH, R^1I, anhydrous DMF ($R^2 = R^1$ for **76**−**78**; R^2 = H for **79**).

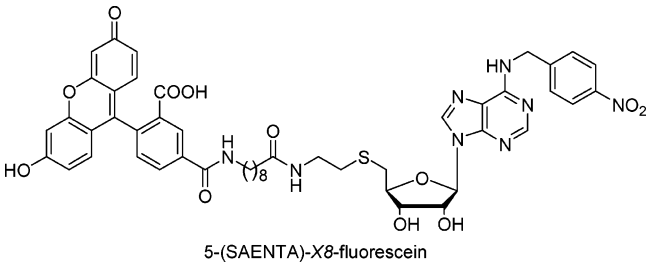


Figure 2. Structure of SAENTA-fluorescein.

symmetrical, but the conditions in the second reaction step caused a loss of one Cbz group to produce the unsymmetrical compound.

Biological Studies

The compounds and positive controls, dipyridamole, NBMPR, and lidoflazine were subjected to a flow cytometric assay with SAENTA-fluorescein (Figure 2) as the fluorescent probe.[44] Flow cytometry has several advantages over the conventional radioligand binding assays in that it eliminates radiation hazards and disposal problems and allows the use of much fewer cells, as few as 5000 cells compared to 2 million cells per sample for comparable radioligand assays. SAENTA-fluorescein is a NBMPR analogue, and it was used successfully in several studies to determine the ENT1 inhibitory activities of NBMPR analogues.[16,17] Studies with radiolabeled ligands have shown that NBMPR, dipyridamole, and lidoflazine displace each other at the binding sites on the ENT1 transporter.[45−47] Thus, we expected the new compounds would similarly displace SAENTA-fluorescein from the NBMPR binding site on the ENT1 transporter.

Dipyridamole itself is a fluorescent molecule (excitation λ_{max} = 280 nm, emmision λ_{max} = 490 nm),[48] but at the experimental wavelengths sets for SAENTA-fluorescein (exciation λ = 488 nm, emmision λ = 533 nm), dipyridamole and its analogues, with the exception of compounds **9** and **10**, had insignificant absorbance and emission, which did not interfere with the detection of bound SAENTA-fluorescein. Human erythroleukemia K562 cells were used as the ENT1 transporter source for the binding experiments. This cell line expresses high levels of ENT1 protein, with very limited fraction of other nucleoside transporters,[49] and has been used widely for assessing ENT1 binding affinity of compounds.[50−53] Compounds were first screened at 10 μM, and those compounds that showed good inhibitory activities (% inhibition of >40%) were further tested at 10 concentration levels to generate dose-dependent curves from which the IC_{50} values were derived and used to calculate the corresponding K_i values. The inhibitory activities of the highly fluorescent dipyridamole analogues like **9** and **10** could not be determined by this method.

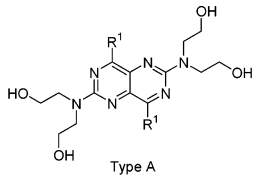
Structure−Activity Relationships

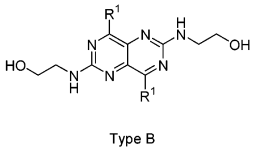
All dipyridamole analogues had the core structure of 2,4,6,8-tetrasubstituted-pyrimido[5,4-*d*]pyrimidine. They maintained the symmetric feature as in the case of dipyridamole, with the exception of compound **8**, which had two different substituents at the 4- and 8-positions of the core pyrimidopyrimidine structure. The ENT1 inhibitory activities are summarized in Tables 1−4. In all the tables, the activities of one negative control (DMSO) and three positive controls, NBMPR, lidoflazine, and dipyridamole, are listed for comparison.

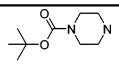
Compounds listed in Table 1 are dipyridamole analogues with ring structures at the 4- and 8-positions of the pyrimidopyrimidine template. Compounds listed in Table 2 are analogues with open-chain tertiary amines at the pyrimidopyrimidine 4- and 8-positions. Compounds listed in Table 3 have primary or secondary amine substituents at the 4- and 8-positions of the core structure. Compounds listed in Table 4 are derivatives of dipyridamole. In this study, NBMPR had a K_i of 0.43 nM, dipyridamole a K_i of 8.18 nM, and lidoflazine a K_i of 279.9 nM, which are in agreement with the literature.

For substituents at the 4- and 8-positions of the pyrimido-[5,4-*d*]pyrimidine, nitrogen-containing monocyclic ring structures usually gave analogues with good inhibitory activities, as

Table 1. Inhibitory Activities of Compounds with Different Ring Systems at the 4- and 8-Positions

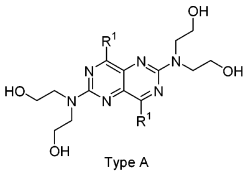

Type A

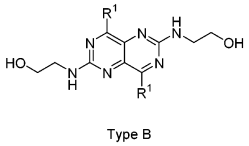

Type B

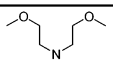
Comp.	Type	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
DMSO	-	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	-	90.0 ± 0.2	4954	279.9
Dipyridamole	A		86.7 ± 0.1	144.8	8.18
1	B		53.5 ± 0.1	12,229	690.9
2	A		85.7 ± 0.3	1,764	99.7
3	B		19.3 ± 1.1	ND	ND
4	A		70.4 ± 0.4	6,956	393
5	B		13.5 ± 0.3	ND	ND
6	A		0.9 ± 0.7	ND	ND
7	A		44.6 ± 2.1	ND	ND
8[b]	A	-	68.8 ± 0.0	7,947	449
9	A		N. D.	ND	ND
10	A		N. D.	ND	ND
11	A		94.4 ± 0.2	15.2	0.86
12	B		28.7 ± 0.1	ND	ND
13	A		93.1 ± 0.3	8.67	0.49
14	B		78.6 ± 0.3	375	21.2
15	A		78.0 ± 0.4	13.6	0.77
16	B		69.3 ± 0.3	672	38
17	A		85.0 ± 0.1	3,416	193
18	B		14.9 ± 1.7	ND	ND
19	A		24.7 ± 0.2	ND	ND
20	B		16.2 ± 1.9	ND	ND

[a] ND = not determined. [b] For structure of compound **8**, see Scheme 1.

Table 2. Inhibitory Activities of Compounds with Open Chain 4- and 8-Position Substituents


Type A

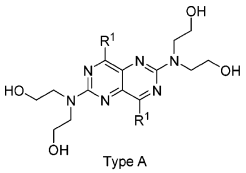

Type B

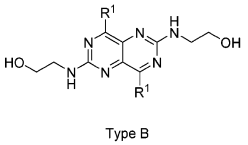
Comp.	Type	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (μM)	K_i (nM)
DMSO	-	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	-	90.0 ± 0.2	4,954	279.9
DP	A		86.7 ± 0.1	144.8	8.18
21	A	Me_2N	49.7 ± 0.1	3,828	216.7
22	B		9.9 ± 0.4	ND	ND
23	A	Et_2N	57.0 ± 0.7	3,831	216.4
24	B		30.4 ± 0.1	ND	ND
25	A	$(n\text{-Propyl})_2N$	41.6 ± 1.4	ND	ND
26	B		13.0 ± 0.1	ND	ND
27	A	$(n\text{-Butyl})_2N$	53.6 ± 2.9	ND	ND
28	B		3.0 ± 4.3	ND	ND
29	A	$(iso\text{-Butyl})_2N$	19.3 ± 1.8	ND	ND
30	B		6.6 ± 0.1	ND	ND
31	A	$(n\text{-Pentyl})_2N$	7.2 ± 1.3	ND	ND
32	B		-4.6 ± 1.9	ND	ND
33	A	$(iso\text{-Pentyl})_2N$	8.2 ± 0.8	ND	ND
34	B		2.7 ± 1.2	ND	ND
35	A		38.4 ± 0.4	ND	ND
36	B		9.9 ± 0.4	ND	ND
37	A		-65.6 ± 1.8	ND	ND
38	B		-8.0 ± 1.6	ND	ND

[a] ND = not determined.

in the case of compounds **2**, **4**, **11**, **13**, and **15**. Increasing ring size from five (compound **2**) to eight (compound **13**) increased inhibitory activity accordingly, with K_i values going from 99.7 to 0.49 nM, about a 200-fold increase in inhibitory activity. Compound **13** was the most active analogue in the series with activity comparable to the activity of one of best ENT1 nucleoside analogue inhibitors, NBMPR (K_i = 0.43 nM). Compared to dipyridamole (K_i = 8.18 nM), compound **13** is 16 times more potent. A ring size of eight was optimal because a further increase in ring size to nine decreased activity as can be seen with compound **15**, which had a K_i of 0.77 nM. The effect of ring size could be due to an increased hydrophobic effect because the piperidine ring in dipyridamole (K_i = 8.18 nM) provided higher inhibitory activity than the morpholino or piperazine rings in compounds **5** (K_i = 6956 nM) and **6** (practically inactive), respectively. The binding pocket at the 4- and 8-positions also has limits on the ring size it can accommodate. Further, not only does the ring size matter but also the ring flexibility is important, with flexible rings affording

Table 3. Inhibitory Activities of Compounds with Free Hydrogen on the Nitrogen of 4- and 8-Position Substituents

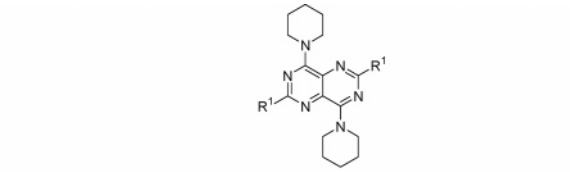

Type A


Type B

Comp.	Type	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
			%Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
DMSO	-	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	-	90.0 ± 0.2	4954	279.9
DP	A		86.7 ± 0.1	144.8	8.18
39	A	NH_2	6.5 ± 0.1	ND	ND
40	A	MeNH	4.1 ± 0.9	ND	ND
41	B		-1.0 ± 0.1	ND	ND
42	A	EtNH	24.0 ± 1.0	ND	ND
43	B		0.7 ± 0.5	ND	ND
44	A	n-PropylNH	79.5 ± 1.0	5,310	300
45	B		5.7 ± 0.3	ND	ND
46	A	iso-PropylNH	81.7 ± 1.4	3,381	191
47	B		14.1 ± 1.2	ND	ND
48	A	n-ButylNH	87.3 ± 0.1	2,407	136
49	B		61.6 ± 0.1	8,655	489
50	A	iso-ButylNH	92.0 ± 0.2	673	38
51	B		24.9 ± 0.1	ND	ND
52	A	tert-ButylNH	81.2 ± 0.1	297	16.8
53	B		28.0 ± 0.1	ND	ND
54	A	n-PentylNH	56.4 ± 0.4	2,476	139.9
55	B		12.5 ± 1.4	ND	ND
56	A	iso-PentylNH	86.8 ± 0.6	2,136	120.7
57	B		7.56 ± 0.1	ND	ND
58	A	tert-PentylNH	94.4 ± 0.2	260	14.7
59	B		16.1 ± 0.3	ND	ND
60	A		48.7± 0.9	7,554	427.6
61	B		6.2 ± 1.3	ND	ND
62	A		84.1 ± 0.3	1,838	104.1
63	B		23.0 ± 2.6	ND	ND
64	A		90.8 ± 0.2	279.7	15.8
65	B		4.9 ± 0.2	ND	ND
66	A		80.9 ± 0.1	940	53.1
67	B		1.4 ± 0.3	ND	ND
68	A		5.8 ± 0.5	ND	ND
69	B		6.6 ± 3.9	ND	N. D.
70	A		11.9 ± 1.8	ND	ND
71	B		3.8 ± 0.2	ND	ND

[a] ND = not determined.

Table 4. Inhibitory Activities of Compounds with Modification at the Hydroxyl Groups of Dipyridamole



Comp.	R^1	ENT1 inhibitory activity in K562 cells determined by flow cytometry		
		% Inhibition at 10μM	IC_{50} (nM)	K_i (nM)
DMSO	-	0.0 ± 0.7	ND[a]	ND
NBMPR	-	97.1 ± 0.4	7.6	0.43
Lidoflazine	-	90.0 ± 0.2	4954	279.9
DP	$N(CH_2CH_2OH)_2$	86.7 ± 0.1	144.8	8.18
72[b]	OCH_2CH_2OH	60.7 ± 0.7	5,746	325.2
73		13.0 ± 0.4	ND	ND
74	$N(CH_2CH_2OOCH_3)_2$	91.3 ± 0.2	145	8.2
75	$N(CH_2CH_2OOCCH_3)_2$	90.4 ± 0.3	302	17.1
76	$N(CH_2CH_2OCH_3)_2$	66.2 ± 1.1	1,621	91.6
77	$N(CH_2CH_2OCH_2CH_3)_2$	8.4 ± 0.1	ND	ND
78	$N(CH_2CH_2OCH_2CH_2CH_3)_2$	2.3 ± 0.4	ND	ND
79		73.8 ± 1.1	76	4.3

[a] ND = not determined. [b] Prepared according to a literature procedure.[40]

higher activity than rigid ring systems. This is evident in comparing the activities of compound **15** (K_i = 13.6 nM) and compound **17** (K_i = 3416 nM). Compounds with *N*-(bis-hydroxyethyl) substituents at the 2- and 6-positions (type A in Tables 1−4) were much more potent than the corresponding *N*-(monohydroxyethyl) substituted analogues (type B in Tables 1−4).

The open-chain analogues (compound **21**−**38**) were less active than the cyclic counterparts. Compounds with carbon chain length from 1 to 4 (compounds **21**, **23**, **25**, and **27**) exhibited low inhibitory activities. Increasing the chain length (compound **31**) or branching it (compounds **29** and **33**) led to a decrease in activity. Compound **35** has polar oxygen atoms in the side chain, which also resulted in low activity. Compound **37** has dibenzylamino groups at the 4- and 8-positions and was inactive. In this set also, the *N*-(monohydroxyethyl) substituted analogues (compounds **22**, **24**, **26**, **28**, **30**, **32**, **34**, **36**, and **38**) were less active than the *N*-(bis-hydroxyethyl) counterparts.

The analogues that contained a primary or secondary amine (compounds **39**−**71**) at the 4- and 8- positions had lower inhibitory activities relative to dipyridamole. The most active compounds in the group, **52**, **58**, and **64**, were only about half as active as dipyridamole. These are analogues with *tert*-butylamino, isopentylamino, and cyclopentylamnio groups at the 4- and 8-positions. Again, analogues with *N*-(monohydroxy-ethyl) substitution were less active than the *N*-(bis-hydroxyethyl) counterparts.

Compounds **72**−**79** are 2- and 6-substituted dipyridamole analogues. The presence of a 2′-hydroxyethoxy group at the 2- and 6-positions (compound **72**) resulted in a steep drop in activity compared to dipyridamole. However, compound **72** exhibited higher activity than compound **1**, the *N*-(monohydroxyethyl) counterpart of dipyridamole. This indicates that a hydrogen atom on the 2- and 6-position nitrogen is unfavorable for potent activity. Compound **73** has the diethanolamino groups at the 2- and 6-postions locked into morpholino rings, and this modification caused a loss of activity. Esterification of dipyridamole (compound **74** and **75**) maintained relatively good activity compared to dipyridamole, which indicates that free hydroxyl groups are not necessary for activity. Esterification introduces additional oxygen atoms, which might participate in additional hydrogen bonding that probably compensates for the loss of activity caused by an increase in lipophilicity. In contrast, ether type lipophilic modification at the same positions caused a decrease in activity as in the case compounds **76**−**78**. Interestingly, compound **79**, which has one free hydroxyl group at the 2- and 6-positions, exhibited a higher potency than dipyridamole. The reasons for the higher potency of **79** relative to dipyridamole are not apparent. Some compounds, namely, **7**, **25**, and **27**, had a percent inhibition above 40%, but no IC_{50} values could be determined because of low solubility.

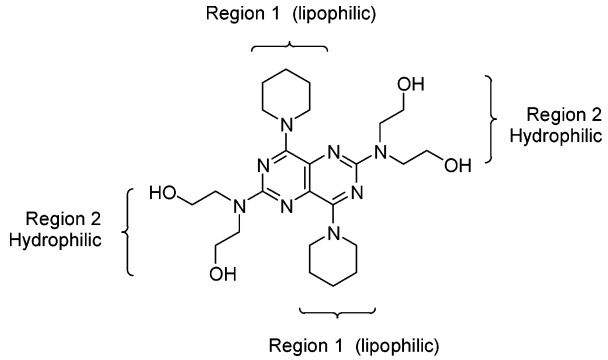


Figure 3. Representative regions for dipyridamole analogues.

These dipyridamole analogues had modifications at two important regions with regard to ENT1 inhibitory activity (see Figure 3). Region 1 should be lipophilic to obtain the highest ENT1 inhibitory activities, with single nitrogen-containing flexible rings being preferred to carbocyclic, morpholine, piperazine, or rigid multicyclic ring systems. For the nitrogen-containing flexible rings, an eight-membered ring is optimal. Region 2 should be a hydrophilic region with the diethanolamino group providing optimal activity, although it is not essential; small lipophilic modifications over the hydroxyl groups are well tolerated.

Conclusion

In this study, a substantial number of dipyridamole analogues were synthesized and explored for their inhibitory activity against ENT1 transporter using a flow cytometric method. Compounds with much higher activity than dipyridamole were identified for the first time, with the best, compound **13,** being 16 times more active than dipyridamole and having activity comparable to that of the potent ENT1 standard inhibitor NBMPR. The study has also revealed important structural determinants for ENT1 inhibitory activity in this series, among which are the requirements for a lipophilic medium to large size nitrogen-containing lipophilic rings at the 4- and 8-positions and hydrophilic, hydrogen-bond acceptor substituents at the 2- and 6-positions. The newly identified higher potency dipyridamole analogue, compound **13**, may facilitate the therapeutic exploitation of the ENT1 inhibitory activity of dipyridamole and related compounds.

Experimental Section

Chemistry. Thin-layer chromatography (TLC) was conducted on silica gel plates (Analtech). Compounds were visualized by UV light (254 and 365 nm). 1D NMR spectra were recorded on a Varian Inova 500 MHz NMR instrument by using $CDCl_3$ or $(CD_3)_2SO$ as solvents and using tetramethylsilane (TMS) as an internal standard. Flash column chromatography was performed on Fisher silica gel (170−400 mesh). Melting points were determined using a Fisher-Johns melting point apparatus and were reported uncorrected. Mass spectra were obtained on a Bruker-HP ESQUIRE ion trap LC/MS-(*n*) system. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. All solvents and reagents were purchased from Aldrich or other major chemical companies and used without further purification. All reactions were carried under argon gas.

2,6-Bis(diethanolamino)-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine or 2,6-Diethanolamino-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine. General Procedure I. To a solution of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (TCPP) (0.27 g, 1 mmol) in anhydrous THF (10 mL), the appropriate amine (4.2 mmol) was added in this first step. The reaction was stirred on an ice−water bath for 20 min, and then water (100 mL) was added to precipitate the reaction intermediate. After drying over P_2O_5, the intermediate was dissolved in DMSO (3 mL). An appropriate amine (diethanolamine, ethanolamine, or morpholine) (3 mL) was added, and the mixture was heated at 150 °C for 6 h with stirring. Then the product was purified by flash silica gel chromatography.

2,6-Bis(diethanolamino)-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine. General Procedure II. To a solution of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (TCPP) (0.27 g, 1 mmol) in anhydrous THF (10 mL), the appropriate Grignard reagent (2.1 mmol) was added at this first step. The reaction was stirred in ice−water bath for 20 min, and then water (100 mL) was added to precipitate the reaction intermediate. After drying over P_2O_5, the intermediate was dissolved in DMSO (3 mL), and diethanolamine (3 mL) was added. The mixture was heated at 150 °C for 6 h with stirring. Then the product was purified by flash silica gel chromatography.

2,6-Bis(dialkoxylethylamino)-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine. General Procedure III. NaH (60% in mineral oil, 0.28 g, 7 mmol) was added to a solution of dipyridamole (0.35 g, 0.69 mmol) in anhydrous DMF (10 mL), and the mixture was stirred at room temperature for 2 h. Then the appropriate alkyl halide (32 mmol) was added, and the mixture was stirred overnight. The reaction mixture was partitioned between CH_2Cl_2 (60 mL) and H_2O (50 mL), and the organic layer was separated. The remaining aqueous solution was extracted with CH_2Cl_2 (20 mL × 2), and all organic solutions were incorporated and dried over anhydrous Na_2SO_4. Then the CH_2Cl_2 was removed under reduced pressure, and the residue was subjected to flash silica gel chromatography for purification of the product.

2,6-Diethanolamino-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (1). Compound **1** was prepared by general procedure I with piperidine (0.41 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. The product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow powdery solid (162 mg, 39%). Mp 152−153 °C; MS (ESI) *m*/*z* 417 $(M + H)^+$, 439 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.016 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.606 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 4.057 (br s, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$CH$_2$), 3.513 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.269 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.641 (br d, 4H, 2 × N(CH$_2$CH$_2$)$_2$C*H*$_2$, J = 4.5 Hz), 1.592 (br d, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$-CH$_2$, J = 4.5 Hz). Anal. ($C_{20}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dipyrrolidinylpyrimido[5,4-*d*]pyrimidine (2). Compound **2** was prepared by general procedure I with pyrroline (0.35 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 15/1) to give a yellow powdery solid (252 mg, 53%). Mp 212−213 °C; MS (ESI) *m*/*z* 477 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.688 (m, 4H, 4 × OH, disappeared after D_2O exchange), 4.119 (br s, 8H, 2 × N(C*H*$_2$-CH$_2$)$_2$), 3.592 (br s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 1.877 (br s, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$). Anal. ($C_{22}H_{36}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dipyrrolidinylpyrimido[5,4-*d*]pyrimidine (3). Compound **3** was prepared by general procedure I with pyrroline (0.35 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 15/1) to give a yellow powdery solid (176 mg, 45%). Mp 219−220 °C; MS (ESI) *m*/*z* 389 $(M + H)^+$, 411 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.774 (t, 2H, 2 × NH, disappeared after D_2O), 4.591 (t, 2H, 2 × OH, disappeared after D_2O exchange), 4.006 (br s, 8H, 2 × N(C*H*$_2$-CH$_2$)$_2$), 3.505 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J = 6 Hz), 3.292 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, J_1 = 6 Hz), 1.863 (br s, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$). Anal. ($C_{18}H_{28}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dimorpholinopyrimido[5,4-*d*]pyrimidine (4). Compound **4** was prepared by general procedure I with morpholine (0.37 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow powdery solid (274 mg, 54%). Mp 205−206 °C; MS (ESI) *m*/*z* 509 $(M + H)^+$, 531 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.689 (t, 4H, 4 × OH, disappeared after D_2O), 4.121 (br s, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$O), 3.715 (t, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$O), 3.573 (br s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$). Anal. ($C_{22}H_{36}N_8O_6$·0.5H_2O) C, H, N.

2,6-Diethanolamino-4,8-dimorpholinopyrimido[5,4-*d*]pyrimidine (5). Compound **5** was prepared by general procedure I with morpholine (0.37 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 15/1) to give a yellow powdery solid (211 mg, 50%). Mp 203−204 °C; MS (ESI) *m*/*z* 421 $(M + H)^+$, 443 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 6.186 (t, 2H, 2 × NH, disappeared after D_2O), 4.619 (t, 2H, 2 × OH, disappeared after D_2O), 4.128 (br s, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$O), 3.708 (t, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$O), 3.504 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH), 3.254 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH). Anal. ($C_{18}H_{18}N_8O_4$·0.5H_2O) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(*N*-methylpiperazino)pyrimido-[5,4-*d*]pyrimidine (6). Compound **6** was prepared by general procedure I with 1-methylpiperazine (0.47 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 1/1) to give a yellow powdery solid (273 mg, 51%). Mp 199−200 °C; MS (ESI) *m*/*z* 535 $(M + H)^+$, 557 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.719 (t, 4H, 4 × OH, disappeared after D_2O), 4.122 (br s, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$NCH$_3$), 3.591 (br s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 2.434 (t, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$NCH$_3$), 2.219 (s, 6H, 2 × CH$_3$). Anal. ($C_{24}H_{42}N_{10}O_4$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(*N*-BOC-piperazino)pyrimido-[5,4-*d*]pyrimidine (7). Compound **7** was prepared by general procedure I with *N*-BOC-piperazine (0.78 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 5/1) to give a yellow powdery solid (304 mg, 43%). Mp 223−224 °C; MS (ESI) *m*/*z* 707 $(M + H)^+$, 729 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.749 (br t, 4H, 4 × OH, disappeared after D_2O), 4.121 (br s, 8H, 2 × N(C*H*$_2$CH$_2$)$_2$*N*-BOC), 3.611 (s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 3.489 (br s, 8H, 2 × N(CH$_2$C*H*$_2$)$_2$*N*-BOC), 1.465 (s, 18H, 6 × CH$_3$). Anal. ($C_{32}H_{54}N_{10}O_8$) C, H, N.

2,6-Bis(diethanolamino)-4-piperazino-8-(*N*-Cbz-piperazino)-pyrimido[5,4-*d*]pyrimidine (8). Compound **8** was prepared by general procedure I with benylpiperazine 1-carboxylate (0.93 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 1.5/1) to give a yellow powdery solid (77 mg, 12%). Mp 133−134 °C; MS (ESI) *m*/*z* 641 $(M + H)^+$, 663 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.388 (d, 3H, Ar−H-3, Ar−H-4, Ar−H-5), 7.335 (m, 2H, Ar−H-2, Ar−H-6), 5.121 (s, 2H, PhC*H*$_2$), 4.699 (t, 4H, 4 × OH, disappeared after D_2O),

4.119 (br s, 4H, N(CH_2CH$_2$)$_2$NH), 4.031 (br s, 4H, N(CH_2CH$_2$)$_2$-NCbz), 3.570 (br s, 21H, 2 × N(CH_2CH_2OH)$_2$, N(CH$_2$CH_2)$_2$NH), 2.789 (br s, 4H, N(CH$_2$CH_2)$_2$NCbz). Anal. ($C_{30}H_{44}N_{10}O_6$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclohexylpyrimido[5,4-*d*]pyrimidine (9). Compound **9** was prepared by general procedure II with cyclehexylmagnesium chloride solution (2.0 M in diethyl ether, 1.05 mL, 2.1 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 24/1) to give a yellow powdery solid (226 mg, 45%). Mp 226−228 °C; MS (ESI) *m*/*z* 503 (M + H)$^+$, 525 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.782 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.717 (br s, 8H, 2 × N(CH_2-CH$_2$OH)$_2$), 3.675 (br s, 8H, 2 × N(CH$_2$CH_2OH)$_2$), 3.602 (m, 2H, 2 × CH(CH$_2$CH$_2$)$_2$CH$_2$), 1.892−1.815 (m, 8H, 2 × CH(CH_2CH$_2$)$_2$-CH$_2$), 1.753 (d, 2H, 2 × CH(CH$_2$CH$_2$)$_2$CH_AH$_B$), 1.589−1.397 (m, 8H, 2 × CH(CH$_2$CH_2)$_2$CH$_2$), 1.283 (m, 2H, 2 × CH(CH$_2$CH$_2$)$_2$-CH$_A$$H_B$). Anal. ($C_{26}H_{42}N_6O_4$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diphenylpyrimido[5,4-*d*]pyrimidine (10). Compound **10** was prepared by general procedure II with phenylmagnesium chloride solution (2.0 M in tetrahydrofuran, 1.05 mL, 2.1 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 9/1) to give a red powdery solid (29 mg, 5.9%). Mp 208−209 °C; MS (ESI) *m*/*z* 491 (M + H)$^+$, 513 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 8.470 (m, 4H, 2 × Ar−H-2, 2 × Ar−H-6), 7.557 (m, 6H, 2 × Ar−H-3, 2 × Ar−H-4, 2 × Ar−H-5), 4.805 (t, 4H, 4 × OH, disappeared after D$_2$O, J = 5 Hz), 3.793 (br s, 8H, 2 × N(CH_2CH$_2$OH)$_2$), 3.708 (t, 8H, 2 × N(CH$_2$CH_2OH)$_2$, J = 5 Hz). Anal. ($C_{26}H_{30}N_6O_4$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dihexamethyleneiminopyrimido-[5,4-*d*]pyrimidine (11). Compound **11** was prepared by general procedure I with hexamethyleneimine (0.48 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 2/1) to give a yellow powdery solid (213 mg, 40%). Mp 212−213 °C; MS (ESI) *m*/*z* 533 (M + H)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.677 (br t, 4H, 4 × OH, disappeared after D$_2$O), 4.129 (br, s, 8H, 2 × N(CH_2CH$_2$CH$_2$)$_2$), 3.572 (s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.775 (br s, 8H, 2 × N(CH$_2$CH_2CH$_2$)$_2$), 1.511 (br s, 8H, 2 × N(CH$_2$CH$_2$CH_2)$_2$). Anal. ($C_{26}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dihexamethyleneiminopyrimido[5,4-*d*]pyrimidine (12). Compound **12** was prepared by general procedure I with hexamethyleneimine (0.48 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$-Cl$_2$/MeOH = 20/1) to give a yellow powdery solid (106 mg, 24%). Mp 166−167 °C; MS (ESI) *m*/*z* 445 (M + H)$^+$, 467 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.787 (q, 2H, 2 × NH, disappeared after D$_2$O, J = 5.5 Hz), 4.592 (t, 2H, 2 × OH, disappeared after D$_2$O, J = 5.5 Hz), 4.112 (br s, 8H, 2 × N(CH_2CH$_2$CH$_2$)$_2$), 3.514 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.246 (t, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.776 (br s, 8H, 2 × N(CH$_2$CH_2CH$_2$)$_2$), 1.500 (br s, 8H, 2 × N(CH$_2$CH$_2$CH_2)$_2$). Anal. ($C_{22}H_{36}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diheptamethyleneiminopyrimido-[5,4-*d*]pyrimidine (13). Compound **13** was prepared by general procedure I with heptamethyleneimine (0.53 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 3/1) to give a yellow powdery solid (219 mg, 39%). Mp 204−205 °C; MS (ESI) *m*/*z* 561 (M + H)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.681 (t, 4H, 4 × OH, disappeared after D$_2$O), 4.091 (br, s, 8H, 2 × N(CH_2CH$_2$CH$_2$)$_2$CH$_2$), 3.576 (br s, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.782 (br s, 8H, 2 × N(CH$_2$CH_2CH$_2$)$_2$CH$_2$), 1.541 (br s, 8H, 2 × N(CH$_2$CH$_2$CH_2)$_2$CH$_2$), 1.479 (br s, 4H, 2 × N(CH$_2$-CH$_2$CH$_2$)$_2$CH_2). Anal. ($C_{28}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diheptamethyleneiminopyrimido[5,4-*d*]pyrimidine (14). Compound **14** was prepared by general procedure I with heptamethyleneimine (0.53 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 2/1) to give a yellow powdery solid (246 mg, 43%). Mp 150−151 °C; MS (ESI) *m*/*z* 573 (M + H)$^+$, 495 (M + Na)$^+$, 511 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.751 (t, 2H, 2 × NH, disappeared after D$_2$O, J = 6 Hz), 4.592 (t, 2H, 2 × OH, disappeared after D$_2$O, J = 5.5 Hz), 4.077 (br, s, 8H, 2 × N(CH_2-CH$_2$CH$_2$)$_2$CH$_2$), 3.508 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.256 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 6 Hz), 1.786 (br s, 8H, 2 × N(CH$_2$CH_2CH$_2$)$_2$CH$_2$), 1.527 (br s, 8H, 2 × N(CH$_2$CH$_2$CH_2)$_2$CH$_2$), 1.468 (br s, 4H, 2 × N(CH$_2$CH$_2$-CH$_2$)$_2$CH_2). Anal. ($C_{24}H_{40}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dioctomethyleneimino-pyrimido-[5,4-*d*]pyrimidine (15). Compound **15** was prepared by general procedure I with octomethyleneimine (0.54 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 2.5/1) to give a yellow powdery solid (65 mg, 11%). Mp 213−214 °C; MS (ESI) *m*/*z* 589 (M + H)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.686 (t, 4H, 4 × OH, disappeared after D$_2$O), 4.055 (br, s, 8H, 2 × N(CH_2CH$_2$CH$_2$CH$_2$)$_2$), 3.602 (m, 16H, 2 × N(CH_2CH_2OH)$_2$), 1.811 (s, 8H, 2 × N(CH$_2$CH_2CH$_2$CH$_2$)$_2$), 1.648 (s, 8H, 2 × N(CH$_2$CH$_2$CH_2CH$_2$)$_2$), 1.451 (s, 8H, 2 × N(CH$_2$CH$_2$-CH$_2$CH_2)$_2$). Anal. Calcd for $C_{30}H_{52}N_8O_4$: C 61.20, H 8.90, N 19.03. Found: C 60.73, H 8.84, N 18.87.

2,6-Diethanolamino-4,8-dioctomethyleneiminopyrimido[5,4-*d*]pyrimidine (16). Compound **16** was prepared by general procedure I with octomethyleneimine (0.54 g, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 2.5/1) to give a yellow powdery solid (60 mg, 12%). Mp 167 °C; MS (ESI) *m*/*z* 501 (M + H)$^+$, 523 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.746 (t, 2H, 2 × NH, disappeared after D$_2$O, J = 6 Hz), 4.601 (t, 2H, 2 × OH, disappeared after D$_2$O, J = 5.5 Hz), 4.039 (br, s, 8H, 2 × N(CH_2CH$_2$CH$_2$CH$_2$)$_2$), 3.514 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.284 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.822 (br s, 8H, 2 × N(CH$_2$CH_2CH$_2$CH$_2$)$_2$), 1.645 (br s, 8H, 2 × N(CH$_2$CH$_2$CH_2-CH$_2$)$_2$), 1.440 (s, 8H, 2 × N(CH$_2$CH$_2$CH$_2$CH_2)$_2$). Anal. ($C_{26}H_{44}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dinontropanopyrimido[5,4-*d*]pyrimidine (17). Compound **17** was prepared by general procedure I with nontropane[54] (0.47 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 10/1) to give a yellow powdery solid (250 mg, 45%). Mp 244−245 °C; MS (ESI) *m*/*z* 557 (M + H)$^+$, 579 (M + Na)$^+$; ^{1}H NMR[56] (DMSO-d_6) δ 6.203 (br s, 2H), 4.908 (br s, 2H), 4.695 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.581 (br s, 16H, 2 × N(CH_2CH_2-OH)$_2$), 1.942−1.455 (series of br s, 20H). Anal. ($C_{28}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dinontropanopyrimido[5,4-*d*]pyrimidine (18). Compound **18** was prepared by general procedure I with nontropane[54] (0.47 g, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 16/1) to give a yellow powdery solid compound **7** (128 mg, 27%). Mp 254−255 °C; MS (ESI) *m*/*z* 469 (M + H)$^+$, 491 (M + Na)$^+$; ^{1}H NMR[57] (DMSO-d_6) δ 6.319 (br s, 2H), 5.901 (t, 2H, 2 × NHCH$_2$CH$_2$OH, disappeared after D$_2$O, J = 5.5 Hz), 4.909 (br s, 2H), 4.584 (t, 2H, 2 × OH, disappeared after D$_2$O, J = 5.5 Hz), 3.515 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.243 (d, 4H, 2 × NHCH_2CH$_2$OH, J = 5.5 Hz), 1.938−1.430 (series of br s, 20H). Anal. ($C_{24}H_{36}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di-(4-azatricyclo[4.3.1.1^{3,8}]-undecane)pyrimido[5,4-*d*]pyrimidine (19). Compound **19** was prepared by general procedure I with 4-azatricyclo[4.3.1.1^{3,8}]-undecane[55] (0.64 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 20/1) to give a yellow powdery solid (262 mg, 41%). Mp 252−253 °C; MS (ESI) *m*/*z* 637 (M + H)$^+$, 659 (M + Na)$^+$; ^{1}H NMR[57] (DMSO-d_6) δ

5.765 (br s, 2H), 4.680 (t, 4H, 4 × OH, disappeared after D_2O), 3.890 (br s, 4H), 3.589 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 2.299 (br s, 2H), 1.959 (t, 4H), 1.929 (br s, 8H), 1.759−1.733 (br d, 4H), 1.604−1.516 (m, 8H). Anal. ($C_{34}H_{52}N_8O_4$·0.5H_2O) C, H, N.

2,6-Diethanolamino-4,8-di(4-azatricyclo[4.3.1.1^{3,8}]undecane)-pyrimido[5,4-*d*]pyrimidine (20). Compound **20** was prepared by general procedure I with 4-azatricyclo[4.3.1.1^{3,8}]undecane[55] (0.64 g, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 2.5/1) to give a yellow powdery solid (198 mg, 36%). Mp 194−196 °C; MS (ESI) *m*/*z* 549 (M + H)$^+$, 571 (M + Na)$^+$; ^{1}H NMR[55] (DMSO-d_6) δ 5.746 (t, 2H, 2 × NH, disappeared after D_2O, J = 6 Hz), 5.720 (br s, 2H), 4.580 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.834 (br s, 4H), 3.505 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.289 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 6 Hz), 2.287 (br s, 2H), 1.951−1.926 (m, 12H), 1.793−1.767 (br d, 4H), 1.605−1.510 (m, 8H). Anal. ($C_{30}H_{44}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(dimethylamino)pyrimido[5,4-*d*]pyrimidine (21). Compound **21** was prepared by general procedure I with dimethylamine solution (2.0 M in tetrahydrofuran, 2.1 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 12/1) to give a yellow powdery solid (174 mg, 41%). Mp 207−208 °C; MS (ESI) *m*/*z* 425 (M + H)$^+$, 447 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.704 (t, 4H, 4 × OH, disappeared after D_2O, J = 5.5 Hz), 3.597 (m, 16H, 2 × N($CH_2CH_2OH)_2$, J = 5.5 Hz), 3.409 (br s, 12H, 4 × CH_3). Anal. ($C_{18}H_{32}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(dimethylamino)pyrimido[5,4-*d*]pyrimidine (22). Compound **22** was prepared by general procedure I with dimethylamine solution (2.0 M in tetrahydrofuran, 2.1 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 9/1) to give a yellow powdery solid (40 mg, 12%). Mp 159−161 °C; MS (ESI) *m*/*z* 337 (M + H)$^+$, 359 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.954 (br s, 2H, 2 × NH, disappeared after D_2O), 4.634 (t, 2H, 2 × OH, disappeared after D_2O), 3.509 (q, 4H, 2 × NHCH_2CH_2OH), 3.371 (br s, 12H, 4 × CH_3), 3.284 (t, 4H, 2 × NHCH_2CH_2OH). Anal. ($C_{14}H_{24}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(diethylamino)pyrimido[5,4-*d*]-pyrimidine (23). Compound **23** was prepared by general procedure I with diethylamine (0.44 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 10/1) to give a yellow powdery solid (187 mg, 39%). Mp 165−166 °C; MS (ESI) *m*/*z* 481 (M + H)$^+$, 503 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.692 (t, 4H, 4 × OH, disappeared after D_2O), 3.914 (br s, 8H, 4 × C$H_2$$CH_3$), 3.589 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 1.205 (t, 12H, 4 × CH_2CH_3). Anal. ($C_{22}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(diethylamino)pyrimido[5,4-*d*]pyrimidine (24). Compound **24** was prepared by general procedure I with diethylamine (0.44 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow powdery solid (122 mg, 31%). Mp 127 °C; MS (ESI) *m*/*z* 393 (M + H)$^+$, 415 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.787 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.599 (t, 2H, 2 × OH, disappeared after D_2O, J = 6 Hz), 3.902 (br s, 8H, 4 × C$H_2$$CH_3$), 3.506 (q, 4H, 2 × NHC$H_2CH_2$OH, J_1 = 6 Hz, J_2 = 6 Hz), 3.265 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.198 (t, 12H, 4 × CH_2CH_3). Anal. ($C_{18}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(dipropylamino)pyrimido[5,4-*d*]pyrimidine (25). Compound **25** was prepared by general procedure I with dipropylamine (0.58 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 18/1) to give a yellow powdery solid (81 mg, 15%). Mp 150−151 °C; MS (ESI) *m*/*z* 537 (M + H)$^+$, 559 (M + Na)$^+$, 575 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.707 (br s, 4H, 4 × OH, disappeared after D_2O), 3.846 (br s, 8H, 4 × C$H_2$$CH_2CH_3$), 3.586 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 1.637 (q, 8H, 4 × CH_2CH_2CH_3, J = 7.5 Hz), 0.878 (t, 12H, 4 × CH_2CH_2CH_3, J = 7.5 Hz). Anal. ($C_{26}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(dipropylamino)pyrimido[5,4-*d*]pyrimidine (26). Compound **26** was prepared by general procedure I with dipropylamine (0.58 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 12/1) to give a yellow powdery solid (85 mg, 19%). Mp 144−145 °C; MS (ESI) *m*/*z* 449 (M + H)$^+$, 471 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.723 (t, 2H, 2 × NH, disappeared after D_2O, J = 6 Hz), 4.618 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.837 (br s, 8H, 4 × C$H_2$$CH_2CH_3$), 3.518 (q, 4H, 2 × NHC$H_2CH_2$-OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.288 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 6 Hz), 1.644 (m, 8H, 4 × CH_2CH_2CH_3, J = 7.5 Hz), 0.876 (t, 12H, 4 × CH_2CH_2CH_3, J = 7.5 Hz). Anal. ($C_{22}H_{40}N_8O_2$) C, H, N.

2,6- Bis(diethanolamino)-4,8-di-(dibutylamino)pyrimido[5,4-*d*]pyrimidine (27). Compound **27** was prepared by general procedure I with dibutylamine (0.71 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 27/1) to give a yellow powdery solid (157 mg, 27%). Mp 126−127 °C; MS (ESI) *m*/*z* 593 (M + H)$^+$, 615 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.705 (t, 4H, 4 × OH, disappeared after D_2O, J = 5 Hz), 3.876 (br s, 8H, 4 × C$H_2$$CH_2CH_2CH_3$), 3.581 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 1.591 (m, 8H, 4 × CH_2CH_2-CH_2CH_3, J = 8 Hz), 1.308 (m, 8H, 4 × CH_2CH_2CH_2CH_3, J_1 = 8 Hz, J_2 = 7.5 Hz), 0.903 (t, 12H, 4 × CH_3, J = 7.5 Hz). Anal. ($C_{30}H_{56}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(dibutylamino)pyrimido[5,4-*d*]pyrimidine (28). Compound **28** was prepared by general procedure I with dibutylamine (0.71 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 5/1) to give a yellow powdery solid (124 mg, 22%). Mp 129−130 °C; MS (ESI) *m*/*z* 505 (M + H)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.687 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.615 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.870 (br s, 8H, 4 × C$H_2$$CH_2CH_2CH_3$), 3.505 (q, 4H, 2 × NHC$H_2CH_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.267 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 6 Hz), 1.596 (m, 8H, 4 × CH_2C$H_2CH_2CH_3$, J = 7.5 Hz), 1.308 (m, 8H, 4 × CH_2CH_2CH_2CH_3, J = 7.5 Hz), 0.907 (t, 12H, 4 × CH_3, J = 7.5 Hz). Anal. ($C_{26}H_{48}N_8O_2$·0.5H_2O) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(diisobutylamino)pyrimido[5,4-*d*]pyrimidine (29). Compound **29** was prepared by general procedure I with diisobutylamine (0.73 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow powdery solid (59 mg, 10%). Mp 169−171 °C; MS (ESI) *m*/*z* 593 (M + H)$^+$, 615 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 4.669 (br s, 4H, 4 × OH, disappeared after D_2O), 3.808 (br s, 8H, 4 × CH_2CH($CH_3)_2$), 3.528 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 1.890 (br s, 4H, 4 × CH_2CH($CH_3)_2$), 0.752 (br s, 24H, 4 × CH_2CH($CH_3)_2$). Anal. ($C_{30}H_{56}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(diisobutylamino)pyrimido[5,4-*d*]-pyrimidine (30). Compound **30** was prepared by general procedure I with diisobutylamine (0.73 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 18/1) to give a yellow powdery solid (212 mg, 42%). Mp 154 °C; MS (ESI) *m*/*z* 505 (M + H)$^+$, 527 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 5.821 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.634 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.881 (br s, 8H, 4 × CH_2CH($CH_3)_2$), 3.532 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.276 (q, 4H, 2 × NHCH_2CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.983 (m, 4H, 4 × CH_2CH($CH_3)_2$, J = 6.5 Hz), 0.840 (d, 24H, 4 × CH_2CH(C$H_3)_2$, J = 6.5 Hz). Anal. ($C_{26}H_{48}N_8O_2$·0.5H_2O) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(dipentylamino)pyrimido[5,4-*d*]pyrimidine (31). Compound **31** was prepared by general procedure I with dipentylamine (0.85 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 20/1) to give a yellow powdery solid (145 mg, 22%). Mp 130−131 °C; MS (ESI) *m/z* 649 $(M + H)^+$, 671 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.706 (t, 4H, 4 × OH, disappeared after D_2O), 3.865 (br s, 8H, 4 × C$H_2$$(CH_2)_3CH_3$), 3.578 (q, 16H, 2 × N($CH_2CH_2OH)_2$), 1.604 (m, 8H, 4 × $CH_2CH_2(CH_2)_2CH_3$), 1.332−1.229 (m, 16H, 4 × $CH_2CH_2(CH_2)_2CH_3$), 0.869 (t, 12H, 4 × CH_3). Anal. ($C_{34}H_{64}N_8O_4$·0.5H_2O) C, H, N.

2,6-Diethanolamino-4,8-di(dipentylamino)pyrimido[5,4-*d*]pyrimidine (32). Compound **32** was prepared by general procedure I with dipentylamine (0.85 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 15/1) to give a yellow powdery solid (26 mg, 4.6%). Mp 128−129 °C; MS (ESI) *m/z* 561 $(M + H)^+$, 583 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.659 (t, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.619 (t, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.860 (br s, 8H, 4 × C$H_2$$(CH_2)_3CH_3$), 3.504 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.269 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 1.609 (m, 8H, 4 × $CH_2CH_2(CH_2)_2CH_3$, J_1 = 7.5 Hz, J_2 = 7 Hz), 1.255 (m, 16H, 4 × $CH_2CH_2(CH_2)_2CH_3$), 0.871 (t, 12H, 4 × CH_3, J = 7 Hz). Anal. ($C_{30}H_{56}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(diisopentylamino)pyrimido-[5,4-*d*]pyrimidine (33). Compound **33** was prepared by general procedure I with diisopentylamine (0.86 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 7/1) to give a yellow powdery solid (156 mg, 24%). Mp 129 °C; MS (ESI) *m/z* 671 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.705 (t, 4H, 4 × OH, disappeared after D_2O, J = 5.5 Hz), 3.864 (br s, 8H, 4 × C$H_2$$CH_2CH(CH_3)_2$), 3.578 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 1.603 (m, 8H, 4 × $CH_2CH_2CH(CH_3)_2$), 1.301 (m, 16H, 4 × $CH_2CH_2CH(CH_3)_2$, 4 × CH_3), 0.868 (t, 12H, 4 × CH_3, J = 7 Hz). Anal. ($C_{34}H_{64}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(diisopentylamino)pyrimido[5,4-*d*]-pyrimidine (34). Compound **34** was prepared by general procedure I with diisopentylamine (0.86 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 15/1) to give a yellow powdery solid (125 mg, 22%). Mp 97−98 °C; MS (ESI) *m/z* 561 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 5.727 (m, 2H, 2 × NH, disappeared after D_2O, J = 5.5 Hz), 4.621 (m, 2H, 2 × OH, disappeared after D_2O, J = 5.5 Hz), 3.858 (br d, 8H, 4 × C$H_2CH_2CH(CH_3)_2$), 3.510 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.269 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.841−1.335 (m, 8H, 4 × $CH_2CH_2CH(CH_3)_2$), 1.321−1.058 (m, 12H, 4 × CH_3), 0.884−0.778 (m, 16H, 4 × $CH_2CH_2CH(CH_3)_2$), 4 × CH_3). Anal. ($C_{30}H_{56}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(bis(2-methoxyethyl)amino)pyrimido[5,4-*d*]pyrimidine (35). Compound **35** was prepared by general procedure I with bis(2-methoxyethyl)amine (0.65 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 10/1) to give a yellow powdery solid (260 mg, 43%). Mp 104−105 °C; MS (ESI) *m/z* 601 $(M + H)^+$, 623 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.685 (br s, 4H, 4 × OH, disappeared after D_2O), 4.145 (br s, 8H, 4 × C$H_2CH_2OCH_3$), 3.594 (t, 8H, 4 × $CH_2CH_2OCH_3$), 3.557 (br s, 16H, 2 × N($CH_2CH_2OH)_2$), 3.260 (s, 12H, 4 × CH_3). Anal. ($C_{26}H_{48}N_8O_8$) C, H, N.

2,6-Diethanolamino-4,8-di(bis(2-methoxyethyl)amino)pyrimido-[5,4-*d*]pyrimidine (36). Compound **36** was prepared by general procedure I with bis(2-methoxyethyl)amine (0.65 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow powdery solid (100 mg, 20%). Mp 68−69 °C; MS (ESI) *m/z* 513 $(M + H)^+$, 535 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 5.905 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O), 4.598 (t, 2H, 2 × $NHCH_2CH_2O$*H*, disappeared after D_2O, J = 5 Hz), 4.125 (br s, 8H, 4 × CH_2CH_2-OCH_3), 3.595 (t, 8H, 4 × $CH_2CH_2OCH_3$), 3.496 (q, 4H, 2 × $NHCH_2CH_2OH$, J = 5 Hz), 3.257 (s, 12H, 4 × CH_3), 3.235 (br s, 4H, 2 × NHCH_2CH_2OH). Anal. ($C_{22}H_{40}N_8O_6$) C, H, N.

2,6-Bis(diethanolamino)-4,8-bis(dibenzylamino)pyrimido[5,4-*d*]pyrimidine (37). Compound **37** was prepared by general procedure I with dibenzylamine (0.83 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 16/1) to give a yellow powdery solid compound **9** (332 mg, 46%). Mp 199 °C; MS (ESI) *m/z* 729 $(M + H)^+$, 751 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.324 (t, 8H, 4 × Ar−H-3, 4 × Ar−H-5, J_1 = 7.5 Hz, J_2 = 7 Hz), 7.261−7.224 (m, 12H, 4 × Ar−H-2, 4 × Ar−H-6, 4 × Ar−H-4), 5.317 (br s, 8H, 4 × CH_2-Ph), 4.564 (t, 4H, 4 × OH, disappeared after D_2O, J = 5 Hz), 3.259 (br d, 16H, 2 × N($CH_2CH_2OH)_2$). Anal. ($C_{42}H_{48}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-bis(dibenzylamino)pyrimido[5,4-*d*]-pyrimidine (38). Compound **38** was prepared by general procedure I with dibenzylamine (0.83 g, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 20/1) to give a yellow powdery solid compound **8** (125 mg, 20%). Mp 215−216 °C; MS (ESI) *m/z* 641 $(M + H)^+$, 663 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.327 (t, 8H, 4 × Ar−H-3, 4 × Ar−H-5, J_1 = 7 Hz, J_2 = 7.5 Hz), 7.289 (d, 8H, 4 × Ar−H-2, 4 × Ar−H-6, J = 7 Hz), 7.247 (t, 4H, 4 × Ar−H-4, J_1 = 7.5 Hz, J_2 = 7 Hz), 5.970 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.279 (br s, 8H, 4 × CH_2Ph), 4.419 (t, 2H, 2 × $NHCH_2$-CH_2O*H*, disappeared after D_2O, J = 5.5 Hz), 3.243 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.927(br s, 4H, 2 × NHCH_2CH_2OH). Anal. ($C_{38}H_{40}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diaminopyrimido[5,4-*d*]pyrimidine (39). Compound **39** was prepared by general procedure I with ammonia solution (7 N in methanol, 0.6 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 7/1) to give a yellow powdery solid (63 mg, 17%). Mp 225−226 °C; MS (ESI) *m/z* 369 $(M + H)^+$, 391 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.194 (br s, 2H, 2 × NH_AH_B, disappeared after D_2O), 6.663 (br s, 2H, 2 × NH_AH_B, disappeared after D_2O), 4.675 (t, 4H, 4 × OH, disappeared after D_2O, J = 4.5 Hz), 3.625 (t, 8H, 2 × N($CH_2CH_2OH)_2$, J = 5 Hz), 3.584 (t, 8H, 2 × N(CH_2CH_2-$OH)_2$, J_1 = 4.5 Hz, J_2 = 5 Hz). Anal. Calcd for $C_{14}H_{24}N_8O_4$: C 45.64, H 6.57, N 30.42. Found: C 45.29, H 6.73, N 29.58.

2,6-Bis(diethanolamino)-4,8-dimethylaminopyrimido[5,4-*d*]-pyrimidine (40). Compound **40** was prepared by general procedure I with methylamine solution (2 M in tetrahydrofuran, 2.1 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 7/1) to give a yellow powdery solid (190 mg, 48%). Mp 213−214 °C; MS (ESI) *m/z* 397 $(M + H)^+$, 419 $(M + Na)^+$, 435 $(M + K)^+$; ^{1}H NMR (DMSO-d_6) δ 7.154 (q, 2H, 2 × N*H*CH_3, disappeared after D_2O, J = 4.5 Hz), 4.675 (br s, 4H, 4 × OH, disappeared after D_2O), 3.676 (t, 8H, 2 × N($CH_2CH_2OH)_2$), 3.619 (t, 8H, 2 × N($CH_2CH_2OH)_2$), 2.949 (d, 6H, 2 × NHCH_3, J = 4.5 Hz). Anal. ($C_{16}H_{28}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dimethylaminopyrimido[5,4-*d*]pyrimidine (41). Compound **41** was prepared by general procedure I with methylamine solution (2 M in tetrahydrofuran, 2.1 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 15/1) to give a yellow powdery solid (176 mg, 57%). Mp 212 °C; MS (ESI) *m/z* 309 $(M + H)^+$, 331 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.120 (q, 2H, 2 × N*H*CH_3, disappeared after D_2O, J = 5 Hz), 5.947 (t, 2H, 2 × N*H*CH_2CH_2-OH, disappeared after D_2O, J = 6 Hz), 4.610 (t, 2H, 2 × $NHCH_2$-CH_2O*H*, disappeared after D_2O, J = 5.5 Hz), 3.530 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.402 (q, 4H, 2 ×

NHCH_2CH$_2$OH, J_1 = 6 Hz, J_2 = 6 Hz), 2.920 (d, 6H, 2 × NHCH_3, J = 5 Hz). Anal. ($C_{12}H_{20}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diethylaminopyrimido[5,4-*d*]pyrimidine (42). Compound **42** was prepared by general procedure I with ethylamine solution (2 M in tetrahydrofuran, 2.1 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 10/1) to give a yellow powdery solid (174 mg, 41%). Mp 188−189 °C; MS (ESI) *m*/*z* 425 (M + H)$^+$, 447 (M + Na)$^+$, 463 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.151 (br s, 2H, 2 × N*H*CH$_2$CH$_3$, disappeared after D$_2$O), 4.690 (s, 4H, 4 × OH, disappeared after D$_2$O), 3.668 (br s, 8H, 2 × N(CH_2CH$_2$OH)$_2$), 3.622 (br s, 8H, 2 × N(CH$_2$CH_2OH)$_2$), 3.469 (br s, 4H, 2 × CH_2CH$_3$), 1.190 (t, 6H, 2 × CH$_2$CH_3). Anal. ($C_{18}H_{32}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diethylaminopyrimido[5,4-*d*]pyrimidine (43). Compound **43** was prepared by general procedure I with ethylamine solution (2 M in tetrahydrofuran, 2.1 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 24/1) to give a yellow powdery solid (205 mg, 61%). Mp 175−176 °C; MS (ESI) *m*/*z* 337 (M + H)$^+$, 357 (M + Na)$^+$, 375 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.057 (br s, 2H, 2 × N*H*CH$_2$CH$_3$, disappeared after D$_2$O), 5.941 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 6 Hz), 4.621 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O, J = 5.5 Hz), 3.532 (q, 4H, 2 × NHCH$_2$CH_2OH, J = 5.5 Hz), 3.442 (m, 4H, 2 × CH_2CH$_3$, J = 6 Hz), 3.392 (q, 4H, 2 × NHCH_2CH$_2$OH, J = 6 Hz), 1.174 (t, 6H, 2 × CH$_2$CH_3, J = 6 Hz). Anal. ($C_{14}H_{24}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dipropylaminopyrimido[5,4-*d*]pyrimidine (44). Compound **44** was prepared by general procedure I with propylamine (0.35 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 8/1) to give a yellow powdery solid (217 mg, 48%). Mp 145−146 °C; MS (ESI) *m*/*z* 453 (M + H)$^+$, 475 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.154 (t, 2H, 2 × N*H*CH$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 4.701 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.670 (br d, 8H, 2 × N(CH_2CH$_2$OH)$_2$), 3.631 (t, 8H, 2 × N(CH$_2$CH_2OH)$_2$), 3.400 (m, 4H, 2 × CH_2CH$_2$CH$_3$), 1.607 (m, 4H, 2 × CH$_2$CH_2CH$_3$, J = 7.5 Hz), 0.910 (t, 6H, 2 × CH$_2$CH$_2$CH_3, J = 7.5 Hz). Anal. ($C_{20}H_{36}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-dipropylaminopyrimido[5,4-*d*]pyrimidine (45). Compound **45** was prepared by general procedure I with propylamine (0.35 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 10/1) to give a yellow powdery solid (196 mg, 54%). Mp 147−148 °C; MS (ESI) *m*/*z* 365 (M + H)$^+$, 387 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.055 (br s, 2H, 2 × N*H*CH$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 5.957 (br s, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O), 4.636 (br s, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.544 (br s, 4H, 2 × NHCH$_2$CH_2OH), 3.376 (br s, 8H, 2 × NHCH_2-CH$_2$OH, 2 × CH_2CH$_2$CH$_3$), 1.591 (m, 4H, 2 × CH$_2$CH_2CH$_3$, J = 7.5 Hz), 0.901 (t, 6H, 2 × CH$_2$CH$_2$CH_3, J = 7.5 Hz). Anal. ($C_{16}H_{28}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diisopropylaminopyrimido[5,4-*d*]pyrimidine (46). Compound **46** was prepared by general procedure I with isopropylamine (0.36 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 8/1) to give a yellow powdery solid (201 mg, 44%). Mp 188−190 °C; MS (ESI) *m*/*z* 453 (M + H)$^+$, 475 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.547 (d, 2H, 2 × N*H*CH(CH$_3$)$_2$, disappeared after D$_2$O), 4.706 (br s, 4H, 4 × OH, disappeared after D$_2$O), 4.218 (m, 2H, 2 × NHC*H*(CH$_3$)$_2$), 3.662 (t, 8H, 2 × N(CH_2CH$_2$OH)$_2$), 3.620 (br s, 8H, 2 × N(CH$_2$CH_2OH)$_2$), 1.264 (d, 12H, 2 × NHCH-(CH_3)$_2$). Anal. ($C_{20}H_{36}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diisopropylaminopyrimido[5,4-*d*]pyrimidine (47). Compound **47** was prepared by general procedure I with isopropylamine (0.36 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow powdery solid (165 mg, 45%). Mp 167 °C; MS (ESI) *m*/*z* 365 (M + H)$^+$, 387 (M + Na)$^+$, 403 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.590 (d, 2H, 2 × N*H*CH(CH$_3$)$_2$, disappeared after D$_2$O), 6.008 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 6 Hz), 4.646 (br s, 2H, 2 × NHCH$_2$-CH$_2$O*H*, disappeared after D$_2$O), 4.241 (m, 2H, 2 × NHC*H*(CH$_3$)$_2$, J = 6.5 Hz), 3.535 (br s, 4H, 2 × NHCH$_2$CH_2OH), 3.382 (m, 4H, 2 × NHCH_2CH$_2$OH, J = 6 Hz), 1.235 (d, 12H, 2 × NHCH(CH_3)$_2$ J = 6.5 Hz). Anal. ($C_{16}H_{28}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dibutylaminopyrimido[5,4-*d*]pyrimidine (48). Compound **48** was prepared by general procedure I with butylamine (0.42 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 10/1) to give a yellow powdery solid (82 mg, 17%). Mp 117−118 °C; MS (ESI) *m*/*z* 481 (M + H)$^+$, 503 (M + Na)$^+$, 519 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.135 (br s, 2H, 2 × N*H*CH$_2$CH$_2$-CH$_2$CH$_3$, disappeared after D$_2$O), 4.693 (br s, 4H, 4 × OH, disappeared after D$_2$O), 3.658 (br s, 8H, 2 × N(CH_2CH$_2$OH)$_2$), 3.617 (br s, 8H, 2 × N(CH$_2$CH_2OH)$_2$), 3.431 (br s, 4H, 2 × NHCH_2-CH$_2$CH$_2$CH$_3$), 1.580 (m, 4H, 2 × NHCH$_2$CH_2CH$_2$CH$_3$, J = 7 Hz), 1.350 (m, 4H, 2 × NHCH$_2$CH$_2$CH_2CH$_3$, J_1 = 7 Hz, J_2 = 7.5 Hz), 0.919 (t, 6H, 2 × NHCH$_2$CH$_2$CH$_2$CH_3, J = 7.5 Hz). Anal. Calcd for $C_{22}H_{40}N_8O_4$·0.5H$_2$O: C 53.86, H 8.63, N 22.84. Found: C 54.32, H 8.34, N 22.72.

2,6-Diethanolamino-4,8-dibutylaminopyrimido[5,4-*d*]pyrimidine (49). Compound **49** was prepared by general procedure I with butylamine (0.42 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow powdery solid (70 mg, 18%). Mp 127−128 °C; MS (ESI) *m*/*z* 393 (M + H)$^+$, 415 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.018 (br s, 2H, 2 × N*H*CH$_2$CH$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 5.938 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 5.5 Hz), 4.618 (br s, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.529 (m, 4H, 2 × NHCH$_2$CH_2OH), 3.404 (m, 4H, 2 × NHCH_2CH$_2$CH$_2$CH$_3$, J = 7 Hz), 3.380 (m, 4H, 2 × NHCH_2CH$_2$OH, J = 5.5 Hz), 1.556 (m, 4H, 2 × NHCH$_2$CH_2CH$_2$-CH$_3$, J_1 = 7 Hz, J_2 = 7.5 Hz), 1.345 (m, 4H, 2 × NHCH$_2$CH$_2$CH_2-CH$_3$, J_1 = 7.5 Hz, J_2 = 7 Hz), 0.921 (t, 6H, 2 × NHCH$_2$CH$_2$-CH$_2$CH_3, J = 7 Hz). Anal. ($C_{18}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diisobutylaminopyrimido[5,4-*d*]pyrimidine (50). Compound **50** was prepared by general procedure I with isobutylamine (0.36 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 9/1) to give a yellow powdery solid (58 mg, 12%). Mp 162−163 °C; MS (ESI) *m*/*z* 481 (M + H)$^+$, 503 (M + Na)$^+$, 519 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.145 (br s, 2H, 2 × N*H*CH$_2$CH(CH$_3$)$_2$, disappeared after D$_2$O), 4.703 (br s, 4H, 4 × OH, disappeared after D$_2$O), 3.649 (d, 8H, 2 × N(CH_2CH$_2$OH)$_2$), 3.619 (d, 8H, 2 × N(CH$_2$CH_2OH)$_2$), 3.278 (t, 4H, 2 × NHCH_2CH(CH$_3$)$_2$, J = 6.5 Hz), 1.957 (m, 2H, 2 × NHCH$_2$C*H*(CH$_3$)$_2$, J_1 = 6.5 Hz, J_2 = 7 Hz), 0.917 (d, 12H, 2 × NHCH$_2$CH(CH_3)$_2$, J = 7 Hz). Anal. ($C_{22}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diisobutylaminopyrimido[5,4-*d*]pyrimidine (51). Compound **51** was prepared by general procedure I with isobutylamine (0.36 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 12/1) to give a yellow powdery solid (78 mg, 20%). Mp 141−142 °C; MS (ESI) *m*/*z* 393 (M + H)$^+$, 415 (M + Na)$^+$, 431 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.019 (t, 2H, 2 × N*H*CH$_2$CH(CH$_3$)$_2$, disappeared after D$_2$O), 5.980 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 5.5 Hz), 4.631 (t, 2H, 2 × NHCH$_2$-CH$_2$O*H*, disappeared after D$_2$O, J = 5.5 Hz), 3.537 (q, 4H, 2 × NHCH$_2$CH_2OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.377 (q, 4H, 2 × NHCH_2CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 3.255 (t, 4H, 2 × NHCH_2-CH(CH$_3$)$_2$, J = 6.5 Hz), 1.954 (m, 2H, 2 × NHCH$_2$C*H*(CH$_3$)$_2$, J_1

= 6.5 Hz, J_2 = 7 Hz), 0.914 (d, 12H, 2 × NHCH$_2$CH(C*H*$_3$)$_2$, J = 7 Hz). Anal. Calcd for $C_{18}H_{32}N_8O_2$: C 55.08, H 8.22, N 28.55. Found: C 54.46, H 8.15, N 28.22.

2,6-Bis(diethanolamino)-4,8-di(*tert*-butylamino)pyrimido[5,4-*d*]pyrimidine (52). Compound **52** was prepared by general procedure I with *tert*-butylamine (0.44 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 6/1) to give a yellow powdery solid (173 mg, 36%). Mp 244−245 °C; MS (ESI) *m*/*z* 481 (M + H)$^+$, 503 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.475 (s, 2H, 2 × N*H*C(CH$_3$)$_3$, disappeared after D$_2$O), 4.748 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.643 (br s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 1.474 (s, 18H, 2 × NHC(C*H*$_3$)$_3$). Anal. ($C_{22}H_{40}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(*tert*-butylamino)pyrimido[5,4-*d*]-pyrimidine (53). Compound **53** was prepared by general procedure I with *tert*-butylamine (0.44 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 9/1) to give a yellow powdery solid (190 mg, 48%). Mp 154 °C; MS (ESI) *m*/*z* 393 (M + H)$^+$, 415 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.456 (s, 2H, 2 × N*H*C(CH$_3$)$_3$, disappeared after D$_2$O), 6.135 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 6 Hz), 4.652 (br s, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.536 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J = 5.5 Hz), 3.338 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.477 (s, 18H, 2 × NHC(C*H*$_3$)$_3$). Anal. ($C_{18}H_{32}N_8O_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diamylaminopyrimido[5,4-*d*]pyrimidine (54). Compound **54** was prepared by general procedure I with amylamine (0.49 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 12/1) to give a yellow powdery solid (190 mg, 37%). Mp 128 °C; MS (ESI) *m*/*z* 509 (M + H)$^+$, 531 (M + Na)$^+$, 547 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.139 (br s, 2H, 2 × N*H*CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$, disappeared after D$_2$O), 4.690 (br s, 4H, 4 × OH, disappeared after D$_2$O), 3.662 (br s, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.624 (d, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 3.423 (br s, 4H, 2 × NHC*H*$_2$CH$_2$(CH$_2$)$_2$CH$_3$), 1.598 (m, 4H, 2 × NHCH$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), 1.320 (m, 8H, 2 × NHCH$_2$CH$_2$(C*H*$_2$)$_2$CH$_3$), 0.882 (t, 6H, 2 × NHCH$_2$CH$_2$(CH$_2$)$_2$C*H*$_3$). Anal. ($C_{24}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-diamylaminopyrimido[5,4-*d*]pyrimidine (55). Compound **55** was prepared by general procedure I with amylamine (0.49 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 17/1) to give a yellow powdery solid (155 mg, 37%). Mp 147−148 °C; MS (ESI) *m*/*z* 421 (M + H)$^+$, 443 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.032 (t, 2H, 2 × N*H*CH$_2$CH$_2$(CH$_2$)$_2$CH$_3$, disappeared after D$_2$O), 5.931 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O), 4.620 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.530 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH), 3.382 (m, 8H, 2 × NHC*H*$_2$-CH$_2$(CH$_2$)$_2$CH$_3$, 2 × NHC*H*$_2$CH$_2$OH), 1.582 (m, 4H, 2 × NHCH$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), 1.309 (m, 8H, 2 × NHCH$_2$CH$_2$(C*H*$_2$)$_2$-CH$_3$), 0.882 (t, 6H, 2 × NHCH$_2$CH$_2$(CH$_2$)$_2$C*H*$_3$). Anal. ($C_{20}H_{36}N_8O_2$·0.5H$_2$O) C, H, N.

2,6-Bis(diethanolamino)-4,8-diisopentylaminopyrimido[5,4-*d*]-pyrimidine (56). Compound **56** was prepared by general procedure I with isopentylamine (0.49 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 22/1) to give a yellow powdery solid (346 mg, 68%). Mp 124−125 °C; MS (ESI) *m*/*z* 509 (M + H)$^+$, 531 (M + Na)$^+$, 547 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.131 (t, 2H, 2 × N*H*CH$_2$CH$_2$CH-(CH$_3$)$_2$, disappeared after D$_2$O), 4.679 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.666 (t, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.621 (d, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 3.454 (q, 4H, 2 × NHC*H*$_2$CH$_2$CH(CH$_3$)$_2$), 1.621 (m, 2H, 2 × NHCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.505 (m, 4H, 2 × NHCH$_2$C*H*$_2$-CH(CH$_3$)$_2$), 0.936 (d, 12H, 2 × NHCH$_2$CH$_2$CH(C*H*$_3$)$_2$). Anal. ($C_{24}H_{44}N_8O_4$·0.5H$_2$O) C, H, N.

2,6-Diethanolamino-4,8-diisopentylaminopyrimido[5,4-*d*]pyrimidine (57). Compound **57** was prepared by general procedure I with isopentylamine (0.49 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 30/1) to give a yellow powdery solid (235 mg, 56%). Mp 132−133 °C; MS (ESI) *m*/*z* 421 (M + H)$^+$, 443 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.999 (br s, 2H, 2 × N*H*CH$_2$CH$_2$CH(CH$_3$)$_2$, disappeared after D$_2$O), 5922 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O), 4.621 (br s, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.538 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH), 3.422 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH), 3.384 (m, 4H, 2 × NHC*H*$_2$CH$_2$CH(CH$_3$)$_2$), 1.614 (m, 2H, 2 × NHCH$_2$CH$_2$C*H*(CH$_3$)$_2$), 1.490 (m, 4H, 2 × NHCH$_2$C*H*$_2$-CH(CH$_3$)$_2$), 0.931 (d, 12H, 2 × NHCH$_2$CH$_2$CH(C*H*$_3$)$_2$). Anal. ($C_{20}H_{36}N_8O_2$·H$_2$O) C, H, N.

2,6-Bis(diethanolamino)-4,8-di(*tert*-amylamino)pyrimido[5,4-*d*]pyrimidine (58). Compound **58** was prepared by general procedure I with *tert*-amylamine (0.49 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow powdery solid (245 mg, 48%). Mp 212−213 °C; MS (ESI) *m*/*z* 509 (M + H)$^+$, 531 (M + Na)$^+$, 547 (M + K)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.432 (s, 2H, 2 × N*H*C-(CH$_3$)$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 4.757 (d, 4H, 4 × OH, disappeared after D$_2$O), 3.637 (s, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 1.841 (q, 4H, 2 × NHC(CH$_3$)$_2$C*H*$_2$CH$_3$, J = 7.5 Hz), 1.415 (s, 12H, 2 × NHC(C*H*$_3$)$_2$CH$_2$CH$_3$), 0.833 (t, 6H, 2 × NHC(CH$_3$)$_2$CH$_2$C*H*$_3$, J = 7.5 Hz). Anal. ($C_{24}H_{44}N_8O_4$) C, H, N.

2,6-Diethanolamino-4,8-di(*tert*-amylamino)pyrimido[5,4-*d*]pyrimidine (59). Compound **59** was prepared by general procedure I with *tert*-amylamine (0.49 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 18/1) to give a yellow powdery solid (196 mg, 47%). Mp 167−168 °C; MS (ESI) *m*/*z* 421 (M + H)$^+$, 443 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.391 (s, 2H, 2 × N*H*C(CH$_3$)$_2$CH$_2$CH$_3$, disappeared after D$_2$O), 6.143 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 6 Hz), 4.653 (br s, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.552 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J = 5.5 Hz), 3.328 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, J_1 = 5.5 Hz, J_2 = 6 Hz), 1.881 (q, 4H, 2 × NHC(CH$_3$)$_2$C*H*$_2$CH$_3$, J = 7.5 Hz), 1.414 (s, 12H, 2 × NHC(C*H*$_3$)$_2$CH$_2$CH$_3$), 0.814 (t, 6H, 2 × NHC(CH$_3$)$_2$CH$_2$C*H*$_3$, J = 7.5 Hz). Anal. Calcd for $C_{20}H_{36}N_8O_2$·0.5H$_2$O: C 55.92, H 8.68, N 26.09. Found: C 56.02, H 8.67, N 25.62.

2,6-Bis(diethanolamino)-4,8-dicyclopropylaminopyrimido[5,4-*d*]pyrimidine (60). Compound **60** was prepared by general procedure I with cyclopropylamine (0.31 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 14/1) to give a yellow powdery solid (220 mg, 49%). Mp 225 °C; MS (ESI) *m*/*z* 449 (M + H)$^+$, 471 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 7.060 (d, 2H, 2 × N*H*CH(CH$_2$)$_2$, disappeared after D$_2$O, J = 3.5 Hz), 4.688 (s, 4H, 4 × OH, disappeared after D$_2$O), 3.681 (t, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.627 (d, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 2.796 (m, 2H, 2 × NHC*H*(CH$_2$)$_2$), 0.783 (m, 4H, 2 × NHCH(CH$_2$)$_2$−H$_{2a,3a}$), 0.622 (m, 4H, 2 × NHCH(CH$_2$)$_2$−H$_{2e,3e}$). Anal. ($C_{20}H_{32}N_8O_4$·0.25H$_2$O) C, H, N.

2,6-Diethanolamino-4,8-dicyclopropylaminopyrimido[5,4-*d*]-pyrimidine (61). Compound **61** was prepared by general procedure I with cyclopropylamine (0.31 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 17/1) to give a yellow powdery solid (156 mg, 43%). Mp 199 °C; MS (ESI) *m*/*z* 361 (M + H)$^+$, 383 (M + Na)$^+$; ^{1}H NMR (DMSO-d_6) δ 6.983 (d, 2H, 2 × N*H*CH(CH$_2$)$_2$, disappeared after D$_2$O, J = 3.5 Hz), 5.003 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, J = 6 Hz), 4.633 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O, J_1 = 5 Hz, J_2 = 5.5 Hz), 3.525 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.396 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, J = 6 Hz), 2.869 (m, 2H, 2 × NHC*H*(CH$_2$)$_2$),

0.748 (m, 4H, 2 × NHCH(CH$_2$)$_2$−H$_{2a,3a}$), 0.633 (m, 4H, 2 × NHCH(CH$_2$)$_2$−H$_{2e,3e}$). Anal. (C$_{16}$H$_{24}$N$_8$O$_2$·0.25H$_2$O) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclobutylaminopyrimido[5,4-*d*]pyrimidine (62). Compound **62** was prepared by general procedure I with cyclobutylamine (0.37 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 16/1) to give a yellow powdery solid (212 mg, 45%). Mp 222−223 °C; MS (ESI) *m*/*z* 477 (M + H)$^+$, 499 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 6.992 (br s, 2H, 2 × N*H*CH(CH$_2$)$_2$CH$_2$, disappeared after D$_2$O), 4.701 (s, 4H, 4 × OH, disappeared after D$_2$O), 4.467 (br s, 2H, 2 × NHC*H*(CH$_2$)$_2$CH$_2$), 3.615 (br d, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$, 2.295 (m, 4H, 2 × NHCH(CH$_2$)$_2$CH$_2$−H$_{2a,4a}$), 2.196 (m, 4H, 2 × NHCH(CH$_2$)$_2$CH$_2$−H$_{2e,4e}$), 1.729 (m, 4H, 2 × NHCH(CH$_2$)$_2$C*H*$_2$). Anal. (C$_{22}$H$_{36}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-dicyclobutylaminopyrimido[5,4-*d*]pyrimidine (63). Compound **63** was prepared by general procedure I with cyclobutylamine (0.37 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 19/1) to give a yellow powdery solid (183 mg, 47%). Mp 189−190 °C; MS (ESI) *m*/*z* 389 (M + H)$^+$, 411 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 7.052 (d, 2H, 2 × N*H*CH(CH$_2$)$_2$CH$_2$, disappeared after D$_2$O, *J* = 8 Hz), 5.971 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, *J* = 5.5 Hz), 4.645 (t, 2H, 2 × NHCH$_2$-CH$_2$O*H*, disappeared after D$_2$O, *J*$_1$ = 4.5 Hz, *J*$_2$ = 5.5 Hz), 4.555 (m, 2H, 2 × NHC*H*(CH$_2$)$_2$CH$_2$, *J*$_1$ = 8 Hz, *J*$_2$ = 8.5 Hz), 3.533 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, *J*$_1$ = 6 Hz, *J*$_2$ = 5.5 Hz), 3.405 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, *J*$_1$ = 5.5 Hz, *J*$_2$ = 6 Hz), 2.272 (m, 4H, 2 × NHCH(CH$_2$)$_2$CH$_2$−H$_{2a,4a}$), 2.097 (m, 4H, 2 × NHCH(CH$_2$)$_2$CH$_2$−H$_{2e,4e}$), 1.675 (m, 4H, 2 × NHCH(CH$_2$)$_2$C*H*$_2$). Anal. (C$_{18}$H$_{28}$N$_8$O$_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclopentylaminopyrimido[5,4-*d*]pyrimidine (64). Compound **64** was prepared by general procedure I with cyclopentylamine (0.42 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 10/1) to give a yellow powdery solid (97 mg, 19%). Mp 211−212 °C; MS (ESI) *m*/*z* 505 (M + H)$^+$, 527 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 6.619 (d, 2H, 2 × N*H*CH(CH$_2$CH$_2$)$_2$, disappeared after D$_2$O), 4.704 (br s, 4H, 4 × OH, disappeared after D$_2$O), 4.296 (m, 2H, 2 × NHC*H*(CH$_2$CH$_2$)$_2$), 3.657 (t, 8H, 2 × N(C*H*$_2$-CH$_2$OH)$_2$), 3.615 (d, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$), 2.016 (m, 4H, 2 × NHCH(CH$_{ax}$*H*$_{eq}$CH$_2$)$_2$), 1.720 (m, 4H, 2 × NHCH(CH$_2$-CH$_{ax}$*H*$_{eq}$)$_2$), 1.587 (m, 8H, 2 × NHCH(CH$_2$C*H*$_{ax}$H$_{eq}$)$_2$, 2 × NHCH-(C*H*$_{ax}$H$_{eq}$CH$_2$)$_2$). Anal. (C$_{24}$H$_{40}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-dicyclopentylaminopyrimido[5,4-*d*]pyrimidine (65). Compound **65** was prepared by general procedure I with cyclopentylamine (0.42 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 18/1) to give a yellow powdery solid (175 mg, 42%). Mp 203−204 °C; MS (ESI) *m*/*z* 417 (M + H)$^+$, 439 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 6.678 (d, 2H, 2 × N*H*CH(CH$_2$CH$_2$)$_2$, disappeared after D$_2$O), 6.028 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O, *J* = 6 Hz), 4.651 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O, *J* = 5 Hz), 4.337 (q, 2H, 2 × NHC*H*(CH$_2$CH$_2$)$_2$), 3.534 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, *J* = 5 Hz), 3.380 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, *J* = 6 Hz), 1.998 (m, 4H, 2 × NHCH(CH$_{ax}$*H*$_{eq}$-CH$_2$)$_2$), 1.708 (m, 4H, 2 × NHCH(CH$_2$CH$_{ax}$*H*$_{eq}$)$_2$), 1.559 (m, 8H, 2 × NHCH(CH$_2$C*H*$_{ax}$H$_{eq}$)$_2$, 2 × NHCH(C*H*$_{ax}$H$_{eq}$CH$_2$)$_2$). Anal. (C$_{20}$H$_{32}$N$_8$O$_2$·0.5H$_2$O) C, H, N.

2,6-Bis(diethanolamino)-4,8-dicyclohexylaminopyrimido[5,4-*d*]pyrimidine (66). Compound **66** was prepared by general procedure I with cyclohexylamine (0.48 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 12/1) to give a yellow powdery solid (320 mg, 60%). Mp 198−199 °C; MS (ESI) *m*/*z* 533 (M + H)$^+$, 555 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 6.586 (d, 2H, 2 × N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$, disappeared after D$_2$O), 4.708 (t, 4H, 4 × OH, disappeared after D$_2$O), 3.891 (m, 2H, 2 × NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 3.637 (m, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$), 1.916 (m, 4H, 2 × NHCH(CH$_{ax}$*H*$_{eq}$CH$_2$)$_2$-CH$_2$), 1.729 (m, 4H, 2 × NHCH(CH$_2$CH$_{ax}$*H*$_{eq}$)$_2$CH$_2$), 1.593 (m, 2H, 2 × NHCH(CH$_2$CH$_2$)$_2$CH$_{ax}$*H*$_{eq}$), 1.442−1.330 (m, 8H, 2 × NHCH(CH$_2$C*H*$_{ax}$H$_{eq}$)$_2$CH$_2$, 2 × NHCH(C*H*$_{ax}$H$_{eq}$CH$_2$)$_2$CH$_2$), 1.248 (m, 2H, 2 × NHCH(CH$_2$CH$_2$)$_2$C*H*$_{ax}$H$_{eq}$). Anal. (C$_{26}$H$_{44}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-dicyclohexylaminopyrimido[5,4-*d*]pyrimidine (67). Compound **67** was prepared by general procedure I with cyclohexylamine (0.48 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 15/1) to give a yellow powdery solid (44 mg, 10%). Mp 163 °C; MS (ESI) *m*/*z* 445 (M + H)$^+$, 467 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 6.589 (d, 2H, 2 × N*H*CH(CH$_2$CH$_2$)$_2$CH$_2$, disappeared after D$_2$O), 6.033 (t, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O), 4.632 (t, 2H, 2 × NHCH$_2$CH$_2$O*H*, disappeared after D$_2$O), 3.915 (q, 2H, 2 × NHC*H*(CH$_2$CH$_2$)$_2$CH$_2$), 3.525 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH), 3.355 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH), 1.901 (m, 4H, 2 × NHCH(CH$_{ax}$*H*$_{eq}$CH$_2$)$_2$CH$_2$), 1.735 (m, 4H, 2 × NHCH-(CH$_2$CH$_{ax}$*H*$_{eq}$)$_2$CH$_2$), 1.600 (m, 2H, 2 × NHCH(CH$_2$CH$_2$)$_2$CH$_{ax}$*H*$_{eq}$), 1.399−1.304 (m, 8H, 2 × NHCH(CH$_2$C*H*$_{ax}$H$_{eq}$)$_2$CH$_2$, 2 × NHCH-(C*H*$_{ax}$H$_{eq}$CH$_2$)$_2$CH$_2$), 1.225 (m, 2H, 2 × NHCH(CH$_2$CH$_2$)$_2$C*H*$_{ax}$H$_{eq}$). Anal. (C$_{22}$H$_{36}$N$_8$O$_2$) C, H, N.

2,6-Bis(diethanolamino)-4,8-diphenylaminopyrimido[5,4-*d*]pyrimidine (68). Compound **68** was prepared by general procedure I with aniline (0.39 mL, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 14/1) to give a yellow powdery solid (186 mg, 36%). Mp 209−210 °C; MS (ESI) *m*/*z* 519 (M − H)$^-$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 8.918 (s, 2H, 2 × N*H*Ar, disappeared after D$_2$O), 7.916 (d, 4H, 2 × Ar−H-2, 2 × Ar−H-6, *J* = 8 Hz), 7.404 (t, 4H, 2 × Ar−H-3, 2 × Ar−H-5, *J* = 8 Hz), 7.105 (t, 2H, 2 × Ar−H-4), 4.753 (t, 4H, 4 × OH, disappeared after D$_2$O, *J* = 5.5 Hz), 3.789 (br s, 8H, 2 × N(C*H*$_2$CH$_2$OH)$_2$), 3.689 (q, 8H, 2 × N(CH$_2$C*H*$_2$OH)$_2$, *J* = 5.5 Hz). Anal. (C$_{26}$H$_{32}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-diphenylaminopyrimido[5,4-*d*]pyrimidine (69). Compound **69** was prepared by general procedure I with aniline (0.39 mL, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 16/1) to give a yellow powdery solid compound **4** (138 mg, 32%). Mp 233−234 °C; MS (ESI) *m*/*z* 433 (M + H)$^+$, *m*/*z* 455 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 8.937 (s, 2H, 2 × N*H*Ar, disappeared after D$_2$O), 8.010 (d, 4H, 2 × Ar−H-2, 2 × Ar−H-6, *J* = 8 Hz), 7.388 (t, 4H, 2 × Ar−H-3, 2 × Ar−H-5, *J*$_1$ = 7.5 Hz, *J*$_2$ = 8.5 Hz), 7.092 (t, 2H, 2 × Ar−H-4, *J*$_1$ = 7.5 Hz, *J*$_2$ = 7 Hz), 6.564 (br s, 2H, 2 × N*H*CH$_2$CH$_2$OH, disappeared after D$_2$O), 4.694 (t, 2H, 2 × OH, disappeared after D$_2$O, *J* = 5.5 Hz), 3.613 (q, 4H, 2 × NHC*H*$_2$CH$_2$OH, *J*$_1$ = 6 Hz, *J*$_2$ = 5.5 Hz), 3.526 (q, 4H, 2 × NHCH$_2$C*H*$_2$OH, *J*$_1$ = 5.5 Hz, *J*$_2$ = 6 Hz). Anal. Calcd for C$_{22}$H$_{24}$N$_8$O$_2$·0.5H$_2$O): C 59.85, H 5.71, N 25.38. Found; C 60.31, H 5.69, N 25.23.

2,6-Bis(diethanolamino)-4,8-bis(dibenzylamino)pyrimido[5,4-*d*]pyrimidine (70). Compound **70** was prepared by general procedure I with dibenzylamine (0.83 g, 4.2 mmol) at the first step and with diethanolamine (3 mL, 30 mmol) at the second step. Product was purified by flash silica gel chromatography (CH$_2$Cl$_2$/MeOH = 16/1) to give a yellow powdery solid compound **9** (332 mg, 46%). Mp 199 °C; MS (ESI) *m*/*z* 729 (M + H)$^+$, 751 (M + Na)$^+$; ^{1}H NMR (DMSO-*d*$_6$) *δ* 7.324 (t, 8H, 4 × Ar−H-3, 4 × Ar−H-5, *J*$_1$ = 7.5 Hz, *J*$_2$ = 7 Hz), 7.261−7.224 (m, 12H, 4 × Ar−H-2, 4 × Ar−H-6, 4 × Ar−H-4), 5.317 (br s, 8H, 4 × C*H*$_2$-Ph), 4.564 (t, 4H, 4 × OH, disappeared after D$_2$O, *J* = 5 Hz), 3.259 (br d, 16H, 2 × N(C*H*$_2$C*H*$_2$OH)$_2$). Anal. (C$_{42}$H$_{48}$N$_8$O$_4$) C, H, N.

2,6-Diethanolamino-4,8-bis(dibenzylamino)pyrimido[5,4-*d*]pyrimidine (71). Compound **71** was prepared by general procedure I with dibenzylamine (0.83 g, 4.2 mmol) at the first step and with ethanolamine (3 mL, 50 mmol) at the second step. Product was

purified by flash silica gel chromatography (CH_2Cl_2/MeOH = 20/1) to give a yellow powdery solid compound **8** (125 mg, 20%). Mp 215−216 °C; MS (ESI) *m*/*z* 641 $(M + H)^+$, 663 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 7.327 (t, 8H, 4 × Ar−H-3, 4 × Ar−H-5, J_1 = 7 Hz, J_2 = 7.5 Hz), 7.289 (d, 8H, 4 × Ar−H-2, 4 × Ar−H-6, J = 7 Hz), 7.247 (t, 4H, 4 × Ar−H-4, J_1 = 7.5 Hz, J_2 = 7 Hz), 5.970 (t, 2H, 2 × N*H*CH_2CH_2OH, disappeared after D_2O, J = 5.5 Hz), 4.279 (br s, 8H, 4 × C*H*$_2$Ph), 4.419 (t, 2H, 2 × $NHCH_2$-CH_2O*H*, disappeared after D_2O, J = 5.5 Hz), 3.243 (q, 4H, 2 × $NHCH_2CH_2OH$, J_1 = 6 Hz, J_2 = 5.5 Hz), 3.927(br s, 4H, 2 × $NHCH_2CH_2OH$). Anal. ($C_{38}H_{40}N_8O_2$) C, H, N.

2,6-Di-(2′-hydroxyethoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (72). Compound **72** was prepared according to a literature procedure.[43]

2,6-Dimorpholino-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (73). Compound **73** was prepared by general procedure I with piperidine (0.41 mL, 4.2 mmol) at the first step and with morpholine (3 mL, 34 mmol) at the second step. Product was purified by flash silica gel chromatography (hexane/acetone = 10/1) to give a yellow powdery solid (173 mg, 37%). Mp 203−204 °C; MS (ESI) *m*/*z* 469 $(M + H)^+$, 491 $(M + Na)^+$; ^{1}H NMR (DMSO-d_6) δ 4.076 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.663 (t, 8H, 2 × $N(CH_2CH_2)O$, J = 5 Hz), 3.542 (t, 8H, 2 × $N(CH_2CH_2)O$, J = 5 Hz), 1.664 (br d, 4H, 2 × $N(CH_2CH_2)_2CH_2$, J = 4.5 Hz), 1.608 (br d, 8H, 2 × $N(CH_2CH_2)_2CH_2$, J = 4.5 Hz). Anal. ($C_{24}H_{36}N_8O_2$) C, H, N.

2,6-Bis[*N*,*N*-di-(2′-formyloxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (74). Dipyridamole (0.51 g, 1 mmol) was dissolved in formic acid (10 mL, 0.25 mole). The mixture was stirred at reflux for 6 h, and then the solvent was evaporated under reduced pressure. The residue was dissolved in methylene chloride (50 mL) and washed with 10% $NaHCO_3$ solution, and then the organic layer was separated and dried by anhydrous Na_2SO_4. After the solvent was removed, the residue was subjected to flash silica gel chromatography (hexane/acetone = 6:1) to give a yellow fluorescent powdery compound **74** (0.567 g, 92%). Mp 129−130 °C (lit[25] 128−130 °C); MS (ESI) *m*/*z* 639 $(M + Na)^+$, 617 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 8.225 (s, 4H, 4 × CHO), 4.289 (t, 8H, 2 × $N(CH_2CH_2OCHO)_2$, J = 5.5 Hz), 4.052 (m, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.784 (t, 8H, 2 × $N(CH_2CH_2OCHO)_2$, J = 5.5 Hz), 1.646 (m, 4H, 2 × $N(CH_2CH_2)_2CH_2$), 1.599 (m, 8H, 2 × $N(CH_2CH_2)_2CH_2$). Anal. ($C_{28}H_{40}N_8O_8$) C, H, N.

2,6-Bis[*N*,*N*-di-(2′-acetoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (75). In an ice−water bath, acetyl chloride (1.45 mL, 20 mmol) was added to a solution of dipyridamole (0.51 g, 1 mmol) and a catalytic amount of DMAP in anhydrous THF (30 mL). The mixture was stirred for 3 h, and then the solvent was evaporated under reduced pressure. The residue was dissolved in methylene chloride (50 mL) and washed with 10% $NaHCO_3$ solution, and then the organic layer was separated and dried by anhydrous Na_2SO_4. After the solvent was removed, the residue was subjected to flash silica gel chromatography (hexane/acetone = 10:1) to give a yellow fluorescent needle-like compound **75** (0.64 g, 95%). Mp 121−122 °C (lit[25] 123−124 °C); MS (ESI) *m*/*z* 695 $(M + Na)^+$, 673 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.191 (t, 8H, 2 × $N(CH_2CH_2OCOCH_3)_2$, J = 5.5 Hz), 4.046 (m, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.746 (t, 8H, 2 × $N(CH_2CH_2OCOCH_3)_2$, J = 5.5 Hz), 1.982 (s, 12H, 4 × CH_3), 1.649 (m, 4H, 2 × $N(CH_2CH_2)_2CH_2$), 1.598 (m, 8H, 2 × $N(CH_2CH_2)_2CH_2$). Anal. ($C_{32}H_{48}N_8O_8$) C, H, N.

2,6-Bis[*N*,*N*-di-(2′-methoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (76). Compound **76** was prepared by general procedure III with MeI (2 mL, 32 mmol) as the alkyl halide. The residue was subjected to flash silica gel chromatography (hexane/acetone = 8:1) to give yellow fluorescent powdery compound **56** (247 mg, 64%). Mp 64−65 °C; MS (ESI) *m*/*z* 583 $(M + Na)^+$, 561 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.052 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.766 (t, 8H, 2 × $N(CH_2CH_2OCH_3)_2$, J = 6 Hz), 3.574 (t, 8H, 2 × $N(CH_2CH_2OCH_3)_2$, J = 6 Hz), 3.351 (s, 12H, 4 × CH_3), 1.683 (s, 12H, 2 × $N(CH_2CH_2)_2CH_2$). Anal. ($C_{28}H_{48}N_8O_4$) C, H, N.

2,6-Bis[*N*,*N*-di-(2′-ethoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (77). Compound **77** was prepared by general procedure III with ethyl bromide (2.4 mL, 32 mmol) as the alkyl halide. The residue was subjected to flash silica gel chromatography (hexane/acetone = 12:1) to give yellow fluorescent powdery compound **57** (520 mg, 84%). Mp 58−59 °C; MS (ESI) *m*/*z* 639 $(M + Na)^+$, 617 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.049 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.776 (br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_3)_2$), 3.624 (br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_3)_2$), 3.500 (q, 8H, 4 × CH_2CH_3), 1.708 (br s, 12H, 2 × $N(CH_2CH_2)_2CH_2$), 1.194 (t, 12H, 4 × CH_2CH_3). Anal. ($C_{32}H_{56}N_8O_4$) C, H, N.

2,6-Bis[*N*,*N*-di-(2′-propoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (78). Compound **78** was prepared by general procedure III with propyl bromide (2.9 mL, 32 mmol) as the alkyl halide. The residue was subjected to flash silica gel chromatography (hexane/acetone = 13:1) to give yellow fluorescent powdery compound **58** (570 mg, 85%). Mp 32−34 °C; MS (ESI) *m*/*z* 695 $(M + Na)^+$, 673 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.056 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.779(br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_2CH_3)_2$), 3.616 (br s, 8H, 2 × $N(CH_2CH_2OCH_2CH_2CH_3)_2$), 3.399(t, 8H, 4 × $CH_2CH_2CH_3$, J = 6.5 Hz), 1.699 (br s, 12H, 2 × $N(CH_2CH_2)_2CH_2$), 1.578 (m, 8H, 4 × $CH_2CH_2CH_3$, J_1 = 6.5 Hz, J_2 = 7.5 Hz), 0.915 (t, 12H, 4 × $CH_2CH_2CH_3$, J = 7.5 Hz). Anal. ($C_{36}H_{64}N_8O_4$) C, H, N.

2,6-[*N*-(2-Hydroxyethyl)-*N*-(2-isopropoxyethyl)amino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (79). Compound **79** was prepared by general procedure III with isopropyl bromide (3 mL, 32 mmol) as the alkyl halide. The residue was subjected to flash silica gel chromatography (hexane/acetone = 2:1) to give yellow fluorescent powdery compound **58** (326 mg, 55%). Mp 56−58 °C; MS (ESI) *m*/*z* 611 $(M + Na)^+$, 589 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 4.574 (m, 2H, 2 × OH, disappeared after D_2O), 4.058 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 3.631 (t, 4H, 2 × C*H*$_2$OH), 3.570 (br s, 8H, 2 × $(CH_3)_2CHOCH_2CH_2NCH_2CH_2OH$), 3.525 (m, 6H, 2 × $(CH_3)_2CHOCH_2$), 1.646 (br s, 4H, 2 × $N(CH_2CH_2)_2CH_2$), 1.597 (br s, 8H, 2 × $N(CH_2CH_2)_2CH_2$), 1.075 (d, 12H, 4 × CH_3). Anal. ($C_{30}H_{52}N_8O_4$) C, H, N.

Flow Cytometric Assays. The compounds were tested to determine their ENT1 nucleoside transporter binding ability by a flow cytometric assay.[44] Briefly, human leukemia K562 cells growing in RPMI 1640 medium were washed once, resuspended at 1.6 × 10^6 cells/mL in phosphate-buffered saline at pH 7.4, and incubated with 5-(SAENTA)-X8-fluorescein (30 nM) in the presence or absence of varying concentrations of test compounds at room temperature for 45 min. Flow cytometric measurements of cell-associated fluorescence were then performed with a FACSCalibur instrument (Becton Dickinson, San Jose, CA) equipped with a 15 mW argon laser (Molecular Resources Flow Cytometry Facility, University of Tennessee Health Sciences Center). In each assay, 5000 cells were analyzed from suspensions of 4 × 10^5 cells/mL. The units of fluorescence were arbitrary channel numbers. Percentage (%) of control (i.e., ENT1 transporter-specific fluorescence in the presence of SAENTA-fluorescein without test compounds) was calculated for each sample by

$$\%\ \text{inhibition} = \left[100 - \frac{(SF_s)}{(SF_f)}\right] \times 100 \qquad (1)$$

where SF_s is the ENT1 transporter-specific fluorescence of test samples and whre SF_f is the ENT1 transporter-specific fluorescence of the SAENTA-fluorescein ligand standard in mean channel numbers. The results were fed into the PRISM program (GraphPad, San Diego, CA) to derive concentration-dependent curves. From these curves, the IC_{50} values were obtained and used to calculate inhibition constants (K_i):

$$K_i = \frac{IC_{50}}{1 + [L]/K_L} \qquad (2)$$

where [L] and K_L are the concentration and the K_d value of the SAENTA-fluorescein, respectively.[57] The K_i values were used to compare the abilities of the new compounds to displace the ENT1 transporter-specific ligand 5-(SAENTA)-X8-fluorescein[24] and, for that matter, their affinity for the ENT1 transporter.

Acknowledgment. Financial support from the National Cancer Institute (Grant Nos. CA101856 and CA105327) and the National Institute of Allergy and Infectious Diseases (Grant No. AI065372) is hereby acknowledged.

Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Griffith, D. A.; Jarvis, S. M. Nucleoside and Nucleobase Transport Systems of Mammalian Cells. *Biochim. Biophys. Acta* **1996**, *1286*, 153−181.
(2) Kong, W.; Engel, K.; Wang, J. Mammalian Nucleoside Transporters. *Curr. Drug Metab.* **2004**, *5*, 63−84.
(3) Baldwin, S. A.; Beal, P. R.; Yao, S. Y. M.; King, A. E.; Cass, C. E.; Young, J. D. The Equilibrative Nucleoside Transporter Family, SLC29. *Pfluegers Arch.* **2004**, *447*, 735−743.
(4) Gray, J. H.; Owen, R. P.; Giacomini, K. M. The Concentrative Nucleoside Transporter Family, SLC28. *Pflugers Arch.* **2004**, *447*, 728−734.
(5) Fritzgerald, G. A. Dipyridamole. *N. Engl. J. Med.* **1987**, *316*, 1247−1257.
(6) Abd-Elfattah, A. S.; Wechsler, A. S. Separation between Ischemic and Reperfusion Injury by Site Specific Entrapment of Endogenous Adenosine and Inosine Using NBMPR and EHNA. *J. Cardiovasc. Surg.* **1994**, *9* (Suppl.), 387−396.
(7) Abd-Elfattah, A.-S. A.; Hoehner, J.; Wechsler, A. S. Identification of Nucleoside Transport Binding Sites in the Human Myocardium. *Mol. Cell. Biochem*. **1998**, *180*, 105−110.
(8) Van Belle, H. Nucleoside Transport Inhibition: A Therapeutic Approach to Cardioprotection via Adenosine? *Cardiovasc. Res.* **1993**, *27*, 68−76.
(9) Deckert, J.; Morgan, P. F.; Marangos, P. J. Adenosine Uptake Site Heterogeneity in the CNS? Uptake Inhibitors as Probes and Potential Neuropharmaceuticals. *Life Sci.* **1988**, *42*, 1331−1345.
(10) Geiger, J. D.; Parkinson, F. E.; Kowaluk, E. A. Regulators of Endogenous Adenosine Levels as Therapeutic Agents. In *Purinergic Approaches in Experimental Therapeutics*; Jacobson, K. A., Jarvis, M. F., Eds.; Wiley-Liss: New York, 1997; pp 55−84.
(11) Ohta, A.; Stkovski, M. The Role of G-Protein-Coupled Adenosine Receptors in Downregulation of Inflammation and Protection from Tissue Damage. *Nature* **2001**, *414*, 916−920.
(12) Tew, K. D.; Houghton, P. J.; Houghton, J. A. *Preclinical and Clinical Modulation of Anticancer Drugs*; CRC Press: Boca Raton, FL, 1993; Chapter 7.
(13) Buolamwini, J. K. Nucleoside Transport Inhibitors: Structure−Activity Relationships and Potential Therapeutic Applications. *Curr. Med. Chem.* **1997**, *4*, 35−66.
(14) Williams, E. F. Radioreceptor Characterization of Cardiovascular Nucleoside Transporters. *SAAS Bull. Biochem. Biotechnol.* **1996**, *9*, 51−56.
(15) Hoehner, J.; Wechsler, A. S.; Abd-Elfattah, A.-S. Nucleoside Transport in the Human Myocardium. *Dev. Cardiovasc. Med*. **1996**, *181*, 209−218.
(16) Zhu, Z.; Furr, J.; Buolamwini; J. K. Synthesis and Flow Cytometric Evaluation of Novel 1,2,3,4-Tetrahydroisoquinoline Conformationally Constrained Analogs of Nitrobenzylmercaptopurine Riboside (NBMPR) Designed for Probing Its Conformation When Bound to the *es* Nucleoside Transporter. *J. Med. Chem.* **2003**, *46*, 831−837.
(17) Gupte, A.; Buolamwini, J. K. Novel Halogenated Nitrobenzylthioinosine Analogs as *es* Nucleoside Transporter Inhibitors. *Bioorg. Med. Chem.* **2004**, *14*, 2257−2260.
(18) Hammond, J. R. Interaction of a Series of Draflazine Analogues with Equilibrative Nucleoside Transporters: Species Differences and Transporter Subtype Selectivity. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 373−382.
(19) Cass, C. E.; Muzik, H.; Paterson, R. P. Combination Therapy of Mouse Leukemia L1210 by 1-β-D-Arabinofuranosylcytosine and 6-[(4-Nitrobenzyl)thio]-9-β-D-Ribofuranosylpurine. *Cancer Res.* **1975**, *35*, 1187−1193.
(20) Benedict, W. F.; Baker, M. S.; Haroun, L.; Choi, E.; Ames, B. N. Mutagenicity of Cancer Chemotherapeutic Agents in the *Salmonella*/Microsome Test. *Cancer Res.* **1977**, *37*, 2209−2213.
(21) Elion, G. B. Biochemistry and Pharmacology of Purine Analogs. *Fed. Proc.* **1967**, *26*, 898−904.
(22) Grover, G. J.; Sleph, P. G. The Cardioprotective Effects of R 75231 and Lidoflazine Are Not Caused by Adenosine A_1 Receptor Activation. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 90−96.
(23) Mustafa, S. J.; Nakagawa, Y. Relaxing Effects of Dipazep and Lidoflazine in Dog Cerebral and Renal Arteries Independent of Adenosine. *Pharmacology* **1988**, *37*, 75−84.
(24) Kates, R. A.; Dorsey, L. M.; Kaplan, J. A.; Hatcher, C. R.; Guyton, R. A. Pretreatment with Lidoflazine, a Calcium-Channel Blocker. *J. Thorac. Cardiovasc. Surg.* **1983**, *85*, 278−286.
(25) Harker, L. A.; Kadatz, R. A.; Gmbh, K. T. Mechanism of Action of Dipyridamole. *Thromb. Res.* **1983**, Suppl. IV, 39−46.
(26) Rivey, M. P.; Alexander, M. R.; Taylor, J. W. Dipyridamole: A Critical Evaluation. *Drug Intell. Clin. Pharm.* **1984**, *18*, 869−880.
(27) Weber, G.; Prajda, N. Targeted and Non-targeted Actions of Anticancer Drugs. *Adv. Enzyme Regul.* **1994**, *34*, 71−89.
(28) Boyer, C. R.; Karjian, P. L.; Wahl, G. M.; Pegram, M.; Neuteboom, S. T. C. Nucleoside Transport Inhibitors, Dipyridamole and *p*-Nitrobenzylthioinosine, Selectively Potentiate the Antitumor Activity of NB1011. *Anti-Cancer Drugs* **2002**, *13*, 29−36.
(29) Grem, J. L. Biochemical Modulation of Fluorouracil by Dipyridamole: Preclinical and Clinical Experience. *Semin. Oncol.* **1992**, *19* (Suppl. 3), 56−65.
(30) Smith, P. G.; Marshman, E.; Newell, D. R.; Curtin, N. J. Dipyridamole Potentiates the in Vitro Activity of MTA (LY231514) by Inhibition of Thymidine Transport. *Br. J. Cancer* **2000**, *82*, 924−930.
(31) Sherman, I. W. Biochemistry of *Plasmodium* (Malarial Parasites). *Microbiol. Rev.* **1979**, *43*, 453−495.
(32) Gero, A. M.; O'Sullivan, W. J. Purines and Pyrimidines in Malarial Parasites. *Blood Cells* **1990**, *16*, 467−484.
(33) el Kouni, M. H. Potential Chemotherapeutic Targets in the Purine Metabolism of Parasites. *Pharmacol. Ther*. **2003**, *99*, 283−309.
(34) Carter, N. S.; Landfear, S. M.; Ullmam, B. Nucleoside Transporters of Parasitic Protozoa. *Trends Parasitol*. **2001**, *17*, 145.
(35) el Kouni, M. H.; Guarcello, V.; Al Safarjalani, O. N.; Naguib, F. N. M. Metabolism and Selective Toxicity of 6-Nitrobenzylthioinosine in *Toxoplasma gondii*. *Antimicrob. Agents Chemother*. **1999**, *43*, 2437−2443.
(36) Akaki, M.; Nakano, Y.; Ito, Y.; Nagayasu, E.; Aikawa, M. Effects of Dipyridamole on *Plasmodium falciparum*-Infected Erythrocytes. *Parasitol. Res.* **2002**, *88*, 1044−1050.
(37) Kadatz, R. The Pharmacology of 2,6-bis(diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-*d*)-pyrimidine, a new compound with coronary dilatory properties. *Arzneim.-Forsch.* **1959**, *9*, 39−45.
(38) Fischer, F. G.; Roch, W.; Roch, J.; Kottler, A. Substituted Pyrimido-[5,4-*d*]-pyrimidines. U.S. Patent 3,031,450, 1962.
(39) Roch, J.; Beisenherz, G.; an der Riss, B. Basic-Substituted Pyrimido-[5,4-*d*]-pyrimidines. U.S. Patent 3,074,928, 1963.
(40) Kaminsky, D.; Lutz, W. B.; Lazarus, S. Some Congeners and Analogs of Dipyridamole. *J. Med. Chem.* **1966**, *9*, 610−612.
(41) Roch, J.; Erich, M.; Narr, B.; Machleidt, H. *S* African Patent 6,-905,504, 1970.
(42) Northen, J. S.; Boyle, F. T.; Clegg, W.; Curtin, N. J.; Edwards, A. J.; Griffin, R. J.; Golding, B. T. Controlled Stepwise Conversion of 2,4,6,8-Tetrachloropyrimido-[5,4-*d*]pyrimidine into 2,4,6,8-Tetrasubstituted Pyrimido[5,4-*d*]-pyrimidines. *J. Chem. Soc., Perkin Trans. 1* **2002**, 108−115.
(43) Curitn, N. J.; Barlow, H. C.; Bowman, K. J.; Calvert, A. H.; Davison, R.; Golding, B. T.; Huang, B.; Loughlin, P. J.; Newell, D. R.; Smith, P. G.; Griffin, R. J. Resistance-Modifying Agents. 11. Pyrimido-[5,4-*d*]pyrimidine Modulators of Antitumor Drug Activity. Synthesis and Structure−Activity Relationships for Nucleoside Transport Inhibition and Binding to α_1-Acid Glycoprotein. *J. Med. Chem.* **2004**, *47*, 4905−4922.
(44) Buolamwini, J. K.; Wiley, J. S.; Robins, M. J.; Craik, J. D.; Cass, C. E.; Gati, W. P.; Paterson, A. R. P. Conjugates of Fluorescein and SAENTA (5′-*S*-(2-Aminoethyl)-N^6-(4-nitrobenzyl)-5′-thioadenosine): Flow Cytometry Probes for the *Es* Transporter Elements of the Plasma Membrane. *Nucleosides Nucleotides* **1994**, *13*, 737−751.
(45) Hammond, J. R. Kinetic Analysis of Ligand Binding to the Ehrlich Cell Nucleoside Transporter: Pharmacological Characterization of Allosteric Interaction with the [^{3}H]Nitrobenzylthioinosine Binding Site. *Mol. Pharmacol.* **1991**, *39*, 771−779.
(46) Jones, K. W.; Hammond, J. R. Heterogeneity of [^{3}H]Dipyridamole Binding to CNS Membrances: Correlation with [^{3}H]Nitrobenzylthioinosine Binding and [^{3}H]Uridine Influx Studies. *J. Neurochem.* **1992**, *59*, 1363−1371.

(47) Deckert, J.; Hennemann, A.; Bereznai, B.; Fritze, J.; Vock, R.; Marangos, P. J.; Riederer, P. (^{3}H)Dipyridamole and (^{3}H)Nitrobenzylthioinosine Binding Sites at the Human Parietal Cortex and Erythrocyte Adenosine Transporter: A Comparison. *Life Sci.* **1994**, *55*, 1675−1682.
(48) Vargas, F.; Rivas, C.; Fuentes, A.; Tse, Cheng, A.; Velutini, G. The Photochemistry of Dipyridamole. *J. Photochem. Photobiol.* **2002**, *153*, 237−243.
(49) Boleti, H.; Coe, I. R.; Baldwin, S. A.; Young, J. D.; Cass, C. E. Molecular Identification of the Equilibrative NBMPR-sensitive (*es*) Nucleoside Transporter and Demonstration of an Equilibrative NBMPR-insensitive (*ei*) Transport Activity in Human Erythroleukemia (K562) Cells. *Neuropharmacology* **1997**, *36*, 1167−1179.
(50) Huang, Y.; Anderle, P.; Bussey, K. J.; Barbacioru, C.; Shankavaram, U.; Dai, Z.; Reinhold, W. C.; Papp, A.; Weinstein, J. N.; Sadee, W. Membrane Transporters and Chennels: Role of the Transportome in Cancer Chemosensitivity and Chemoresistance. *Cancer Res.* **2004**, *64*, 4294−4301.
(51) Addo, J. K.; Buolamwini, J. K. Design, Synthesis, and Evaluation of 5′-S-Aminoethyl-N(6)-azidobenzyl-5′-thioadenosine Biotin Conjugate: A Bifunctional Photoaffinity Probe for the es Nucleoside Transporter. *Bioconjugate Chem.* **2004**, *15*, 536−540.
(52) Huang, M.; Wang, Y.; Cogut, S. B.; Mitchell, B. S.; Graves, L. M. Inhibition of Nucleoside Transport by Protein Kinase Inhibitors. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 753−760.
(53) Huang, M.; Wang, Y.; Collins, M.; Gu, J. J.; Mitchell, B. S.; Graves, L. M. Inhibition of Nucleoside Transport by p38 MARK Inhibitors. *J. Biol. Chem.* **2002**, *277*, 28364−28367.
(54) Agoston, G. E.; Wu, J. H.; Izenwasser, S.; George, C.; Katz, J.; Kline, R. H.; Newman, A. H. Novel N-Substituted 3α-[Bis(4′-fluorophenyl)-methoxyltropane Analogs: Selective Ligands for the Dopamine Transporter. *J. Med. Chem.* **1997**, *40*, 4329−4339.
(55) Georgiev, V. S.; Kinsolving, C. R. Substituted 4-Azatricyclo[4.3.1.1^{3,8}]undecane Compounds. U.S. Patent 4,557,865, 1985.
(56) Williams, D. E.; Craig, K. S.; Patrick, B.; McHardy, L. M.; Soest, R. V.; Roberge, M.; Andersen, R. J. Motuporamines, Anti-Invasion and Anti-Angiogenic Alkaloids from the Marine Sponge *Xestospongia exigua* (Kirkpatrick): Isolation, Structure Elucidation, Analog Synthesis, and Conformational Analysis. *J. Org. Chem.* **2002**, *67*, 245−258.
(57) Cheng, Y.; Prusoff, W. H. Relationship between the Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes 50 Percent Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

JM070311L