Resistance-Modifying Agents. 11.[1] 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

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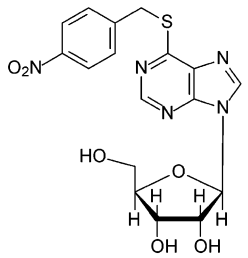
The cardiovascular and antithrombotic agent dipyridamole (DP) has potential therapeutic utility as a modulator of the activity of antimetabolite antitumor agents by virtue of its inhibition of nucleoside transport. However, the activity of DP can be compromised by binding to the acute phase serum protein, α_1-acid glycoprotein (AGP). Analogues of DP were synthesized and evaluated as inhibitors of ^{3}H-thymidine uptake into L1210 leukamia cells in the presence and absence of 5 mg/mL AGP. Compounds with potency similar to that of DP were identified where the piperidino substituents at the 4,8-positions were replaced by 4′-methoxybenzylamino, 3′,4′-dimethoxybenzylamino, or piperonylamino groups. Replacement of the diethanolamino groups at the 2,6-positions of DP by alkylamino or alkoxy substituents was tolerated, although at least one oxygen-bearing function (hydroxyl or alkoxy) was required in the side chain for activity comparable to that of DP. Whereas AGP completely ablated the activity of DP, the majority of the newer compounds synthesized retained significant activity in the presence of excess AGP, although replacement of the piperidino groups at the 4,8-positions by *N*-methylbenzylamino substituents did, in some cases, restore susceptibility to AGP. Selected compounds have been demonstrated to prevent rescue from antifolate cytotoxicity, mediated by nucleoside salvage.

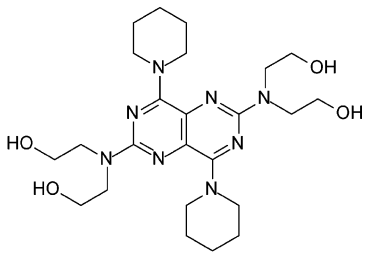
Introduction

Antitumor agents that act through inhibition of de novo purine and pyrimidine biosynthesis (antimetabolites) remain effective drugs for the chemotherapy of cancer.[2] The classical antifolate methotrexate (MTX) is a component of regimens for the treatment of hematological malignancies and certain solid tumors, while more recently the folate analogues raltitrexed and pemetrexed have shown activity in the treatment of colorectal carcinoma and mesothelioma, respectively.[3−6] The pyrimidine derivative 5-fluorouracil (5-FU) is also used extensively for the treatment of colorectal, breast, and head and neck cancer, and capecitabine, an orally active 5-FU prodrug, has recently been approved.[7] A unifying feature of antimetabolite drugs is a net depletion of the intracellular nucleotide pools, which accounts for the cytotoxicity of these agents. One mechanism by which tumor resistance to antimetabolite drugs may arise involves the salvage of extracellular preformed nucleobases and nucleosides, resulting in repletion of nucleotide pools and hence circumvention of inhibition of de novo nucleotide biosynthesis. Thus, salvage of thymidine and hypoxanthine reduces the cytotoxicity of antifolate inhibitors of de novo thymidylate and purine nucleotide biosynthesis, respectively.[8−12] In addition, the antitumor activity of antifolates against tumors deficient in nucleoside and nucleobase salvage enzymes has been shown to be markedly greater than against salvage-competent tumors.[12−14]

The initial step in the nucleoside salvage pathway involves transport across the plasma membrane, the principal route being carrier-mediated facilitated diffusion by the equilibrative transporters ENT1 and ENT2, formerly known as es and ei based on their sensitivity and insensitivity, respectively, to inhibition by the nucleoside analogue nitrobenzylthioinosine (NBT).[15,16] Inhibitors of salvage pathways have a number of potential therapeutic applications, both as single agents and in combination with other drugs.[15,17,18] In particular, a dual inhibitor of ENT1 and ENT2 should increase the antitumor activity of inhibitors of de novo nucleotide synthesis by blocking the uptake of nucleosides required for salvage. Additionally, parasitic protozoa, including *Leishmania* and *Trypanosoma* species, are unable to undertake de novo purine biosynthesis and are entirely dependent upon salvage pathways for



Nitrobenzylthioinosine (NBT)



Dipyridamole (DP)

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survival.[19,20] These pathogens would be especially vulnerable to a selective nucleoside transport (NT) inhibitor.

The cardiovascular and antithrombotic agent dipyridamole (DP) functions as an indirect purinergic agonist by inhibiting the cellular reuptake of adenosine, and this is thought to account, at least in part, for the activity of the drug.[21] In contrast to NBT, DP inhibits both ENT1 and ENT2 transporters and has been employed successfully to potentiate the in vitro cytotoxicity of a number of antifolate antitumor agents.[22−27] However, clinical studies of antimetabolites in combination with DP have been disappointing, and the drug did not markedly increase either antiproliferative toxicity or antitumor activity in a number of studies.[27−33] DP binds avidly to the serum protein α_1-acid glycoprotein (AGP), the levels of which can be elevated in the plasma of cancer patients.[34,35] As a consequence, free levels of DP may not reach those necessary to inhibit nucleoside transport adequately, and this effect on potency has been clearly demonstrated in experimental systems, employing physiological concentrations of AGP.[36]

The in vitro studies referred to above suggest that NT inhibitors may have a role as potentiators of antimetabolite drug cytotoxicity, provided that the problem of AGP binding can be addressed. In this paper we describe the synthesis and biological evaluation of pyrimido[5,4-*d*]pyrimidine-based NT inhibitors and the elucidation of structure−activity relationships for NT inhibition and AGP binding. These studies have resulted in the identification of NT inhibitors that are at least as potent as DP and that retain activity in the presence of supraphysiological concentrations of AGP. A preliminary account of part of this work has been published previously.[37]

Chemistry

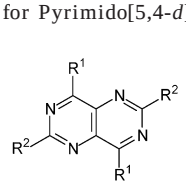
The structures and physicochemical properties of the compounds evaluated as nucleoside transport inhibitors are recorded in Table 1. Pyrimido[5,4-*d*]pyrimidines bearing identical substituents at the 2,6- and 4,8-positions were readily synthesized from 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (**8**), prepared from the disodium salt of commercially available 1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8-(3*H*,7*H*)-tetrone by a literature procedure.[38] This pattern of substitution is possible because reaction rates greatly favor displacements at the 4- and 8-positions over the 2- and 6-positions of **8**. Accordingly, all of the compounds described in this paper have identical substituents at the 2,6-positions and also at the 4,8-positions. However, we have recently developed a methodology to enable the introduction of different groups at the 2-, 4-, 6-, and 8-positions on the pyrimidopyrimidine scaffold.[39] Several of the compounds described in this paper have a stereogenic center in the group at the 2,6- or 4,8-positions. Where racemic precursors were used, it is expected that a mixture of three stereoisomers is obtained: (*R*,*R*), its enantiomer (*S*,*S*), and the meso form (*R*,*S*). Because the second substitution of a particular moiety is unlikely to be strongly influenced by the chiral group already present, these isomers are expected to be formed in approximately a 1:1:2 ratio. The ^{1}H NMR data of such mixtures were qualitatively in accord with this prediction. No attempt was made to separate these mixtures. In selected cases, single stereoisomers were obtained by the use of a monochiral precursor.

Treatment of **8** with an excess of the appropriate amine in THF at room temperature gave the required 4,8-disubstituted-2,6-dichloropyrimidopyrimidines (**9**−**24**) in excellent yields, with no evidence of tri- or tetrasubstitution occurring (Scheme 1). For reactions with benzylamines, the addition of finely powdered K_2CO_3 was found to reduce the incidence of side reactions. Introduction of the 2,2-dimethyl-1,3-dioxolane-4-methoxy group at the 4,8-positions to furnish **25** was achieved by reaction of **8** with the alkoxide of racemic 2,2-dimethyl-1,3-dioxolane-4-methanol (solketal). Pyrimidopyrimidines unsubstituted at the 2,6-positions (**26**, **27**) were prepared by reductive dehalogenation of the appropriate 2,6-dichloro-4,8-disubstituted derivatives (**9**, **12**), with no debenzylation of **27** being observed under the conditions employed (Pd/H_2/KOH). The target tetrasubstituted pyrimidopyrimidines bearing amine substituents at the 2,6-positions (**29**−**54**) were generally accessible from the corresponding 2,6-dichloro-4,8-disubstituted derivatives (**9**−**25**) by treatment with an excess of the amine at 100−150 °C. However, the lower boiling point of diethylamine necessitated the use of a bomb reaction vessel for the preparation of 2,6-bis-diethylamino-4,8-dipiperidinopyrimidopyrimidine (**28**). Methylation of the hydroxyl functions of the 2,6-bis-diethanolamino groups of DP was effected by direct reaction of the tetraalkoxide of DP with iodomethane in DMF, affording **55** in good yield.

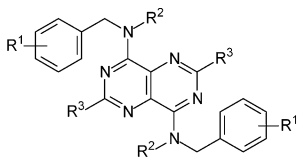
The synthesis of 4,8-dipiperidinopyrimidopyrimidines with alkoxy substituents at the 2,6-positions (**56**−**68**) was readily achieved by heating the 2,6-dichloro derivative **9** with the appropriate sodium alkoxide under reflux, employing the corresponding alcohol as solvent. While this approach was also successful for the preparation of the 2′-hydroxyethoxy and 3′-hydroxypropoxy derivatives (**69** and **70**), introduction of the 2′-hydroxypropoxy substituent required prior 4-methoxybenzyl (pmb) protection of the 2-hydroxy group of propane-1,2-diol, with subsequent hydrogenolysis affording the required pyrimidopyrimidine (**71**). (*S*)-2-(4′-Methoxybenzyloxy)propan-1-ol (**2**) was prepared in two steps from ethyl (*S*)-lactate and used for the synthesis of **71** as a single stereoisomer (*S*,*S*). Reaction of **9** with the alkoxides of racemic or monochiral solketal furnished compounds **72**−**74**, which were smoothly converted into the required pyrimidopyrimidines bearing 2′,3′,-dihydroxypropoxy groups at the 2,6-positions (**75**−**77**) on treatment with 1 M hydrochloric acid. Methylation of **76** with iodomethane in DMF gave (*R*,*R*)-2,6-bis(2′,3′-dimethoxypropoxy)pyrimidopyrimidine (**68**) in excellent yield.

Analogous reactions of the 2,6,-dichloro-4,8-di-*N*-benzyl-*N*-methylpyrimidopyrimidines (**18**−**20**) with alkoxides furnished the 2,6-dialkoxy-4,8-di-*N*-benzyl-*N*-methylpyrimidopyrimidines (**78**−**85**) although, where appropriate, prior monoprotection of diols as their triisopropylsilyloxy ethers was found to improve yields in most cases. 2-Triisopropylsilyloxypropan-1-ol (**3**) was not accessible via the general literature procedure[40] employed for the synthesis of the other monosilylated diols and was prepared by ozonolysis−reductive cleavage[41] of the

Table 1. Physical and Synthetic Data for Pyrimido[5,4-*d*]pyrimidines



A



B

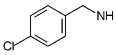
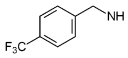
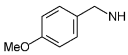
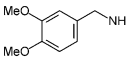
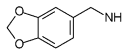
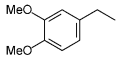
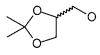
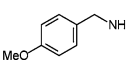
compd	type	R^1	R^2	R^3	method[a]	*solvent system*[b]	yield (%)	mp (°C)	formula	anal[c]
(DP)	A			–	–	–	–	165-166	$C_{24}H_{41}N_8O_4$	C, H, N
8	A	Cl	Cl	–	–	–	39	218-220[d]	$C_6Cl_4N_4$	C, H, N
9	A		Cl	–	I	*A*	93	240-241[e]	$C_{16}H_{20}Cl_2N_6$	C, H, N
10	A		Cl	–	I	*B*	71	217-220	$C_{16}H_{20}Cl_2N_6O_2$	C, H, N
11	A		Cl	–	I	*B*	74	158-162	$C_{14}H_{20}Cl_2N_6O_4$	C, H, N
12	A		Cl	–	II	*B*	93	228-230[f]	$C_{20}H_{16}Cl_2N_6$	C, H, N
13	A		Cl	–	II	*B*	68	299-301	$C_{22}H_{14}Cl_4N_6$	C, H, N
14	A		Cl	–	II	*B*	81	284-285	$C_{24}H_{14}Cl_2F_6N_6$	C, H, N
15	A		Cl	–	II	*B*	93	186-188	$C_{22}H_{20}Cl_2N_6O_2$	C, H, N
16	A		Cl	–	II	*B*	83	206-208	$C_{24}H_{24}Cl_2N_6O_2$	C, H, N
17	A		Cl	–	II	*B*	67	256-258	$C_{22}H_{16}Cl_2N_6O_4$	C, H, N
18	B	H	Me	Cl	II	*B*	72	185-187	$C_{22}H_{20}Cl_2N_6$	C, H, N
19	B	4-MeO	Me	Cl	II	*B*	87	147-149	$C_{24}H_{24}Cl_2N_6O_2$	C, H, N
20	B	3,4-$(MeO)_2$	Me	Cl	II	*B*	43	200-202	$C_{26}H_{28}Cl_2N_6O_4$	C, H, N
21	B	H		Cl	II	*B*	87	141-143	$C_{34}H_{28}Cl_2N_6$	C, H, N
22	B	3,4-$(MeO)_2$		Cl	II	*B*	74	214-216	$C_{38}H_{36}Cl_2N_6O_4$	C, H, N
23	B	3,4-$(MeO)_2$		Cl	II	*B*	68	197-199	$C_{42}H_{44}Cl_2N_6O_8$	C, H, N
24	A		Cl	–	II	*E*	7	143-144	$C_{24}H_{20}Cl_2N_6$	C, H, N
25	A		Cl	–	–	*F*	72	200-201	$C_{18}H_{22}Cl_2N_4O_6$	C, H, N
26	A		H	–	–	–	64	114-116	$C_{16}H_{22}N_6$	C, H, N
27	A		H	–	–	*G*	61	185-186	$C_{20}H_{18}N_6$	C, H, N
28	A		NEt_2	–	–	*H*	90	78-80	$C_{24}H_{40}N_8$	C, H, N
29	A			–	III	*E*	41	178-180	$C_{22}H_{36}N_8O_4$	C, H, N
30	A			–	III	*F*	74	204-208	$C_{24}H_{40}N_8O_6$	C, H, N
31	A			–	III	*B*	62	180-181[g]	$C_{24}H_{40}N_8O_4$	C, H, N
32	A			–	III	*E*	31	220-221	$C_{24}H_{28}N_8O_2$	C, H, N
33	A			–	III	*B*	35	161-163	$C_{26}H_{32}N_8O_4$	C, H, N

Table 1 (Continued)

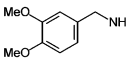
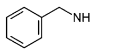
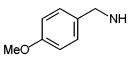
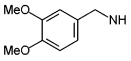
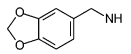
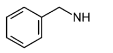
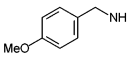
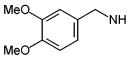
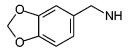
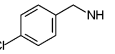
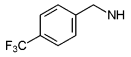
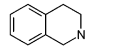
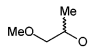
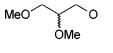
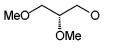
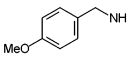
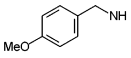
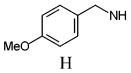
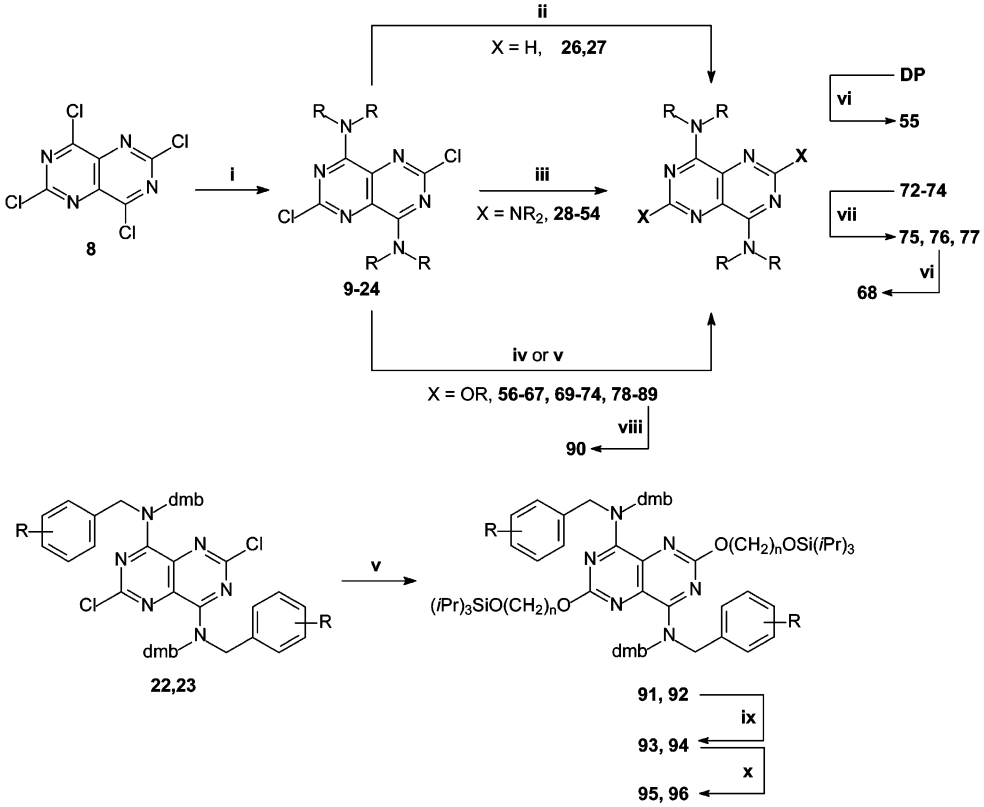
compd	type	R^1	R^2	R^3	method[a]	*solvent system*[b]	yield (%)	mp (°C)	formula	anal[c]
34	A			–	III	*E*	31	186-188	$C_{28}H_{36}N_8O_6$	C, H, N
35	A			–	III	*C*	58	202-203	$C_{26}H_{32}N_8O_2$	C, H, N
36	A			–	III	*C*	40	138-140	$C_{28}H_{36}N_8O_4$	C, H, N
37	A			–	III	*C*	54	154-156	$C_{30}H_{40}N_8O_6$	C, H, N
38	A			–	III	*C*	56	170-172	$C_{28}H_{32}N_8O_6$	C, H, N
39	A			–	III	*C*	64 75 40	207-208	$C_{28}H_{36}N_8O_4$	C, H, N
40	A			–	III	*C*	31	204-205	$C_{30}H_{40}N_8O_6$	C, H, N
41	A			–	III	*B*	13	176-179	$C_{32}H_{44}N_8O_8$	C, H, N
42	A			–	III	*B*	28	190-192	$C_{30}H_{36}N_8O_8$	C, H, N
43	A			–	III	*B*	51	223-224	$C_{28}H_{34}Cl_2N_8O_4$	C, H, N
44	A			–	III	*A*	93	194-196	$C_{30}H_{34}F_6N_8O_4$	C, H, N
45	A			–	III	*F*	17	141-142	$C_{32}H_{40}N_8O_4$	C, H, N
46	B	H	Me		III	*E*	15	158-160	$C_{26}H_{32}N_8O_2$	C, H, N
47	B	4-MeO	Me		III	*G*	2	165-167	$C_{28}H_{36}N_8O_4$	C, H, N
48	B	3,4-$(MeO)_2$	Me		III	*C*	15	179-181	$C_{30}H_{40}N_8O_6$	C, H, N
49	B	H	Me		III	*C*	33	160-162	$C_{28}H_{36}N_8O_2$	C, H, N
50	B	4-MeO	Me		III	*E*	40	140-142	$C_{30}H_{40}N_8O_4$	C, H, N
51	B	3,4-$(MeO)_2$	Me		III	*B*	45	159-161	$C_{32}H_{44}N_8O_6$	C, H, N
52	B	H	Me		III	*F*	5	161-163	$C_{30}H_{40}N_8O_4$	C, H, N
53	B	4-MeO	Me		III	*E*	35	190-192	$C_{32}H_{44}N_8O_6$	C, H, N
54	B	3,4-$(MeO)_2$			III	*B*	52		$C_{46}H_{56}N_8O_8$	C, H, N
55	A			–	–	*J*	69	55-57	$C_{28}H_{48}N_8O_4$	C, H, N
56	A		MeO	–	IV	*C*	46	130-131	$C_{18}H_{26}N_6O_2$	C, H, N
57	A		EtO	–	IV	*C*	84	142-143	$C_{20}H_{30}N_6O_2$	C, H, N
58	A		*n*-PrO	–	IV	*C*	42	127-129	$C_{22}H_{34}N_6O_2$	C, H, N
59	A		*i*-PrO	–	IV	*C*	58	167-169	$C_{22}H_{34}N_6O_2$	C, H, N
60	A			–	IV	*C*	54	114-116	$C_{24}H_{38}N_6O_2$	C, H, N
61	A			–	IV	*H*	98	60-63	$C_{26}H_{42}N_6O_2$	C, H, N
62	A			–	IV	*C*	63	105-106	$C_{26}H_{42}N_6O$	C, H, N
63	A			–	IV	*E*	92	181-183	$C_{30}H_{46}N_6O_2$	C, H, N
64	A		CH_2=$CHCH_2O$	–	IV	*C*	40	109-110	$C_{22}H_{30}N_6O_2$	C, H, N
65	A			–	V	*C*	34	127-129	$C_{22}H_{34}N_6O_4$	C, H, N

Table 1 (Continued)

compd	type	R^1	R^2	R^3	method[a]	*solvent system*[b]	yield (%)	mp (°C)	formula	anal[c]
66	A			–	IV	*E*	22	91-93	$C_{24}H_{38}N_6O_4$	C, H, N
67	A			–	V	*F*	57	57-58	$C_{26}H_{42}N_6O_6$	C, H, N
68	A			–	–	*C*	90	63-64	$C_{26}H_{42}N_6O_6$	C, H, N
69	A			–	V	*E*	45	148-150	$C_{20}H_{30}N_6O_4$	C, H, N
70	A			–	V	*C*	51	154-156	$C_{22}H_{34}N_6O_4$	C, H, N
71	A			–	IV	–	10	128-129	$C_{22}H_{34}N_6O_4$	C, H, N
72	A			–	V	*B*	99	145-147	$C_{28}H_{42}N_6O_6$	C, H, N
73	A			–	V	*B*	91	148-150	$C_{28}H_{42}N_6O_6$	C, H, N
74	A			–	V	*B*	92	142-144	$C_{28}H_{42}N_6O_6$	C, H, N
75	A			–	–	*B*	42	175-178	$C_{22}H_{34}N_6O_6$	C, H, N
76	A			–	–	*B*	46	147-149	$C_{22}H_{34}N_6O_6$	C, H, N
77	A			–	–	*B*	40	149-152	$C_{22}H_{34}N_6O_6$	C, H, N
78	B	H	Me		V	*C*	40	119-120	$C_{28}H_{34}N_6O_2$	C, H, N
79	B	H	Me		VI	*C*	36	174-176	$C_{26}H_{30}N_6O_4$	C, H, N
80	B	4-MeO	Me		VI	*B*	8	168-170	$C_{28}H_{34}N_6O_6$	C, H, N
81	B	H	Me		V	*C*	65	109-111	$C_{28}H_{34}N_6O_4$	C, H, N
82	B	H	Me		VI	*I*	60	148-150	$C_{28}H_{34}N_6O_4$	C, H, N
83	B	4-MeO	Me		VI	*E*	21	125-127	$C_{30}H_{38}N_6O_6$	C, H, N
84	B	3,4-$(MeO)_2$	Me		V	*C*	11	153-154	$C_{32}H_{42}N_6O_8$	C, H, N
85	B	H	Me		V	*C*	43	116-118	$C_{28}H_{34}N_6O_4$	C, H, N
86	A			–	IV	*D*	18	189-191	$C_{26}H_{30}N_6O_6$	C, H, N
87	A			–	V	*C*	20	149-150	$C_{28}H_{34}N_6O_6$	C, H, N
88	A			–	V	*D*	22	190-192	$C_{28}H_{34}N_6O_6$	C, H, N
89	B	H			V	*C*	49	154-155	$C_{40}H_{42}N_6O_4$	C, H, N
90	A			–	–	*I*	26	137-139	$C_{26}H_{30}N_6O_4$	C, H, N
91	B	3,4-$(MeO)_2$			V	*H*	40	81-83	$C_{64}H_{94}N_6O_{12}Si_2$	C, H, N
92	B	3,4-$(MeO)_2$			V	*A*	65	77-79	$C_{66}H_{98}N_6O_{12}Si_2$	C, H, N
93	B	3,4-$(MeO)_2$			–	*B*	68	196-198	$C_{46}H_{54}N_6O_{12}$	C, H, N
94	B	3,4-$(MeO)_2$			–	*B*	83	170-172	$C_{48}H_{58}N_6O_{10}$	C, H, N
95	A			–	–	*H*	36	216-218	$C_{30}H_{38}N_6O_8$	C, H, N
96	A			–	–	*I*	89	123-125	$C_{30}H_{38}N_6O_8$	C, H, N

[a] See the Experimental Section. [b] Recrystallization solvents: A, $CHCl_3$−petroleum ether; B, EtOAc−petroleum ether; C, MeOH−H_2O; D, Propan-2-ol. Chromatography solvents: E, petroleum ether/EtOAc (4:1); F, $CHCl_3$/EtOAc (3:1); G, EtOAc/petroleum ether (3:1); H, DCM/EtOAc (24:1); I, $CHCl_3$/MeOH (95:5); J, DCM/MeOH (9:1). [c] Except where stated, all compounds were analyzed for C, H, N. Unless stated, analytical results were within 0.4% of theoretical value. [d] Lit.[38] mp, 255−258 °C. [e] Lit.[38] mp, 241−242 °C. [f] Lit.[38] mp, 229−230 °C. [g] Lit.[38] mp, 182−184 °C.

Scheme 1[a]



[a] Reagents: (i) R_2NH, THF, 25 °C; (ii) Pd/C, H_2, THF, 25 °C; (iii) R_2NH, 100−150 °C; (iv) Na, ROH, reflux; (v) NaH, ROH, THF, reflux; (vi) NaH, MeI, DMF, 25 °C; (vii) 1M HCl, THF, 25 °C; (viii) AcCl,/MeOH, Pd/C, H_2, THF, 25 °C; (ix) TBAF, THF, 25 °C; (x) TFA, 25 °C.

triisopropylsilyloxy derivative of 3-buten-2-ol. Reactions of alkoxides with 2,6-dichloropyrimidopyrimidines substituted with benzylamino groups at the 4,8-positions (**15** and **16**) were problematical. Thus, direct treatment of **15** with the alkoxides of ethane-1,2-diol, propane-1,3-diol, and (*S*)-propane-1,2-diol gave the target pyrimidopyrimidines **86**−**88**, respectively, in poor yield following extensive purification. This was attributed, at least in part, to deprotonation of the 4,8-benzylamino NH functions by the alkoxide to generate a delocalized anion, which deactivated the 2,6-positions to nucleophilic displacement, and may also have given rise to unwanted side reactions. Clearly, deprotonation cannot arise with a tertiary amine at the 4,8-positions and would account for the success of reactions with alkoxides employing pyrimidopyrimidines with piperidino (**56**−**77**) or *N*-benzylmethylamino (**78**−**85**) groups at these positions.

Protection of the vulnerable NH group as a tertiary amine, prior to introduction of the requisite alkoxy functions at the 2,6-positions, would overcome the problem, provided that the protecting group could be removed subsequently. This approach was investigated using the 2,6-dichloro-4,8-bis(dibenzylamino)pyrimidopyrimidine (**21**), which reacted smoothly with sodium 2-methoxyethoxide to give **89** in reasonable yield. However, attempts to selectively monodebenzylate at the 4,8-positions by catalytic hydrogenation in acidic MeOH afforded only a poor yield of the required 4,8-dibenzylaminopyrimidopyrimidine **90**, together with mono- and tri-benzylated products. To enable a better discrimination between the required benzylamino or substituted benzylamino group and the N-protecting functionality, we investigated the use of the 3,4-dimethoxybenzylamino (dmb) group.[42,43] Treatment of benzylamine or 3,4-dimethoxybenzylamine with 3,4-dimethoxybenzaldehyde and reduction of the resulting imines **4** and **5** with sodium borohydride gave the required *N*-benzyl-*N*-3,4-dimethoxybenzylamine derivatives **6** and **7**, which were reacted directly with **8** to furnish the *N*-dmb-protected 4,8-dibenzylaminopyrimidopyrimidines **22** and **23**. Selective removal of the dmb functionality was demonstrated with **54**, prepared from **22** and diethanolamine, which gave authentic 2,6-bis(diethanolamino)-4,8-dibenzylaminopyrimidopyrimidine **39** on treatment with DDQ or TFA. Analogous treatment of **23** with the alkoxides of 2-triisopropylsilyloxyethanol and 3-triisopropylsilyloxypropan-1-ol afforded the fully protected tetrasubstituted pyrimidopyrimidines **91** and **92**, respectively. Removal of the silyl groups with TBAF gave the corresponding dmb-protected 2,6-di-(hydroxyalkoxy)pyrimidopyrimidines **93** and **94**. Final monodeprotection with TFA removed only one dmb group from each of the 4,8-positions to give the target pyrimidopyrimidines **95** and **96**. This reaction sequence proved to be optimal because initial desilylation at the 2,6-positions was found to confer sufficient solubility in THF to facilitate subsequent TFA-mediated removal of the dmb group.

Structure−Activity Relationships

The biological properties of selected pyrimidopyrimidines are shown in Table 2. The core pyrimido[5,4-*d*]-pyrimidine template and 2,6/4,8 symmetrical substitution pattern of DP was retained throughout these studies, and initial structural modifications were sought

Table 2. Inhibition of ^{3}H-thymidine Uptake into L1210 Cells by Pyrimido[5,4-*d*]pyrimidine Nucleoside Transport Inhibitors in the Absence and Presence of α_1-Acid Glycoprotein

	inhibition of ^{3}H-thymidine uptake in the absence or presence of AGP					inhibition of ^{3}H-thymidine uptake in the absence or presence of AGP			
compd	% inhibition (μM)[a]	IC_{50} (μM)[b]	% inhibition (μM) plus 5 mg/mL AGP[c]	% reduction in activity by 5 mg/mL AGP	compd	% inhibition (μM)[a]	IC_{50} (μM)[b]	% inhibition (μM) plus 5 mg/mL AGP[c]	% reduction in activity by 5 mg/mL AGP
DP	80 ± 8 (1)	0.34 ± 0.06	4 ± 11 (1)[d,e]	87	**58**	37 ± 11 (10)	52		
	99 ± 2 (10)		13 ± 12 (10)[d,e]	96		60 ± 9 (100)			
8	inactive[f]				**59**	23 ± 12 (10)			
12	inactive[f]				**60**	25 ± 4 (10)			
26	inactive[f]				**61**	inactive[g]			
27	40 ± 17 (10)				**64**	52 ± 7 (10)			
28	inactive[g,h]				**65**	20 ± 6 (1)	4.6		
29	18 ± 13 (1)	3.7, 5.3				63, 68 (10)			
	55 ± 14 (10)		20 ± 12 (10)[d]	64	**66**	62 ± 10 (10)			
30	28 ± 10 (1)	2.6				96 ± 7 (100)			
	50 ± 8 at (10)				**67**	38 ± 17 (1)	1.88 ± 1.0		
31	34 ± 11 (1)					87 ± 7 (10)		56 ± 10 (10)[d]	36
	74 ± 10 (10)				**68**	100 (10)[h]			
32	46 ± 14 (1)	0.75			**69**	13 ± 6 (1)	6.3		
33	10 ± 8 (0.1)	0.93, 1.2				61 ± 6 (10)			
	56 ± 6 (1)		35 ± 17 (1)[d]	40	**70**	35 ± 5 (1)			
34	29 ± 10 (0.1)	0.50, 0.60				76, 100 (10)			
	62 ± 9 (1)		44 ± 8 (1)[d]	23	**71**	47 ± 8 (1)	0.90, 1.1	20 ± 10 (1)[d]	55
35	33 ± 9 (1)	3.0, 4.0				92 ± 10 (10)		69 ± 9 (10)[d]	25
	84 ± 7 (10)				**73**	47 ± 5 (1)	1.1, 1.2		
36	21 ± 9 (0.1)	0.70, 0.90				87 ± 8 (10)		62 ± 12 (10)[d]	29
	58 ± 11 (1)		32 ± 6 (1)[d]	45	**74**	76 ± 6 (3)			
37	38 ± 12 (0.1)	0.40 ± 0.17				100 (10)			
	83 ± 8 (1)		73 ± 7 (1)[d]	12	**75**	32 ± 17 (10)		34, 41 (10)	NS
38	69 ± 6 (1)	0.26 ± 0.09	64 ± 9 (1)	NS[i]		83 ± 11 (100)		80 ± 8 (100)	NS
	90 ± 4 (10)				**76**	37 ± 17 (10)	8.8, 9.8	29 ± 4 (10)[d]	22
39	51 ± 14 (1)	0.60, 1.0	21 ± 5 (1)[d]	44		91 ± 7 (100)			
	91 ± 9 (10)		75 ± 13 (10)[d]	18	**77**	32 ± 12 (10)			
40	65 ± 11 (1)	0.25 ± 0.08	46 ± 8 (1)[d]	29		87 ± 4 (100)			
	96 ± 8 (10)		89 ± 14 (10)	NS	**78**	8 ± 1 (10)			
41	73 ± 10 (1)	0.27 ± 0.1	53 ± 7 (1)[d]	25	**79**	49 ± 7 (1)	0.80, 1.0, 1.9	22 ± 11 (1)[d]	55
	98 ± 5 (10)		93 ± 10 (10)	NS		87 ± 8 (10)			
42	73 ± 7 (1)		47 ± 8 (1)[d]	36	**80**	27 ± 16 (0.1)	0.43, 0.65		
43	53 ± 15 (1)	1.1				74 ± 9 (1)		17 ± 20 (1)[d]	77
	92 ± 14 (10)				**81**	52 ± 19 (1)	1.4		
44	32 ± 11 (1)					77, 100 (10)			
	60 ± 7 (10)				**82**	55 ± 10 (1)		9 ± 17 (1)[d]	84
45	72 ± 22 (1)	0.72 ± 0.38			**83**	38 ± 1 (0.1)	0.22 ± 0.03		
	100 (10)		69 ± 8 (10)[d]	31		78 ± 12 (1)		32 ± 10 (1)[d]	59
48	57 ± 12 (1)				**84**	54 ± 2 (1)	0.75		
	84 ± 5 (10)				**85**	17 ± 8 (1)	3.8, 5.9		
49	24 ± 5 (0.1)	0.70, 0.80				68 ± 8 at (10)			
	56 ± 9 (1)		12 ± 21 (1)[d]	77	**86**	40 ± 19 (1)	0.67, 1.2	28 ± 25 (1)	NS
50	39, 33 (1)		5 ± 4 (1)	86		88 ± 11 (10)			
51	73 ± 9 (1)		57 ± 12 (1)[d]	22	**87**	63 ± 6 (1)	0.46, 0.82	49 ± 7 (1)[d]	21
53	27 ± 12 (0.1)	0.84, 1.0			**88**	68 ± 14 (1)[h]			
	71 ± 10 (1)		40 ± 20 (1)[d]	46	**89**	9 ± 10 (1)			
55	29 ± 15 (1)	2.07 ± 0.71				15 ± 4 (10)			
	86 ± 11 (10)		43 ± 8 (10)[d]	50	**90**	31 ± 19 (1)	2.4		
56	39 ± 7 (10)					78 ± 4 (10)			
57	35 ± 4 (10)				**95**	56 ± 6 (1)		30 ± 8 (1)[d]	46
					96	71 ± 5 (1)		47 ± 7 (1)[d]	34

[a] Mean ± SD of three or more separate determinations. [b] IC_{50} value interpolated from concentration−inhibition curves. [c] Mean ± SD of three or more separate determinations in the presence of 5 mg/mL human AGP. [d] Significantly different from the level of inhibition observed in the absence of 5 mg/mL AGP ($p \leq 0.05$). [e] Not significantly different from no drug control. [f] No activity at 100 μM. [g] No activity at 10 μM. [h] Limit of solubility. [i] NS no significant reduction in inhibition by AGP. Statistical analyses were performed by the Student's *t*-test (two-tailed) paired or unpaired as appropriate.

to define the contribution of the 2,6- and 4,8-substituents to NT inhibitory activity. As previously reported,[1,37] DP was found in the current studies to be a potent NT inhibitor with an IC_{50} value of 0.34 μM for inhibition of ^{3}H-thymidine uptake into L1210 murine leukemia cells. Consistent with our previous observations,[1,37] the addition of 5 mg/mL AGP essentially abolished the activity of DP, reducing the inhibition of ^{3}H-thymidine uptake at DP concentrations of 1 and 10 μM by 87% and 96%, respectively. 2,4,6,8-Tetrachloropyrimidopyrimidine (**8**) was inactive, as were the 4,8-dipiperidino (**26**) and 4,8-dibenzylamino (**12**) derivatives, which retain chloro groups at the 2,6-positions. Interestingly, modest but significant inhibitory activity was observed for the 4,8-dibenzylamino derivative **27** in which H atoms are present at the 2,6-positions, suggesting that benzy-

lamino might substitute for piperidino at the pyrimidopyrimidine 4,8-positions. Hydroxylation at the piperidine 3′-position (**30**) reduced potency approximately 7-fold compared with DP, while transposition of the 2,6- and 4,8-substituents of DP was also detrimental, with the isomeric **31** having an IC_{50} value in the range 1−10 μM. These observations indicated that the DP pharmacophore is intolerant of dramatic structural modifications and suggested that defined molecular interactions arise between the DP pharmacophore and hitherto poorly characterized binding sites on the nucleoside transporters. Subsequent investigations were thus focused on more subtle structural modifications at the 2,6- and 4,8-positions and also the possibility of replacing the 4,8-piperidino groups by substituted benzylamino groups. The effect of replacing the 2,6-amino functions of DP by alkoxy substituents was also studied in the expectation that reducing the basicity of DP ($pK_a = 6.4$) might lower the avidity of binding to AGP.

Preliminary evidence for the importance of the side chain hydroxyl functions of DP was provided by the 2,6-diethylamino derivative **28**, which was inactive at 10 μM, while simple alkoxy substitution at the 2,6-positions gave compounds **56**−**61** with IC_{50} values greater than 10 μM. The introduction of allyloxy groups at the 2,6-positions (**64**) resulted in a modest increase in potency compared with simple alkoxy substitution (**58**). Although methylation of the hydroxyl substituents (**55**) or replacement of diethanolamino by 2′,3′-dihydroxypropylamino (**29**) reduced potency compared with the parent DP, these derivatives nevertheless exhibited IC_{50} values in the low micromolar range, prompting further SAR studies.

Pyrimidopyrimidines with 2′,3′-dihydroxypropoxy groups at the 2,6-positions (**75**−**77**) retained activity ($IC_{50} \approx 10$ μM). An increase in potency was observed with the 2′-hydroxyethoxy or 3′-hydroxypropoxy derivatives (**69** and **70**) and especially with the 2′-hydroxypropoxy derivative **71** ($IC_{50} \approx 1$ μM). Alkylation of the side chain hydroxyl functions (**65**−**68**) was also tolerated without detriment to activity (compare **65** with **69**, and **67**/**75** with **68**/**76**), as was incorporation of the alkoxy groups within a dioxolane ring (**73** and **74**). Taken together, these data suggest that a minimum of one hydroxyl or one alkoxy group is required in the side chain at the pyrimidopyrimidine 2,6-positions. Where evaluated (**67**/**68**, **73**/**74**, **75**−**77**), the stereochemistry (*R* or *S*) at the 2′-position did not appear to influence NT-inhibitory activity. Compared with DP and the 2,3-dihydroxypropylamino derivative (**29**), compounds with substituted alkoxy groups at the 2,6-positions (**67**, **71**, **73**, **75**, **76**) are less susceptible to AGP binding. Indeed, for the 2′,3′-dihydroxypropoxy derivative **75**, there was no significant reduction in activity in the presence of 5 mg/mL AGP.

As expected, the modest potency observed for the 4,8-dibenzylaminopyrimidopyrimidine **27** was enhanced when hydroxyalkylamino groups were introduced at the 2,6-positions (**32**, **35**), this effect being most pronounced with a 2′-hydroxyethylamino substituent (**32**, $IC_{50} = 0.75$ μM). Substitution by alkoxy groups on the benzylamino ring of **32** and **35** improved potency still further. Thus, whereas a 4′-methoxybenzylamino group (**33**, **36**) was tolerated, 3′,4′-dimethoxybenzylamino (**34**, **37**) or piperonylamino (**38**) functions at the 4,8-positions conferred potency at least comparable with that of DP, inhibitors bearing a 2′-hydroxypropylamino group at the 2,6-positions (**37**, **38**) proving especially potent. Crucially, alkoxybenzylamino substitution at the 4,8-positions attenuated AGP binding. Thus, whereas the activity of the 4′-methoxybenzylamino derivatives **33** and **36** was reduced by 40% and 45%, respectively, by AGP, the 3′,4′-dimethoxybenzylamino derivatives **34** and **37** were less susceptible again (23% and 12% reduction, respectively). Importantly, the piperonylamino derivative **38** retained inhibitory activity comparable to that of DP and **37** in the presence of 5 mg/mL AGP, with no detectable reduction in potency being observed.

Analogous studies with 4,8-dibenzylamino and substituted dibenzylamino groups on pyrimidopyrimidines bearing the 2,6-bis-diethanolamino motif of DP (**39**−**45**) followed a general trend similar to that observed for 2′-hydroxyethylamino or 2′-hydroxypropylamino substituents at the pyrimidipyrimidine 2,6-positions. Thus, the submicromolar NT-inhibitory activity of the 4,8-dibenzylamino derivative **39** was further enhanced by alkoxy group substitition on the benzyl ring, with compounds **40**−**42** exhibiting NT-inhibitory activity at least comparable with that of DP. Derivatives bearing a 4′-chloro- (**43**) or 4′-trifluoromethyl (**44**) group on the benzyl ring were somewhat less potent. The respectable activity of the benzylamino "ring-constrained" analogue **45** ($IC_{50} = 0.72 \pm 0.38$ μM), notwithstanding the increased bulk of the molecule, suggests a hydrophobic binding pocket of substantial size or flexibility.

Perhaps more importantly, several of these inhibitors again showed a markedly reduced susceptibility to the effects of AGP compared with DP. Thus, at concentrations of 10 μM, the NT-inhibitory activity of **40** was reduced by less than 10% in the presence of 5 mg/mL AGP while this concentration of AGP had no significant effect on the activity of **41**. Although the activity of **45** was reduced by approximately 30% in the presence of AGP, indicative of some binding, the compound clearly has a reduced affinity for the AGP compared with DP. On the basis of these very encouraging results, compounds **37**, **38**, **40**, and **41** were selected for more detailed biological evaluation.

The possibility that a 4,8-tertiary amine function on the pyrimidopyrimidine (e.g., piperidino in DP) might contribute to AGP binding was investigated through the synthesis and evaluation of derivatives bearing *N*-benzylmethylamino- or N-substituted benzylmethylamino groups at the pyrimidine 4,8-positions (**46**−**53**). No clear SARs could be identified for NT inhibition, with both an enhancement (**35** versus **49**) and reduction (**40** versus **53**) of potency being observed. However, tertiary amino groups at the 4,8-positions did appear to augment AGP binding where a direct comparison could be made, for example, the greater reduction in activity seen for the *N*-methyl derivatives **50** and **51** (86% and 22%, respectively) in the presence of AGP compared with the parent inhibitors **36** and **37** (45% and 12%, respectively).

Finally, having established that pyrimidopyrimidines with substituted alkoxy groups at the 2,6-positions exhibit good NT-inhibitory activity and a reduced af-

finity for AGP and that alkoxy-substituted benzylamino groups at the 4,8-positions confer potent inhibitory activity, this substituent pattern was combined with a view to further optimize biological activity. Replacement of 2′-hydroxyethylamino by 2′-hydroxyethoxy at the 2,6-positions of inhibitors with 4′-methoxybenzyl (**33** and **86**) or 3′,4′-dimethoxybenzyl (**34** and **95**) groups at the 4,8-positions had a negligible effect on potency, although replacing 2′-hydroxypropylamino by 2′-hydroxypropoxy at the 2,6-positions of pyrimidopyrimidines bearing a 4′-methoxybenzyl group at the 4,8-positions did enhance potency (compare **36** and **88**). The nature of the hydroxyalkoxy group at the 2,6-positions of 4,8-bis(4′-methoxybenzylamino)pyrimidopyrimidines also had little effect on activity, compounds **86**−**88** proving approximately equipotent with IC_{50} values of 1 μM or less. A comparison of the 4,8-bis(4′-methoxybenzylamino) (**86**, **87**) and 4,8-bis(3′,4′-dimethoxybenzylamino) (**95**, **96**) derivatives reveals a similar trend, with homologation of the hydroxyalkoxy groups at the 2,6-positions having no marked effect on potency.

Derivatives that combined hydroxyalkoxy groups at the 2,6-positions with tertiary amines at the 4,8-positions (**78**−**85**) were in some instances more potent inhibitors of nucleoside transport than those bearing secondary amines. For example, compound **83**, with *N*-(4′-methoxybenzyl)methylamino groups at the 4,8-positions, was approximately 2-fold more active than the corresponding 4,8-bis(4′-methoxybenzylamino) derivative **87**. However, consistent with our earlier observations, benzylmethylamino- or N-substituted benzylmethylamino substitution was found to enhance AGP binding, the activities of pyrimidopyrimidines **80**, **82**, and **83** being reduced by 77%, 84%, and 59%, respectively, in the presence of 5 mg/mL AGP. Where direct comparisons were possible, the effect of AGP again appeared to be more pronounced for tertiary amines than the corresponding secondary amines (compare **80** with **86**, and **83** with **87**).

In summary, our SAR studies have demonstrated that replacement of the piperidino groups at the 4,8-positions of DP by 4′-methoxybenzylamino, 3′,4′-dimethoxybenzylamino, or piperonylamino substituents affords derivatives with potency comparable to that of the parent compound. Substitution of the diethanolamino groups at the pyrimidopyrimidine 2,6-positions of DP by monoalkylamino or alkoxy substituents is tolerated, although at least one hydroxyl or alkoxy function is required in the side chain for activity comparable to that of the parent compound. The influence of AGP on the activity of the majority of the derivatives studied was less pronounced than observed for DP, with compounds bearing a substituted benzylamino group at the pyrimidopyrimidine 4,8-positions proving particularly resistant. However, the introduction of a tertiary amino function (*N*-methylbenzylamino or substituted *N*-methylbenzylamino) at the 4,8-positions did in some cases restore susceptibility to the effects of AGP.

Conclusions

The aims of the study described in this paper were to develop pyrimidopyrimidine-based nucleoside transport inhibitors, which retain the potency of DP in the absence of significant AGP binding. Structure−activity studies conducted at the pyrimidopyrimidine 2,6- and 4,8-positions have resulted in the identification of alternative substituents that confer potency and have demonstrated that inhibition of nucleoside transport and AGP binding are properties that can be divorced. Accordingly, a number of compounds with potency comparable to that of DP, but only limited susceptibility to the effects of AGP, have been identified. Compounds **40**, **55**, **67**, and **73** have been shown to prevent thymidine rescue from the cytotoxic effects of the thymidylate synthase inhibitor nolatrexed in vitro, with **40** proving at least equipotent with DP in these resistance modulation experiments.[44] Compounds **37**, **40**, and **41** have also been the subject of further detailed biological evaluation, which has shown that they can overcome the ability of thymidine and hypoxanthine to prevent the growth inhibitory activity of the new antifolate drug pemetrexed (MTA).[1] Their ability to reverse thymidine and hypoxanthine rescue was directly related to their NT inhibitory potency (Figure 1). Furthermore, as predicted from the data described in this paper, AGP has only a limited effect on the cellular pharmacology of these compounds (Figure 2). Thus, a 20 ± 3% and 46 ± 7% reduction in activity was observed for **37** and **41**, respectively, on inhibition of rescue by AGP, whereas no significant effect was observed for **40**. By contrast, the activity of DP was essentially abolished in the presence of this acute phase serum protein.

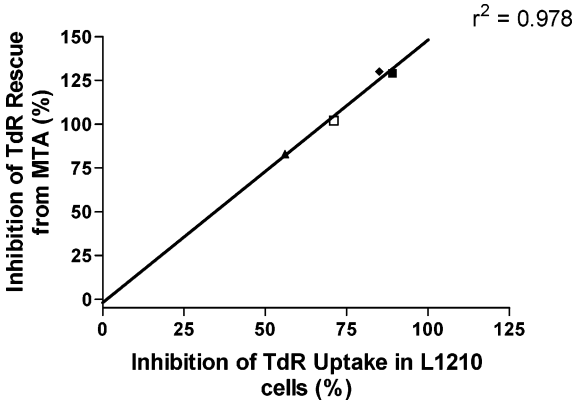


Figure 1. Relationship between the inhibition of TdR rescue from pemetrexed (MTA)-induced growth inhibition in A549 cells and the inhibition of TdR uptake in L1210 cells by 1 μM DP (■), **40** (▲), **41** (□), or **37** (♦). The line is that given by linear regression analysis.

Preliminary pharmacokinetic and pharmacodynamic studies of **37** and **38** have demonstrated that plasma concentrations could be achieved that result in the inhibition of ^{3}H-thymidine uptake into tumors in vivo.[1] Further structural optimization of these promising new compounds is ongoing, with a view to identifying a candidate for clinical evaluation as a resistance modifier in antimetabolite cancer chemotherapy.

Experimental Section

Melting points were obtained on a Kofler hot stage apparatus and are uncorrected. Infrared spectra (IR) were recorded as KBr disks on a Nicolet 20 PC Fourier transform spectrometer. Mass spectra were determined on a Kratos MS80 spectrometer in electron impact (EI) mode or fast atom bombardment (FAB) mode using a *m*-nitrobenzyl alcohol matrix. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at 200 and 50 MHz,

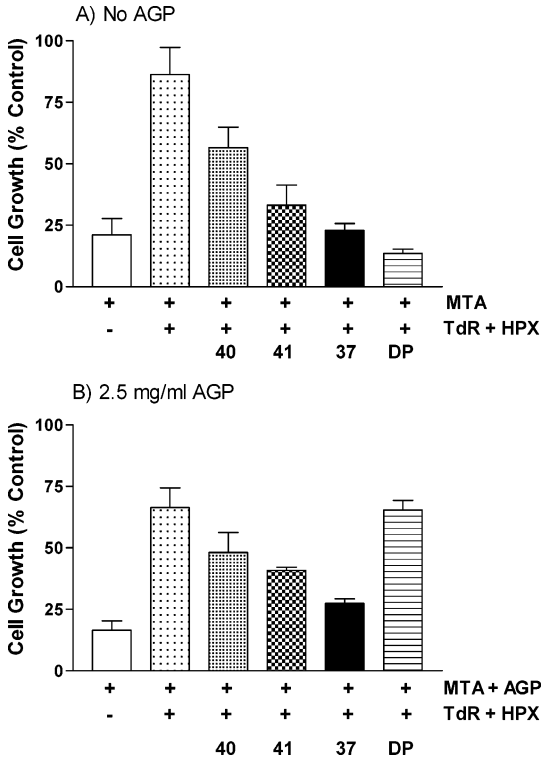


Figure 2. Growth of A549 cells exposed to 7 μM pemetrexed (MTA) + 1 μM thymidine (TdR) + 10 μM hypoxanthine (HPX) + 1 μM **40**, **41**, **37**, or DP in the (A) absence or (B) presence of 2.5 mg/mL AGP for three cell doublings. Data are mean values and SD of three independent experiments.

respectively, on a Bruker WP 200 spectrometer employing TMS or the solvent as the internal standard. Unless indicated otherwise, spectra were recorded in [$^2H_6$]DMSO as solvent. NH signals appeared as broad singlets (br s) exchangeable with D_2O.

The TLC systems employed Merck 1.05554 aluminum sheets precoated with Kieselgel $60F_{254}$ (0.2 mm) as the adsorbent and were visualized with UV light at 254 and 365 nm. Chromatography was conducted under medium pressure on silica (Kieselgel 60, 240−400 mesh). Elemental analyses were performed in house on a Carlo-Erba Instrumentazione 1106 analyzer or by Butterworth Laboratories, Middlesex, U.K., and are within ±0.4% of theory unless otherwise specified. Reagents were purchased from Aldrich Chemical Co., Gillingham, Dorset, U.K., and used as received unless otherwise stated. TBAF refers to a 1 M solution of tetrabutylammonium fluoride in THF. EtOH and MeOH were dried using Mg/I_2 and stored over 4 Å molecular sieves. Diethyl ether and tetrahydrofuran were predried over $CaCl_2$ and distilled from sodium/benzophenone. THF employed for reductive dehalogenation reactions was redistilled from $LiAlH_4$. Petroleum ether refers to that fraction in the boiling range 40−60 °C.

Ethyl (*S*)-2-*O*-(4′-Methoxybenzyl)lactate (1). To a stirred mixture of ethyl (*S*)-lactate (0.12 g, 1 mmol) and freshly prepared silver(I) oxide (0.37 g, 1.6 mmol) in THF (5 mL), was added *p*-methoxybenzyl chloride (0.23 g, 1.5 mmol) in THF (5 mL). The mixture was stirred for 8 h at 25 °C, and solids were removed by filtration under reduced pressure and washed with THF. The solvent was evaporated in vacuo and the product was purified by chromatography on silica, employing petroleum ether/EtOAc (9:1) as eluent, to afford the title compound as a colorless oil (0.99 g, 42%). ^{1}H NMR δ 1.39 (d, 3H, C*H*$_3$-CH), 1.45 (t, 3H, OCH_2CH_3), 3.78 (s, 3H, ArOCH_3), 4.00 (q, 1H, CH$_3$C*H*), 4.19 (q, 2H, OCH_2CH$_3$), 4.31 (d, 1H, ArCH_2), 4.60 (d, 1H, ArCH_2), 6.85 (d, 2H, ArH-*3*, *H-5*), 7.23 (d, 2H, ArH-*2*, *H-6*); MS (EI) *m*/*z* 196 (M$^+$, 93%).

(*S*)-2-*O*-(4′-Methoxybenzyl)propan-1,2-diol (2). A solution of **1** (0.95 g, 4 mmol) in anhydrous THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (0.15 g, 4 mmol) in anhydrous THF (10 mL), and the mixture was stirred for 15 min at 25 °C. After sequential addition of H_2O (1 mL), NaOH (2.0 M, 3 mL), and H_2O (1 mL), the suspension was filtered and washed with THF, and the combined solvents were removed in vacuo to give the title compound as a pale-yellow oil (0.83 g, 98%). ^{1}H NMR δ 1.14 (d, 3H, C*H*$_3$CH), 3.55 (m, 2H, CH_2OH), 3.55 (m, 1H, CH$_2$O*H*), 3.78 (s, 3H, ArOCH_3), 4.39 (d, 1H, ArCH_2), 4.55 (d, 1H, ArCH_2), 6.85 (d, 2H, ArH-*3*, *H-5*), 7.25 (d, 2H, ArH-*2*, *H-6*).

2-Triisopropylsilyloxypropan-1-ol (3). A solution of 2-triisopropylsilyloxy-3-butene[41] (3.87 g, 17.9 mmol) in CH_2Cl_2 (30 mL) was ozonized at −20 °C for 2 h. After removal of excess ozone, the reaction mixture was cooled to 0 °C and a solution of sodium borohydride (5.56 g, 146 mmol) in 50% aqueous EtOH (40 mL) was added cautiously. The mixture was refluxed for 3 h and cooled, and H_2O (30 mL) was added. After extraction with CH_2Cl_2 (4 × 25 mL), the combined solvents were dried (Na_2SO_4) and removed in vacuo. The product was purified by chromatography on silica with petroleum ether/EtOAc (9:1) as eluent to yield the title compound (2.14 g, 51%) as a colorless oil. IR 3387, 2959, 2944 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.98 (m, 21H, 21 × ^{i}Pr-*H*), 1.01 (d, 3H, CH$_3$), 2.50 (br s, 1H, OH), 3.36 (dd, 1H, CHCH_2OH), 3.62 (dd, 1H, CHCH_2OH), 3.80 (m, 1H, C*H*CH$_2$OH); MS (EI) *m*/*z* 233 (MH$^+$). Anal. ($C_{12}H_{28}O_2$-Si) C, H.

***N*-Benzyl-*N*-3,4-dimethoxybenzylimine (4).** Benzylamine (0.98 mL, 9.0 mmol) was added dropwise to a solution of 3,4-dimethoxybenzaldehyde (1.50 g, 9.0 mmol) in dry EtOH (15 mL) under a nitrogen atmosphere. The pale-yellow solution was refluxed for 1 h and allowed to cool to room temperature, and the solvents were removed in vacuo to afford a brown oil. Trituration with MeOH gave the imine **4** (2.19 g, 95%) as a pale-yellow solid, mp 50−52 °C. IR 3001, 2959, 2837 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 6H, 2 × OCH_3), 4.79 (s, 2H, PhCH_2), 7.17 (m, 8H, 8 × Ar-*H*), 8.28 (s, 1H, PhC*H*=N); MS (EI) *m*/*z* 256 (MH$^+$). Anal. ($C_{16}H_{17}NO_2$) C, H, N.

***N*-Benzyl-*N*-3,4-dimethoxybenzylamine (5).** To a stirred solution of **4** (1.00 g, 3.92 mmol) in dry MeOH (10 mL) under N_2 was added $NaBH_4$ (0.15 g, 3.92 mmol) in portions over 15 min. The reaction mixture was heated under reflux for 1 h and cooled, and solvents were removed in vacuo to give a pale-yellow liquid, which was suspended in the minimum amount of H_2O. The aqueous suspension was acidified to pH 3 with aqueous HCl (0.5 M) and washed with ether (5 × 30 mL), and the aqueous layer was basified to pH 12 with aqueous NaOH (5 M) and extracted with ether (5 × 40 mL). The combined organic layer was removed in vacuo to furnish **5** (0.85 g, 84%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.80 (br s, 1H, N*H*), 3.74 (s, 2H, (MeO)$_2$PhCH_2), 3.79 (s, 2H, PhCH_2NH), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.86 (m, 3H, 3 × Ar-*H*), 7.30 (m, 5H, 5 × Ar-*H*).

***N,N*-Bis(3,4-Dimethoxybenzyl)imine (6).** Reaction of 3,4-dimethoxybenzylamine (1.80 mL, 12.0 mmol) with 3,4-dimethoxybenzaldehyde (2.00 g, 12.0 mmol) as described for **4** above afforded **6** as a white solid (3.69 g, 97%), mp 77−79 °C. IR 3009, 2925, 2839, 1636 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.67 (s, 2H, PhCH_2), 8.21 (s, 1H, PhC*H*=N); MS (EI) *m*/*z* 316 (MH$^+$). Anal. ($C_{18}H_{21}O_4N$) C, H, N.

***N,N*-Bis(3,4-Dimethoxybenzyl)amine (7).** Treatment of **6** (3.50 g, 11.1 mmol) with sodium borohydride (0.42 g, 11.1 mmol) in MeOH, as described for **4**, gave **7** as a white solid (3.10 g, 88%), mp 69−71 °C. IR 2961, 2839, 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.51 (br s, 1H, N*H*), 3.68 (s, 4H, 2 × PhCH_2), 3.81 (s, 6H, 2 × OCH$_3$), 3.82 (s, 6H, 2 × OCH$_3$), 6.78 (m, 6H, 6 × Ar-*H*); MS (EI) *m*/*z* 317 (M$^+$). Anal. ($C_{18}H_{23}O_4N$) C, H, N.

2,4,6,8-Tetrachloropyrimido[5,4-*d*]pyrimidine (8).[38,39] To an aqueous solution of sodium hydroxide (0.1 M, 200 mL) was added 1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8-(3*H*,-7*H*)-tetrone (2 g, 10 mmol), and the mixture was boiled for 20 min. After addition of H_2O (150 mL), the solution was filtered hot and was cooled to give the disodium salt as light-yellow needles, which were collected and dried, (1.86 g, 78%). The

finely powdered disodium salt (3 g, 12.5 mmol) was added to a solution of PCl_5 (15 g, 72 mmol) in $POCl_3$ (125 mL, 1.34 mol) with stirring. The mixture was heated under reflux for 8 h, and excess $POCl_3$ was removed under reduced pressure. The yellow-brown residue was carefully added to ice−water (50−60 g), and the solids were collected by filtration, washed with H_2O until acid-free, and dried over KOH at 100 °C. Soxhlet extraction of the residual solid with chloroform (200 mL) and evaporation of the solvent afforded the tetrachloropyrimidopyrimidine (**8**) as yellow platelets: ^{13}C NMR δ 141.9, 157.8, 165.8; MS (EI) *m/z* 268 (M^+, 80%), 236 (100%).

2,6-Dichloro-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidines (9−11). Method I. General Procedure. To a stirred solution of **8** (1.0 mol equiv) in dry THF (15−20 mL) was added the appropriate amine (4.0 mol equiv). The mixture was stirred at room temperature under N_2 until TLC analysis confirmed the absence of starting materials. H_2O (20−30 mL) was added, and the mixture was stirred for a further 20 min. The resultant precipitate was collected by filtration, and the product was purified by chromatography on silica and/or by recrystallization from an appropriate solvent.

2,6-Dichloro-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (9).[38] Compound **9** was prepared from **8** (0.54 g, 2 mmol) and piperidine (0.8 mL, 8 mmol) according to method I. ^{1}H NMR δ 1.70 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 4.18 (s, 8H, 2 × $CH_2NCH_2(CH_2)_3$); MS (EI) *m/z* 366, 368, 370 (9:6:1, M^+).

2,6-Dichloro-4,8-di-(3′-hydroxypiperidino)pyrimido[5,4-*d*]pyrimidine (10). Compound **10** was prepared from **8** (0.2 g, 0.74 mmol) and (*rac*)-3-hydroxypiperidine (0.4 g, 3.9 mmol) according to method I. ^{1}H NMR δ 1.61 (m, 4H, 2 × 5′-H_2), 1.96 (m, 4H, 2 × 4′-H_2), 3.72 (m, 8H, 2 × 2′-H_2, 2 × 6′-H_2), 4.60 (m, 2H, 2 × 3′-*H*), 5.08 (s, 2H, 2 × O*H*); MS (EI) *m/z* 398.

2,6-Dichloro-4,8-bis(diethanolamino)pyrimido[5,4-*d*]pyrimidine (11). Compound **11** was prepared from **8** (0.54 g, 2 mmol) and diethanolamine (0.77 mL, 8 mmol) according to method I. ^{1}H NMR δ 3.82 (s, 8H, 2 × $N(CH_2CH_2OH)_2$), 4.39 (br s, 8H, 2 × $NH(CH_2CH_2OH)_2$, 4.86 (br s, 4H, 4 × OH); MS (EI) *m/z* 407 (M^+).

2,6-Dichloro-4,8-dibenzylaminopyrimido[5,4-*d*]pyrimidines (12−24). Method II. General Procedure. The appropriate benzylamine (5 mol equiv) was added to a stirred solution of **8** (1 mol equiv) in dry THF (15−20 mL) containing K_2CO_3 (7.5 mol equiv). The reaction mixture was stirred (20 min to 1 h) at room temperature under N_2 until TLC analysis confirmed the absence of starting materials. H_2O (20−30 mL) was added, the reaction mixture was stirred for 20 min, and the precipitated solid was collected by filtration. Chromatography on silica and/or recrystallization from an appropriate solvent afforded the target compound.

2,6-Dichloro-4,8-dibenzylaminopyrimido[5,4-*d*]pyrimidine (12). Compound **12** was synthesized according to method II from **8** (0.27 g, 1.0 mmol) and benzylamine (0.44 mL, 4.0 mmol). IR 3386, 3234, 1570 cm^{-1}; ^{1}H NMR δ 4.75 (d, 4H, 2 × PhCH_2NH), 7.26 (t, 2H, 2 × NH), 7.33 (m, 10H, 10 × Ar-*H*); MS (EI) *m/z* 410, 412, 414 (9:6:1).

2,6-Dichloro-4,8-di-(4′-chlorobenzylamino)pyrimido[5,4-*d*]pyrimidine (13). Compound **13** was prepared as described in method II from **8** (0.14 g, 0.50 mmol) and 4-chlorobenzylamine (0.26 mL, 2 mmol). IR 3150 cm^{-1}; ^{1}H NMR δ 4.66 (d, 4H, 2 × CH_2Ar), 7.42 (s, 8H, 8 × Ar-*H*), 9.41 (t, 2H, 2 × N*H*); MS (EI) *m/z* 480 (M^+).

2,6-Dichloro-4,8-di-(4′-trifluoromethylbenzylamino)pyrimido[5,4-*d*]pyrimidine (14). Compound **9** was synthesized according to method II from **8** (0.14 g, 0.50 mmol) and 4-trifluoromethylbenzylamine (0.28 mL, 2 mmol). ^{1}H NMR δ 4.71 (d, 4H, 2 × CH_2Ar), 7.63 (d, 4H, 4 × Ar-*H*), 7.77 (d, 4H, 4 × Ar-*H*), 9.51 (t, 2H, 2 × NH); MS (EI) *m/z* 545 ($M^+ - 1$).

2,6-Dichloro-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (15). Compound **15** was synthesized according to method II from **8** (0.14 g, 0.50 mmol) and 4-methoxybenzylamine (0.27 mL, 2.0 mmol). IR 3319, 3032, 1574 cm^{-1}; ^{1}H NMR δ 3.92 (s, 6H, 2 × OCH_3, 4.76 (s, 4H, 2 × NHCH_2Ph), 6.98 (d, 4H, 2 × Ar*H-2, H-6*, *J* = 8.5), 7.40 (d, 4H, 2 × Ar*H-3, H-5*, *J* = 8.5), 9.40 (br s, 2H, 2 × NH); MS (EI) *m/z* 470, 472,474 (9:6:1, M^+).

2,6-Dichloro-4,8-bis-(3′,4′-dimethoxybenzylamino)-pyrimido[5,4-*d*]pyrimidine (16). Compound **16** was prepared according to method II from **8** (0.40 g, 1.48 mmol) and 3,4-dimethoxybenzylamine (1.11 mL, 7.4 mmol). IR 3287, 2957, 2934, 2838, 1579 cm^{-1}; ^{1}H NMR δ 3.86 (s, 6H, 2 × OCH_3), 3.87 (s, 6H, 2 × OCH_3), 4.67 (d, 4H, 2 × PhCH_2, *J* = 5.8), 6.84 (m, 6H, 6 × Ar-*H*), 7.13 (t, 2H, 2 × N*H*, *J* = 5.6); MS (EI) *m/z* 530, 532, 534 (9:6:1, M^+).

2,6-Dichloro-4,8-di-(3′,4′-dioxymethylenebenzylamino)-pyrimido[5,4-*d*]pyrimidine (17). Compound **17** was prepared from **8** (0.50 g, 1.85 mmol) and piperonylamine (1.15 mL, 9.3 mmol) according to method II. IR 3313, 2904, 2779, 1578 cm^{-1}; ^{1}H NMR δ 4.62 (s, 4H, 2 × PhCH_2), 6.07 (s, 4H, 2 × OCH_2O), 6.94 (s, 4H, 2 × Ar*H-5, H-6*), 7.04 (s, 2H, 2 × Ar *H-2*), 9.28 (br s, 2H, 2 × NH); MS (EI) *m/z* 498, 500, 502 (M^+, 9:6:1).

2,6-Dichloro-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido-[5,4-*d*]pyrimidine (18). Compound **18** was synthesized following method II from **8** (0.30 g, 1.11 mmol) and *N*-benzylmethylamine (0.71 mL, 5.6 mmol). IR 3025, 2979, 2872, 1547 cm^{-1}; ^{1}H NMR δ 3.49 (br s, 6H, 2 × NCH_3), 5.49 (br s, 4H, 2 × PhCH_2), 7.25 (s, 10H, 10 × Ar-*H*); MS (EI) *m/z* 438, 440, 442 (M^+, 9:6:1).

2,6-Dichloro-4,8-di-(*N*-4′-methoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (19). Compound **19** was prepared as detailed in method II from **8** (0.65 g, 2.42 mmol) and *N*-(4′-methoxybenzyl)-*N*-methylamine (1.78 g, 12.1 mmol). IR 2925, 2854, 1545 cm^{-1}; ^{1}H NMR δ 1.23 (s, 6H, 2 × NCH_3), 3.60 (br s, 4H, 2 × CH_2PhOMe), 3.78 (s, 6H, 2 × OCH_3), 6.84 (d, 4H, 2 × Ar *H-2*, *H-6*, *J* = 8.8), 7.26 (d, 4H, 2 × Ar *H-3*, *H-5*, *J* = 8.8); MS (EI) *m/z* 498, 500, 502 (M^+, 9:6:1).

2,6-Dichloro-4,8-bis(*N*-3′,4′-dimethoxy-*N*-methylaminobenzyl)pyrimido[5,4-*d*]pyrimidine (20). Compound **20** was prepared from **8** (0.60 g, 2.23 mmol) and *N*-methyl-*N*-3,4-dimethoxybenzylamine (2.02 g, 11.2 mmol) according to method II. IR 2959, 2941, 1507 cm^{-1}; ^{1}H NMR δ 3.44 (br s, 6H, 2 × NCH_3), 3.81 (s, 12H, 4 × OCH_3), 5.25 (br s, 4H, 2 × PhCH_2), 6.82 (m, 6H, 6 × Ar-*H*); MS (EI) *m/z* 558, 560, 562 (9:6:1, M^+).

2,6-Dichloro-4,8-bis(dibenzylamino)pyrimido[5,4-*d*]pyrimidine (21). Treatment of **8** (0.27 g, 1.0 mmol) with dibenzylamine (1.54 mL, 8.0 mmol) as detailed in method II afforded compound **21**. ^{1}H NMR δ 4.97 (br s, 4H, 4 × NC*H*(H)-Ar), 5.63 (br s, 4H, 4 × NCH(*H*)Ar), 7.32 (m, 20H, 4 × Ph); MS (EI) *m/z* 590, 592, 594 (9:6:1, M^+).

2,6-Dichloro-4,8-di-(*N*-benzyl-*N*-3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (22). Compound **22** was prepared according to method II from **8** (0.18 g, 0.66 mmol) and *N*-benzyl-*N*-3,4-dimethoxybenzylamine (0.85 g, 3.3 mmol). IR 3063, 2929, 2838, 1517 cm^{-1}; ^{1}H NMR δ 3.83 (s, 6H, 2 × OCH_3), 3.86 (s, 6H, 2 × OCH_3), 4.90 (br s, 4H, 2 × $(MeO)_2C_6H_3CH_2$), 5.46 (br s, 4H, 2 × PhCH_2), 6.95 (m, 6H, 2 × $(MeO)_2C_6H_3$), 7.32 (s, 10H, 10 × Ar-H); MS (EI) *m/z* 710, 712, 714 (9:6:1, M^+).

2,6-Dichloro-4,8-bis[di-*N*,*N*-(3′,4′-dimethoxybenzyl)amino]pyrimido[5,4-*d*]pyrimidine (23). Compound **23** was synthesized according to method II from **8** (0.26 g, 0.98 mmol) and *N*,*N*-3,4-dimethoxybenzylamine (1.24 g, 3.9 mmol). IR 3001, 2933, 2834, 1517, cm^{-1}; ^{1}H NMR δ 3.78 (s, 12H, 4 × OCH_3), 3.81 (s, 12H, 4 × OCH_3), 4.77 (br s, 4H, 2 × $[MeO]_2C_6H_3CH_2$), 5.33 (br s, 4H, 2 × $[MeO]_2C_6H_3CH_2$), 6.78 (m, 12H, 4 × $[MeO]_2C_6H_3CH_2$); MS (EI) *m/z* 830, 832, 834 (9:6:1, M^+).

2,6-Dichloro-4,8-di-(*N*-1,2,3,4-tetrahydroisoquinolyl)-pyrimido[5,4-*d*]pyrimidine (24). Treatment of **8** (0.54 g, 1.85 mmol) with 1,2,3,4-tetrahydroisoquinoline (1.0 mL, 7.5 mmol) as detailed in method II afforded compound **25**. ^{1}H NMR δ 3.00 (t, 4H, 2 × CH_2), 3.31 (t, 4H, 2 × CH_2), 4.24 (s, 4H, 2 × CH_2), 7.22 (m, 8H, 8 × Ar-H); MS (EI) *m/z* 462, 464, 466 (9:6:1, M^+).

2,6-Dichloro-4,8-bis([*R*,*S*]-2′,2′-dimethyl-1′,3′-dioxolane-4′-methoxy)pyrimido[5,4-*d*]pyrimidine (25). To a solution of (*rac*)-2,2-dimethyl-1,3-dioxolane-4-methanol (0.28 mL, 2.2

mmol) in dry THF (20 mL) was added NaH (0.01 g, 2.2 mmol). The suspension was stirred at 50−60 °C for 10 min and cooled to room temperature, and **8** (0.27 g, 1.0 mmol) was added. After a further 30 min, H_2O (50 mL) was added and the mixture was extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were washed with H_2O and dried ($MgSO_4$), and the solvent was removed. Trituration of the residue with EtOH (10 mL) furnished the crude product as a yellow syrup. ^{1}H NMR δ 1.35 and 1.44 (2 × s, 12H, 4 × CH_3), 3.92 (m, 2H), 4.16 (m, 2H), 4.62 (m, 6H); MS (EI) *m*/*z* 461, 463, 465 (9:6:1, M^+).

4,8-Dipiperidinopyrimido[5,4-*d*]pyrimidine (26).[38] To a solution of **9** (0.37 g, 1 mmol) in THF, freshly redistilled from $LiAlH_4$ (45 mL), was added KOH pellets (0.2 g) and 10% Pd on carbon (0.2 g). After hydrogenation in a Parr apparatus (50 psi) for 2 days, the catalyst was removed by filtration, and the solvent was evaporated to give the product as a white powder. ^{1}H NMR δ 1.66 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 4.14 (s, 8H, 2 × $CH_2NCH_2(CH_2)_3$), 8.38 (s, 2H, 2-H, 6-H); MS (FAB) 299 (MH^+).

4,8-Dibenzylaminopyrimido[5,4-*d*]pyrimidine (27). To a solution of **12** (0.36 g, 0.9 mmol) in THF (10 mL, redistilled from $LiAlH_4$) was added Pd on activated carbon (10%, 0.2 g) and KOH pellets (0.2 g). Hydrogenolysis was conducted in a Parr apparatus (20 psi) for 4 days, with addition of fresh catalyst (0.4 g) after 2 days. After filtration, the solvent was evaporated and the residual solid was purified by chromatography on silica to furnish **27** as a white powder. IR 3358, 3057, 1585 cm^{-1}; ^{1}H NMR δ 4.82 (d, 4H, 2 × CH_2, J = 6.4), 7.43 (m, 10H, 2 × Ph), 8.55 (s, 2H, 2-H, 6-H), 8.97 (t, 2H, 2 × NH, J = 6.4); MS (FAB) *m*/*z* 343 (MH^+).

2,6-Bis(diethylamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (28). A mixture of diethylamine (10 mL) and **9** (0.37 g, 1.0 mmol) in dry THF (10 mL) was heated at 200 °C in a bomb reaction vessel for 4 days. Volatiles were removed under reduced pressure, and the residue was redissolved in H_2O (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with H_2O and dried ($MgSO_4$). Evaporation of the solvent gave a brown liquid, which was purified by chromatography on silica to furnish **28** as a fluorescent yellow solid. ^{1}H NMR δ 1.19 (t, 12H, 2 × NH-$(CH_2CH_3)_2$, J = 7), 1.72 (s, 12H, 2 × $CH_2NHCH_2(CH_2)_3$), 3.56 (q, 8H, 2 × N(CH_2CH_3)$_2$, J = 7), 4.16 (s, 8H, 2 × CH_2NCH_2-$(CH_2)_3$); MS (EI) 440 (M^+).

2,6-Dialkylamino-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidines (29−54). Method III. General Procedure. The appropriate 2,6-dichloro-4,8-disubstituted pyrimido[5,4-*d*]pyrimidine (0.1−1.5 mmol) was dissolved in an excess (1−10 mL) of the required amine, and the mixture was stirred at 100−150 °C, until TLC analysis confirmed the absence of starting materials (typically 24−48 h). After the mixture was cooled, the crude product was precipitated by addition of H_2O (30−50 mL) and collected by filtration and/or extraction with EtOAc. The product was purified by chromatography on silica and/or recrystallization from an appropriate solvent.

2,6-Bis(2′,3′-Dihydroxypropylamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (29). Treatment of **9** (0.15 g, 0.40 mmol) with 3-amino-1,2-propanediol (8 mL) according to method III gave compound **29** as a mixture of stereoisomers. ^{1}H NMR δ 1.74 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.28 (m, 2H, CH_2), 3.44 (m, 6H, 3 × CH_2), 3.72 (m, 2H, 2 × CH), 4.14 (m, 4H, 2 × $CH_2NH(CH_2)_3$) 4.65 (t, 2H, 2 × OH), 4.85 (d, 2H, 2 × OH), 6.09 (t, 2H, 2 × NH); MS (EI) 476 (M^+).

2,6-Bis(diethanolamino)-4,8-di-(3′-hydroxypiperidino)pyrimido[5,4-*d*]pyrimidine (30). Compound **30** was obtained as a mixture of stereoisomers, following method III, from **10** (0.08 g, 0.80 mmol) and diethanolamine (1.2 mL). ^{1}H NMR δ 1.54−1.60 (2 × t, 4H, 2 × 5′-H_2), 1.90 (m, 4H, 2 × 4′-H_2), 3.14 (m, 4H), 3.69 (m, 20H, 2 × N(CH_2CH_2OH)$_2$, 2′-H_2, 6′-H_2), 4.85 (s, 4H), 4.96 (d, 2H, 2 × 3′-H), 5.09 (br s, 2H); MS (FAB) 537 ($M^+ + 1$).

2,6-Dipiperidino-4,8-bis(diethanolamino)pyrimido[5,4-*d*]pyrimidine (31).[38] Compound **31** was prepared from **11** (0.20 g, 0.50 mmol) and piperidine (3.0 mL) according to method III. ^{1}H NMR δ 1.55 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.31−4.03 (m, 24H); ^{13}C NMR δ 24.81, 25.76, 45.86, 52.47, 60.05, 132.02, 154.38, 159.91; MS (EI) 504 (M^+).

2,6-Diethanolamino-4,8-dibenzylaminopyrimido[5,4-*d*]pyrimidine (32). Compound **32** was prepared from **12** (0.32 g, 0.78 mmol) and 2-aminoethanol (2 mL) according to method III. IR 3396, 3058, 2920, 1527 cm^{-1}; ^{1}H NMR δ 3.44 (m, 4H, 2 × $NHCH_2CH_2OH$), 3.58 (m, 4H, 2 × $NHCH_2CH_2OH$), 4.67 (t, 2H, 2 × OH, J = 5.2), 4.74 (d, 4H, 2 × PhCH_2, J = 6.3), 6.10 (t, 2H, 2 × NH, J = 5.8), 7.74 (t, 2H, 2 × NH, J = 5.8); MS (EI) 460 (M^+).

2,6-Diethanolamino-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (33). Compound **33** was prepared by method III from **15** (0.60 g, 1.27 mmol) and 2-aminoethanol (2 mL). IR 3403, 2925, 2861, 1568 cm^{-1}; ^{1}H NMR δ 3.51 (m, 8H, 2 × $NHCH_2CH_2OH$), 3.77 (s, 6H, 2 × OCH_3), 4.59 (d, 4H, 2 × MeOArCH_2, J = 6.0), 5.09 (t, 2H, 2 × OH, J = 6.0), 6.67 (t, 2H, 2 × NH, J = 6.0), 6.84 (d, 4H, 2 × Ar-H-3, Ar-H-5, J = 9.0), 7.24 (d, 4H, 2 × Ar-H-2, Ar-H-6, J = 9.0); MS (EI) 520 (M^+).

2,6-Diethanolamino-4,8-bis(3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (34). Treatment of **16** (0.64 g, 1.21 mmol) with 2-aminoethanol (2 mL) according to method III furnished compound **34**. IR 3401, 3160, 2999, 2931, 1516 cm^{-1}; ^{1}H NMR δ 3.47 (t, 4H, 2 × $NHCH_2CH_2OH$, J = 5.2), 3.60 (t, 4H, 2 × $NHCH_2CH_2OH$, J = 5.0), 3.81 (s, 6H, 2 × OCH_3), 3.82 (s, 6H, 2 × OCH_3), 4.68 (m, 6H, 2 × ArCH_2 and 2 × OH), 6.13 (t, 2H, 2 × NH, J = 5.4), 6.98 (s, 4H, 4 × Ar-H), 7.59 (t, 2H, 2 × NH); MS (EI) 580 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-dibenzylaminopyrimido[5,4-*d*]pyrimidine (35). Compound **35** was prepared as a mixture of stereoisomers from **12** (0.43 g, 1.05 mmol) and (*rac*)-1-amino-2-propanol (3 mL) following method III. IR 3389, 2963, 1522 cm^{-1}; ^{1}H NMR δ 1.16 (d, 6H, 2 × CH_3, J = 6.1), 3.25 (m, 2H, 2 × $CH_3CHOHCH$), 3.40 (m, 2H, 2 × $CH_3CHOHCH$), 3.87 (m, 2H, 2 × $CH_3CHOHCH_2$), 4.74 (m, 6H, 2 × PhCH_2 and 2 × OH), 6.06 (t, 2H, 2 × NH), 7.38 (m, 10H, 10 × Ar-H), 7.77 (t, 2H, 2 × NH); MS (EI) 488 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (36). Compound **36** was synthesized as a mixture of stereoisomers according to method III by the reaction of **15** (0.18 g, 0.40 mmol) with (*rac*)-1-amino-2-propanol (2 mL). IR 3629, 2998, 2900, 2834, 1570 cm^{-1}; ^{1}H NMR δ 1.17 (d, 6H, 2 × CH_3), 3.28 (m, 2H, 2 × $CH_3CHOHCH$), 3.41 (m, 2H, 2 × $CH_3CHOHCH$), 3.82 (m, 8H, 2 × $CH_3CHOHCH_2$ and 2 × OCH_3), 4.68 (d, 4H, 2 × PhCH_2, J = 4.7), 4.76 (d, 2H, 2 × OH, J = 4.1), 6.60 (t, 2H, 2 × NH), 6.98 (d, 4H, 2 × Ar-H-3, Ar-H-5, J = 7.9), 7.39 (d, 4H, 2 × Ar-H-2, Ar-H-6, J = 7.9), 7.64 (t, 2H, 2 × NH); MS (EI) 548 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-bis(3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (37). Treatment of **16** (0.50 g, 0.94 mmol) with (*rac*)-1-amino-2-propanol (4 mL) in accordance with method III gave compound **37** as a mixture of stereoisomers. IR 3404, 2964, 2929, 1515 cm^{-1}; ^{1}H NMR δ 1.16 (d, 2H, 2 × CH_3, J = 6.1), 3.27 (m, 2H, 2 × CH_3-CHOHCH), 3.40 (m, 2H, 2 × $CH_3CHOHCH$), 3.82 (s, 6H, 2 × OCH_3), 3.83 (s, 6H, 2 × OCH_3), 4.67 (d, 4H, 2 × PhCH_2, J = 3.9), 4.76 (d, 2H, 2 × OH, J = 4.0), 6.09 (t, 2H, 2 × NH, J = 5.5), 7.07 (m, 6H, 6 × Ar-H), 7.60 (t, 2H, 2 × NH); MS (EI) 608 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-di-(3′,4′-dioxymethylenebenzylamino)pyrimido[5,4-*d*]pyrimidine (38). Compound **38** was prepared as a mixture of stereoisomers from **17** (0.50 g, 1.13 mmol) and (*rac*)-1-amino-2-propanol (3 mL). IR 3413, 3233, 2666, 2918, 1559 cm^{-1}; ^{1}H NMR δ 1.17 (d, 6H, 2 × CH_3, J = 5.8), 3.27 (m, 2H, 2 × $CH_3CHOHCH$), 3.44 (m, 2H, 2 × $CH_3CHOHCH$), 3.87 (m, 2H, 2 × $CH_3CHOHCH_2$), 4.64 (d, 4H, 2 × PhCH_2, J = 4.2), 4.76 (s, 2H, 2 × OH), 6.07 (m, 6H, 2 × OCH_2O and 2 × NH), 7.00 (m, 6H, 6 × Ar-H), 7.68 (t, 2H, 2 × NH); MS (EI) 576 (M^+).

2,6-Bis(diethanolamino)-4,8-dibenzylaminopyrimido-[5,4-*d*]pyrimidine (39).[38] Compound **39** was prepared from **12** (0.26 g, 0.63 mmol) and diethanolamine (1.0 mL) according to method III. ^{1}H NMR δ 3.67 (m, 16H, 2 × N(CH_2CH_2OH)$_2$),

4.73 (m, 8H, 2 × NHCH_2Ph and 4 × OH), 7.43 (m, 10H, 2 × Ph), 7.91 (t, 2H, 2 × NH); MS (EI) 548 (M^+).

Compound **39** was also synthesized by the following method. To a solution of **54** (0.10 g, 0.12 mmol) in CH_2Cl_2/H_2O (20:1, 10 mL) was added 2,3-dichloro-5,6-dicyanobenzoquinone (0.07 g, 0.30 mmol), and the mixture was stirred at 25 °C for 24 h. Saturated $NaHCO_3$ solution (25 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were washed sequentially with saturated $NaHCO_3$ solution (20 mL) and saturated NaCl solution (20 mL) and dried (Na_2SO_4). The solvents were removed in vacuo to give a yellow oil, which was purified by chromatography on silica, employing CH_2Cl_2/MeOH (95:5) as eluent, to yield **39** identical (^{1}H NMR, MS, and mp) to that prepared by method III above.

A sample of **39** identical (^{1}H NMR, MS, and mp) to that produced above was also obtained by the following method. A solution of **54** (0.13 g, 0.15 mmol) in TFA (2 mL) was heated at 65 °C for 12 h. The residual black solid that remained on removal of solvents was redissolved in saturated $NaHCO_3$ solution (15 mL), the mixture was extracted with EtOAc (4 × 25 mL), and the combined organic layers were dried (Na_2SO_4). The solvents were removed in vacuo to furnish a brown oil which was purified by chromatography on silica to yield **39**, which was identical (^{1}H NMR, MS, and mp) to that prepared by the other methods above.

2,6-Bis(diethanolamino)-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (40). Treatment of **15** (0.12 g, 0.25 mmol) with diethanolamine (1 mL) as detailed in method III afforded compound **40**. ^{1}H NMR δ 3.70 (br d, 16H, 4 × CH_2CH_2OH), 3.79 (s, 6H, 2 × ArOCH_3), 4.67 (d, 4H, 2 × CH_2Ar), 4.76 (t, 4H, 4 × OH), 6.96 (d, 4H, 4 × Ar-H), 7.38 (d, 4H, 4 × Ar-H), 7.82 (t, 2H, 2 × NH); MS (EI) 608 (M^+).

2,6-Bis(diethanolamino)-4,8-bis-[(3′,4′-dimethoxybenzyl)amino]pyrimido[5,4-*d*]pyrimidine (41). Compound **41** was synthesized as described in method III from **16** (0.27 g, 0.5 mmol) and diethanolamine (5 mL). ^{1}H NMR δ 3.71 (br d, 16 H, 4 × CH_2CH_2OH), 3.81 (s, 12H, 4 × ArOCH_3), 4.66 (d, 4H, 2 × CH_2Ar), 4.76 (s, 4H, 4 × O*H*), 6.97 (s, 4H, 4 × Ar-H), 7.10 (s, 2H, 2 × Ar-H), 7.80 (t, 2H, 2 × NH); MS (EI) 668 (M^+).

2,6-Bis(diethanolamino)-4,8-di-(3′,4′-dioxymethylenebenzylamino)pyrimido[5,4-*d*]pyrimidine (42). Reaction of **17** (0.50 g, 0.79 mmol) with diethanolamine (4 mL) according to method III gave compound **42**. IR 3402, 2927, 2888, 1560 cm^{-1}; ^{1}H NMR δ 3.68 (m, 16H, 4 × CH_2CH_2OH), 4.63 (d, 4H, 2 × PhCH_2), 4.75 (t, 4H, 4 × OH), 6.05 (s, 4H, 2 × OCH_2O), 6.96 (m, 6H, 6 × Ar-H), 7.82 (t, 2H, 2 × NH); MS (EI) 636 (M^+).

2,6-Bis(diethanolamino)-4,8-di-(4′-chlorobenzylamino)-pyrimido[5,4-*d*]pyrimidine (43). Compound **43** was prepared by method III from **13** (0.25 g, 0.50 mmol) and diethanolamine (2 mL). ^{1}H NMR δ 3.66 (br d, 16H, 4 × CH_2CH_2OH), 4.70 (d, 4H, 2 × CH_2Ar), 4.70 (d, 4H, 4 × OH), 7.46 (m, 8H, 8 × Ar-H), 7.99 (t, 2H, 2 × NH); MS (EI) 617 ($M^+ - 1$).

2,6-Bis(diethanolamino)-4,8-di-(4′-trifluoromethylbenzylamino)pyrimido[5,4-*d*]pyrimidine (44). Treatment of **14** (0.14 g, 0.25 mmol) with diethanolamine (1 mL) according to method III afforded compound **44**. ^{1}H NMR δ 3.71 (br d, 16H, 4 × CH_2CH_2OH), 4.64 (s, 4H, 4 × OH), 4.81 (d, 4H, 2 × CH_2Ar), 7.63 (d, 4H, 4 × Ar-H), 7.77 (d, 4H, 4 × Ar-H), 8.08 (t, 2H, 2 × NH); MS (EI) 683 ($M^+ - 1$).

2,6-Bis-(diethanolamino)-4,8-di-(*N*-1,2,3,4-tetrahydroisoquinolyl)pyrimidopyrimidine (45). Reaction of compound **24** (0.20 g, 0.4 mmol) with diethanolamine (5 mL), according to method III, gave compound **45**. ^{1}H NMR δ 3.01 (t, 4H, 2 × CH_2), 3.32 (t, 4H, 2 × CH_2), 3.74 (br s, 16H, 4 × CH_2CH_2OH), 4.20 (br s, 4H, 2 × CH_2), 7.21 (m, 8H, 8 × Ar-H); MS (EI) *m*/*z* 600 (M^+).

2,6-Di-(2′-hydroxyethylamino)-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (46). Compound **46** was prepared according to method III from **18** (0.30 g, 0.68 mmol) and 2-aminoethanol (3 mL). IR 3429, 2918, 2883, 1508 cm^{-1}; ^{1}H NMR δ 3.23 (m, 14H, 2 × CH_2CH_2OH and 2 × NCH_3), 4.61 (t, 2H, 2 × NH, J = 5.5), 5.60 (br s, 4H, 2 × PhCH_2), 6.09 (t, 2H, 2 × OH, J = 5.7), 7.39 (m, 10H, 10 × Ar-H); MS (EI) 488 (M^+).

2,6-Di-(2′-hydroxyethylamino)-4,8-di-(*N*-4′-methoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (47). By use of method III, **19** (0.50 g, 1.00 mmol) was reacted with 2-aminoethanol (2 mL) to give compound **47**. IR 3393, 3010, 2929, 2907, 1510 cm^{-1}; ^{1}H NMR δ 3.16 (s, 6H, 2 × NCH_3), 3.41 (m, 4H, 2 × HOCH_2CH_2NH), 3.58 (m, 6H, 2 × HOCH_2CH_2-NH and 2 × OH), 4.99 (br s, 2H, 2 × NH), 5.28 (s, 4H, 2 × ArCH_2), 6.83 (d, 4H, 2 × Ar-H-3, Ar-H-5, J = 8.4), 7.20 (d, 4H, 2 × Ar-H-2, Ar-H-6, J = 8.4); MS (EI) 548 (M^+).

2,6-Di-(2′-hydroxyethylamino)-4,8-bis(*N*-3′,4′-dimethoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (48). Compound **48** was prepared from **20** (0.29 g, 0.52 mmol) and 2-aminoethanol (2 mL) following method III. IR 3416, 3004, 2837, 1517 cm^{-1}; ^{1}H NMR δ 2.96 (m, 4H, 2 × NHCH_2CH_2-OH), 3.30 (s, 6H, 2 × NCH_3), 3.52 (m, 4H, 2 × NHCH_2CH_2-OH), 3.79 (s, 6H, 2 × OCH_3), 3.82 (s, 6H, 2 × OCH_3), 4.67 (t, 2H, 2 × OH, J = 5.3), 5.51 (br s, 4H, 2 × ArCH_2), 6.13 (t, 2H, 2 × NH, J = 5.1), 6.97 (m, 6H, 6 × Ar-H); MS (EI) 608 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-di-(*N*-methylbenzylamino)pyrimido[5,4-*d*]pyrimidine (49). Treatment of **18** (0.44 g, 1.00 mmol) with (*rac*)-1-amino-2-propanol (4 mL) according to method III gave compound **49** as a mixture of stereoisomers. IR 3321, 2961, 2908, 1510 cm^{-1}; ^{1}H NMR δ 0.96 (d, 4H, 2 × CH_3, J = 6.2), 3.02 (m, 2H, 2 × CH$_3$CHOHC*H*), 3.17 (m, 2H, 2 × CH$_3$CHOHC*H*), 3.27 (s, 6H, 2 × NCH_3), 3.43 (s, 1H, OH), 3.73 (m, 2H, 2 × CH$_3$C*H*OHCH_2), 4.64 (d, 1H, OH, J = 4.5), 5.62 (s, 2H, 2 × ArC*H*), 5.63 (s, 2H, 2 × ArC*H*), 6.02 (t, 2H, 2 × NH, J = 5.7), 7.40 (m, 10H, 10 × Ar-H); MS (EI) 516 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-di-(*N*-methyl-*N*-4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (50). Compound **50** was synthesized as a mixture of stereoisomers from **19** (0.60 g, 1.20 mmol) and (*rac*)-1-amino-2-propanol (3 mL) in accordance with method III. IR 3392, 2926, 2869, 2834, 1510, cm^{-1}; ^{1}H NMR δ 0.99 (d, 6H, 2 × CH_3CHOHCH$_2$, J = 6.0), 2.98 (2H, 2 × CH$_3$CHOHC*H*), 3.16 (m, 2H, 2 × CH$_3$-CHOHC*H*), 3.25 (s, 6H, 2 × NCH_3), 3.48 (m, 2H, 2 × CH$_3$C*H*OHCH$_2$), 3.82 (s, 6H, 2 × OCH_3), 4.67 (d, 2H, 2 × OH, J = 4.4), 5.53 (s, 4H, 2 × ArCH_2), 6.04 (t, 2H, 2 × NH), 6.98 (d, 4H, 2 × Ar-H-3, Ar-H-5, J = 8.3), 7.32 (d, 4H, 2 × Ar-H-2, Ar-H-6, J = 8.3); MS (EI) 576 (M^+).

2,6-Di-(2′-hydroxypropylamino)-4,8-bis(*N*-3′,4′-dimethoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (51). Reaction of **20** (0.45 g, 0.80 mmol) with (*rac*)-1-amino-2-propanol (3 mL) following method III furnished compound **51** as a mixture of stereoisomers. IR 3407, 3380, 2975, 2912, 2866, 1517 cm^{-1}; ^{1}H NMR δ 0.98 (d, 6H, 2 × CH_3CHOHCH$_2$, J = 5.5), 2.97 (m, 2H, 2 × CH$_3$CHOHC*H*), 3.26 (m, 8H, 2 × NCH_3 and 2 × CH$_3$CHOHC*H*), 3.80 (m, 14H, 4 × OCH_3 and 2 × CH$_3$C*H*OHCH$_2$), 4.65 (d, 2H, 2 × OH), 5.49 (br s, 4H, 2 × ArCH_2), 6.05 (t, 2H, 2 × NH), 6.98 (m, 6H, 6 × Ar-H); MS (EI) 636 (M^+).

2,6-Bis(diethanolamino)-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (52). Compound **52** was prepared from **18** (0.30 g, 0.68 mmol) and diethanolamine (3 mL) according to method III. IR 3377, 2929, 1533 cm^{-1}; ^{1}H NMR δ 3.31 (s, 6H, 2 × NCH_3), 3.54 (m, 16H, 2 × N[CH_2CH_2-OH]$_2$), 4.73 (t, 4H, 4 × OH), 5.57 (br s, 4H, 2 × ArCH_2), 7.38 (m, 10H, 10 × Ar-H); MS (EI) 576 (M^+).

2,6-Bis(diethanolamino)-4,8-di-(*N*-4′-methoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (53). Compound **53** was synthesized according to method III from **19** (0.20 g, 0.40 mmol) and diethanolamine (2 mL). IR 3245, 2991, 2958, 2919, 1513, cm^{-1}; ^{1}H NMR δ 3.12 (s, 6H, 2 × NCH_3), 3.58 (m, 20H, 2 × N[CH_2CH_2OH]$_2$ and 4 × OH), 3.73 (s, 6H, 2 × OCH_3), 5.22 (s, 4H, 2 × ArCH_2), 6.81(d, 4H, 2 × 3-Ar*H*, 5-Ar*H*, J = 8.6), 7.13 (d, 2H, d, 4H, 2 × 2-Ar*H*, 6-Ar*H*, J = 8.6); MS (EI) 636 (M^+).

2,6-Diethanolamino-4,8-di-(*N*-benzyl-*N*-3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (54). Compound **54** was prepared by method III from **22** and diethanolamine

(5.0 mL). IR 3380, 2929, 1534 cm^{-1}; ^1H NMR δ 3.60 (m, 16H, 2 × N[CH_2CH_2OH]$_2$), 3.79 (s, 6H, 2 × OCH_3), 3.84 (s, 6H, 2 × OCH_3), 4.67 (d, 4H, 4 × OH, J = 5.9), 5.14 (br s, 8H, 4 × PhCH_2), 6.77 (m, 6H, 6 × [MeO]$_2$Ar-*H*), 7.29 (m, 10H, 10 × Ar-*H*).

2,6-Bis[*N*,*N*-di-(2′-methoxy)ethylamino]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (55). NaH (0.20 g, 5 mmol) was added to a solution of dipyridamole (DP) (0.35 g, 0.69 mmol) in dry DMF (10 mL), and the mixture was stirred vigorously at room temperature under N_2 for 1 h. Iodomethane (2 mL) was added, and the reaction mixture was stirred at room temperature for a further 4 h. After cautious addition of H_2O (10 mL), the reaction mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic layers were washed with H_2O (3 × 30 mL) and dried ($MgSO_4$), and the solvent was evaporated in vacuo to give an orange syrup. Chromatography on silica afforded **55** as a highly fluorescent photosensitive yellow powder. ^1H NMR δ 1.66 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.33 (s, 12H, 4 × CH_3), 5.55 (t, 8H, 4 × CH_2), 3.75 (t, 8H, 4 × CH_2), 4.04 (s, 8H, 2 × $CH_2NCH_2(CH_2)_3$); MS (EI) 560 (M^+).

2,6-Dialkoxy-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidines. Method IV. General Procedure. Sodium (5 mmol) was added to a solution of the appropriate alcohol (5 mmol) in THF (5 mL), and the mixture was stirred for 2 h. The appropriate 2,6-dichloro-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine (0.50 mmol) was added, and the mixture was heated under reflux until TLC analysis confirmed the absence of starting materials (typically 24−72 h). After the mixture was cooled, the crude product was precipitated by addition of H_2O (30−50 mL) and collected by filtration and/or extraction with EtOAc. The product was purified by chromatography on silica and/or recrystallization from an appropriate solvent.

2,6-Dialkoxy-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidines. Method V. General Procedure. NaH (60% dispersion in mineral oil, 5 mmol) was washed three times with anhydrous petroleum ether under N_2 and dried. The resulting solid was added to a solution of the appropriate alcohol (5 mmol) in dry THF (5−10 mL), and the mixture was stirred for 10 min, heated to 60 °C for 5 min, and cooled to room temperature. After addition of the required 2,6-dichloro-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine (0.50 mmol) in dry THF (10 mL), the mixture was heated under reflux until TLC analysis confirmed the absence of starting materials (typically 24−72 h). After the mixture was cooled, the crude product was precipitated by addition of H_2O (30−50 mL) and collected by filtration and/or extraction with EtOAc. The product was purified by chromatography on silica and/or recrystallization from an appropriate solvent.

2,6-Dialkoxy-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidines. Method VI. General Procedure. NaH (60% dispersion in mineral oil, 4−6 mol equiv) was washed three times with anhydrous petroleum ether under N_2 and dried. The resulting solid was added to a solution of the appropriate triisopropylsilyloxy alcohol (4−6 mol equiv) in THF (5−10 mL), and the mixture was stirred at 25 °C for 1 h. Following addition of the required 2,6-dichloro-4,8-disubstituted-pyrimido[5,4-*d*]pyrimidine (1 mol equiv) in dry THF (10 mL), the mixture was heated under reflux until TLC analysis confirmed the absence of starting materials (typically 24−72 h). After the mixture was cooled, the crude product was precipitated by addition of H_2O (30−50 mL) and collected by filtration and/or extraction with EtOAc. Without further purification, the product was dissolved in THF (5−10 mL), and TBAF (6−10 mL) was added. The reaction mixture was stirred at 25 °C for 2 h, H_2O (10−20 mL) was added, and the mixture was extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with H_2O (4 × 20 mL) and dried (Na_2SO_4), and solvents were removed in vacuo to furnish the crude product, which was purified by chromatography on silica and/or recrystallization from an appropriate solvent.

2,6-Dimethoxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (56). Compound **56** was prepared from **9** (0.18 g, 0.5 mmol), sodium (0.12 g, 5 mmol), and MeOH (0.16 g, 5 mmol) in accordance with method IV. ^1H NMR δ 1.68 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 3.87 (s, 6H, 2 × OCH_3), 4.18 (br s, 8H, 2 × $N(CH_2)_2$); MS (EI) 358 (M^+).

2,6-Diethoxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (57). Treatment of **9** (0.18 g, 0.5 mmol) with sodium (0.12 g, 5 mmol) and EtOH (0.23 g, 5 mmol) following method IV gave **57**. ^1H NMR δ 1.41 (t, 6H, 2 × OCH_2CH_3), 1.70 (m, 12H, 2 × $NCH_2(CH_2)_3$), 4.25 (br s, 8H, 2 × $N(CH_2)_2$), 4.32 (q, 4H, 2 × OCH_2CH_3); MS (EI) 386 (M^+).

2,6-Dipropoxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (58). Compound **58** was synthesized following method IV from **9** (0.37 g, 1 mmol), sodium (0.23 g, 10 mmol), and propan-1-ol (0.6 g, 10 mmol). ^1H NMR δ 1.00 (t, 6H, 2 × $OCH_2CH_2CH_3$), 1.65 (m, 12H, 2 × $NCH_2(CH_2)_3$), 1.80 (m, 4H, 2 × $OCH_2CH_2CH_3$), 4.15 (m, 8H, 2 × $N(CH_2)_2$), 4.15 (m, 4H, 2 × $OCH_2CH_2CH_3$); MS (EI) 414 (M^+).

2,6-Diisopropoxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (59). Reaction of **9** (0.18 g, 0.5 mmol) with sodium (0.12 g, 5 mmol) and propan-2-ol (0.3 g, 5 mmol) as described in method IV afforded compound **59**. ^1H NMR δ 1.39 (d, 12H, 2 × $OCH(CH_3)_2$), 1.69 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 4.13 (br s, 8H, 2 × $N(CH_2)_2$), 5.10 (sept, 2H, 2 × $OCH(CH_3)_2$); MS (EI) 415 ($M^+ + 1$).

2,6-Di-(2′-methylpropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (60). Compound **60** was prepared as a mixture of stereoisomers from **9** (0.18 g, 0.5 mmol), sodium (0.12 g, 5 mmol), and (*rac*)-2-methylpropan-1-ol (0.37 g, 5 mmol) according to method IV. ^1H NMR δ 1.00 (d, 12H, 2 × $CH(CH_3)_2$), 1.70 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 2.13 (m, 2H, 2 × $OCH_2CH(CH_2)_3$), 4.00 (d, 4H, 2 × OCH_2), 4.14 (br s, 8H, 2 × $N(CH_2)_2$); MS (EI) 460 (M^+).

2,6-Bis(2′-methylbutoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (61). Compound **61** was prepared as a mixture of stereoisomers from **9** (0.2 g, 0.54 mmol), NaH (1.84 g, 46 mmol), and (*rac*)-2-methylbutan-1-ol (5 mL, 46 mmol) following method V. ^1H NMR δ 0.90 (m, 12H, 4 × CH_3), 1.07−1.91 (m, 8H, 4 × CH_2), 1.64 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$, 4.07 (m, 10H, 2 × $CH_2NCH_2(CH_2)_3$ and 2 × CH); MS (EI) 470 (M^+).

2,6-Di-(3′-methylbutoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (62). By use of method IV, compound **62** was prepared from **9** (0.18 g, 0.50 mmol), sodium (0.12 g, 5 mmol), and 3-methylbutan-1-ol (0.44 g, 5 mmol). ^1H NMR δ 0.93 (d, 12H, 2 × $CH_2CH(CH_3)_2$), 1.70 (m, 12H, 2 × $NCH_2(CH_2)_3$), 1.70 (m, 2H, 2 × $CH_2CH(CH_3)_2$), 4.00 (d, 4H, 2 × OCH_2), 4.15 (br s, 8H, 2 × $N(CH_2)_2$), 4.26 (t, 4H, 2 × $OCH_2CH_2CH(CH_3)_2$); MS (EI) 470 (M^+).

2,6-Dicyclohexylmethoxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (63). Compound **63** was prepared from **9** (0.18 g, 0.50 mmol), sodium (0.12 g, 5 mmol), and cyclohexylmethanol (0.65 mL, 5 mmol) utilizing method IV. ^1H NMR δ 1.69 (m, 34H, 2 × (C_6H_{11}) and 2 × $CH_2NCH_2(CH_2)_3$), 4.04 (d, 4H, 2 × CH_2O, J = 6.4), 4.14 (s, 8H, 2 × $CH_2NCH_2(CH_2)_3$); MS (EI) 522 (M^+).

2,6-Diallyloxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (64). Reaction of **9** (0.18 g, 0.5 mmol) with sodium (0.12 g, 5.0 mmol) and allyl alcohol (0.29 g, 5.0 mmol) according to method IV furnished compound **64**. IR 1640 cm^{-1}; ^1H NMR δ 1.69 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 4.15 (br s, 8H, 2 × $N(CH_2)_2$), 4.76 (d, 4H, 2 × OCH_2), 5.24 (m, 4H, 2 × OCH_2CHCH_2), 6.10 (m, 2H, OCH_2CHCH_2); MS (EI) 410 (M^+).

2,6-Di-(2′-methoxyethoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (65). Compound **65** was synthesized from **9** (0.18 g, 0.50 mmol), NaH (0.12 g), and 2-methoxyethanol (0.38 g, 5.0 mmol) according to method V. ^1H NMR δ 1.70 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 3.43 (s, 6H, 2 × OCH_3), 3.77 (t, 4H, 2 × CH_2OCH_3), 4.17 (br s, 8H, 2 × $N(CH_2)_2$), 4.43 (t, 4H, 2 × OCH_2); MS (EI) 446 (M^+).

2,6-Di-(1′-methyl-2′-methoxy)ethoxy-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (66). Treatment of **9** (0.18 g, 0.50 mmol) with sodium (0.12 g, 5 mmol) and (*rac*)-1-methoxy-2-propanol (0.45 g, 5.0 mmol) following method IV afforded compound **66** as a mixture of stereoisomers. ^1H NMR δ 1.26 (d, 6H, 2 × $OCHCH_3$), 1.63 (br s, 12H, 2 × $NCH_2(CH_2)_3$, 3.25

(s, 6H, 2 × OCH_2OCH_3), 3.44 (m, 4H, 2 × CH_2OCH_3), 4.04 (br s, 8H, 2 × $N(CH_2)_2$, 5.05 (m, 2H, 2 × $OCHCH_3$); MS (EI) 474 (M^+).

2,6-Bis(2′,3′-dimethoxypropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (67, Mixture of Stereoisomers). Compound **67** was prepared from **9** (0.07 g, 0.19 mmol), NaH (0.27 g, 6.75 mmol), and (*rac*)-2,3-dimethoxypropanol (0.52 g, 4.33 mmol) according to method V. ^{1}H NMR δ 1.65 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.32 and 3.45 (2xs, 12H, 4 × CH_3), 3.52 (m, 4H, 2 × CH_2), 3.70 (m, 2H, 2 × CH), 4.10 (s, 8H, 2 × $CH_2NCH_2(CH_2)_3$), 4.27 (dd, 4H, 2 × CH_2); MS (EI) 534 (M^+).

2,6-Bis(2′,3′-(*R*)-dimethoxypropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (68). NaH (0.1 g, 2.5 mmol) was added to a solution of **76** (0.24 g, 0.50 mmol) in dry DMF (15 mL). The mixture was stirred vigorously at 25 °C for 20 min, iodomethane (1 mL) was added, and stirring was continued for a further 3 h. After addition of water (20 mL), the mixture was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic layers were washed with H_2O (3 × 30 mL), dried ($MgSO_4$), and filtered, and the solvents were evaporated under reduced pressure to give a yellow syrup. Trituration with MeOH (10 mL) afforded compound **78** as white crystals. ^{1}H NMR δ 1.68 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.35 and 3.49 (2xs, 12H, 4 × CH_3), 3.60 (m, 4H, 2 × CH_2), 3.74 (m, 2H, 2 × CH), 4.14 (s, 8H, 2 × $CH_2NCH_2(CH_2)_3$), 4.30 (m, 4H, 2 × CH_2); MS (EI) 534 (M^+).

2,6-Di-(2′-hydroxyethoxy)-4,8-dipiperidinopyrimido-[5,4-*d*]pyrimidine (69). Compound **69** was prepared according to method V from **9** (0.18 g, 0.50 mmol), ethane-1,2-diol (0.56 mL, 10 mmol), and NaH (0.24 g, 10 mmol). IR 1650 cm^{-1}; ^{1}H NMR δ 1.64 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 2.44 (br s, 2H, 2 × OH), 3.85 (m, 4H, 2 × OCH_2CH_2OH), 4.09 (br s, 8H, 2 × $N(CH_2)_2$), 4.36 (t, 4H, 2 × OCH_2CH_2OH); MS (EI) 418 (M^+).

2,6-Di-(3′-hydroxypropoxy)-4,8-dipiperidinopyrimido-[5,4-*d*]pyrimidine (70). Treatment of **9** (0.18 g, 0.50 mmol) with NaH (0.24 g, 10 mmol) and propane-1,3-diol (0.73 mL, 10 mmol) according to method V gave compound **70**. IR 1650 cm^{-1}; ^{1}H NMR δ 1.69 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 2.03 (m, 4H, 2 × $OCH_2CH_2CH_2OH$), 2.37 (br s, 2H, 2 × OH), 3.73 (t, 4H, 2 × $OCH_2CH_2CH_2OH$), 4.09 (br s, 8H, 2 × $N(CH_2)_2$), 4.45 (t, 4H, 2 × $OCH_2CH_2CH_2OH$); MS (EI) 446 (M^+).

2,6-Di-(2′-(*S*)-hydroxypropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (71). Treatment of **9** (0.18 g, 0.50 mmol) with sodium (0.12 g, 5.0 mmol) and (*S*)-2-*O*-(4′-methoxybenzyl)propan-1-ol (0.98 g, 5 mmol) according to method IV gave (*S*,*S*)-2,6-di-[2′-*O*-[(4′-methoxybenzyl)propoxy]-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine, which was purified by chromatography on silica with petroleum ether/EtOAc (6:1 + 1.5% *N*,*N*-diisopropylethylamine) as eluent and used directly in the next step. The product was dissolved in MeOH (40 mL), 5% Pd on carbon (0.03 g) was added, and the mixture was stirred vigorously under an atmosphere of H_2 for 4 h. After filtration and removal of solvent in vacuo, the crude product was purified by chromatography on silica with petroleum ether/EtOAc (3:1 + 1% *N*,*N*-diisopropylethylamine) as eluent to give **71** as a white crystalline solid. IR 3600 cm^{-1}; ^{1}H NMR δ 1.22 (d, 6H, 2 × CH_3), 1.67 (br s, 12H, 2 × $NCH_2(CH_2)_3$), 2.90 (br s, 2H, 2 × OH), 4.07 (br s, 8H, 2 × $N(CH_2)_2$), 4.09 (m, 2H, 2 × CHOH), 4.20 (d, 4H, 2 × OCH_2); *m*/*z* (EI) 446 (M^+).

2,6-Bis(2′,2′-dimethyl-1′,3′-dioxolane-4′-methoxy)-4,8,-dipiperidinopyrimido[5,4-*d*]pyrimidine (72, Mixture of Stereoisomers). Compound **72** was prepared from **9** (0.37 g, 1 mmol), NaH (1.6 g, 40 mmol), and racemic solketal (5 mL, 40 mmol) according to method V. ^{1}H NMR δ 1.35 and 1.43 (2 × s, 12H, 4 × CH_3), 1.69 (s,12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.88 (q, 4H, 2 × 4′-CH_2), 4.14 (m, 8H, 2 × $CH_2NCH_2(CH_2)_3$), 4.31 (q, 4H, 2 × 1′-CH_2), and 4.50 (m, 2H, 2 × 3′-CH); HRMS (EI) *m*/*z* found 558.3162 [M^+ calcd for $C_{28}H_{42}N_6O_6$ 558.3166].

(*R*,*R*)-2,6-Bis(2′,2′-dimethyl-1′,3′-dioxolane-4′-methoxy)-4,8-dipiperidino[5,4-*d*]pyrimidine (73). Treatment of **9** (0.37 g, 1 mmol) with NaH (1.6 g, 40 mmol) and (*R*)-solketal (5 mL, 40 mmol) as detailed in method V yielded compound **73**. ^{1}H NMR δ 1.36 and 1.44 (2 × s, 12H, 4 × CH_3), 1.70 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$, 3.89 (q, 4H, 2 × 4′-CH_2), 4.14 (m, 8H, 2 × $CH_2NCH_2(CH_2)_3$, 4.35 (q, 4H, 2 × 1′-CH_2), 4.52 (m, 2H, 2 × 3′-CH); MS (EI) 558 (M^+); $[\alpha]^{17}_D$ −5.0° (*c* 4.0% in $CHCl_3$).

(*S*,*S*)-2,6-Bis(2′,2′-dimethyl-1′,3′-dioxolane-4′-methoxy)-4,8-dipiperidinopyrimido-[5,4-*d*]pyrimidine (74). Compound **74** was prepared in a manner similar to that for **73** from **9** (0.37 g, 1.0 mmol), NaH (1.6 g, 40 mmol), and (*S*)-solketal (5 mL, 40 mmol) according to method V. ^{1}H NMR δ 1.38 and 1.45 (2 × s, 12H, 4 × CH_3), 1.70 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.90 (q, 4H, 2 × 4′-CH_2), 4.20 (m, 8H, 2 × $CH_2NCH_2(CH_2)_3$), 4.40 (q, 4H, 2 × 1′-CH_2), 4.55 (m, 2H, 2 × 3′-CH); MS (EI) 558 (M^+); $[\alpha]^{19}_D$ +5.4° (*c* 3.75% in $CHCl_3$).

2,6-Bis(2′,3′-Dihydroxypropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (75, Mixture of Stereoisomers). To a solution of **72** (0.28 g, 0.5 mmol) in THF (5 mL) was added hydrochloric acid (1 M, 5 mL), and the reaction mixture was stirred at 25 °C for 12 h. Solvents were removed in vacuo, and water (50 mL) was added to the residue. The solution was adjusted to pH 8.0 with saturated aqueous $NaHCO_3$ solution, and the solid that was deposited was collected by filtration and dried to give **75** as a white powder. ^{1}H NMR δ 1.76 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.53 (m, 4H, 2 × 1′-CH_2), 3.89 (m, 2H, 2 × 2′-CH), 4.26 (m, 12H, 2 × 3′-CH_2 and 2 × CH_2N CH_2-$(CH_2)_3$), 4.76 (t, 2H, 2 × OH), 5.06 (d, 2H, 2 × OH); MS (EI) 478 (M^+).

(*R*,*R*)-2,6-Bis(2′,3′-Dihydroxypropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (76). Treatment of **73** (0.28 g, 0.5 mmol) with hydrochloric acid (1 M, 5 mL) in a manner identical to that employed for compound **72** furnished compound **76** as pale-yellow plates. ^{1}H NMR δ 1.75 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.52 (t, 4H, 2 × 1′-CH_2), 3.88 (m, 2H, 2 × 2′-CH), 4.25 (m, 12H, 2 × 3′-CH_2 and 2 × $CH_2NCH_2(CH_2)_3$), 4.76 (t, 2H, 2 × OH), 5.03 (d, 2H, 2 × OH); MS (EI) 478 (M^+).

(*S*,*S*)-2,6-Bis(2′,3′-Dihydroxypropoxy)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (77). Treatment of **74** (0.28 g, 0.5 mmol) with hydrochloric acid (1 M, 5 mL) as for compounds **72** and **73** furnished compound **77** as pale-yellow granules. ^{1}H NMR δ 1.77 (s, 12H, 2 × $CH_2NCH_2(CH_2)_3$), 3.53 (t, 4H, 2 × 1′-CH_2), 3.89 (m, 2H, 2 × 2′-CH), 4.29 (m, 12H, 2 × 3′-CH_2 and 2 × $CH_2NCH_2(CH_2)_3$, 4.77 (t, 2H, 2 × OH), 4.08 (d, 2H, 2 × OH).

2,6-Dipropoxy-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (78). Compound **78** was prepared from **18** (0.22 g, 0.5 mmol), NaH (0.12 g, 5 mmol), and propan-1-ol (0.3 g, 5 mmol) in accordance with method V. ^{1}H NMR δ 0.77 (s, 6H, 2 × CH_3), 1.60 (s, 4H, 2 × $OCH_2CH_2CH_3$), 3.28 (s, 6H, 2 × NCH_3), 3.82 (t, 4H, 2 × OCH_2), 5.53 (s, 4H, 2 × NCH_2Ar), 7.26 (m, 10H, 10 × Ar-H); MS (EI) 486 (M^+).

2,6-Di-(2′-hydroxyethoxy)-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (79). Compound **79** was synthesized from **18** (1.14 g, 5.25 mmol), NaH (0.14 g, 5.78 mmol), 2-triisopropylsilyloxyethanol (0.45 g, 5.25 mmol), and TBAF (6 mL) according to method VI. ^{1}H NMR δ 1.98 (br s, 2H, 2 × OH), 3.23 (s, 6H, 2 × NCH_3), 3.63 (t, 4H, 2 × $HOCH_2$-CH_2O, J = 4.5), 3.92 (t, 4H, 2 × $HOCH_2CH_2O$, J = 4.4), 5.41 (s, 4H, 2 × $PhCH_2$), 7.23 (m, 10H, 10 × Ar-H); MS (EI) 490 (M^+).

2,6-Di-(2′-hydroxyethoxy)-4,8-di-(*N*-4′-methoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (80). Treatment of **19** (0.52 g, 1.04 mmol) with NaH (0.11 g, 4.6 mmol), 2-triisopropylsilyloxyethanol (0.91 g, 4.2 mmol), and TBAF (7.08 mL, 7.08 mmol) following method VI gave compound **80**. IR 3474, 3057, 2868, 2835, 1532 cm^{-1}; ^{1}H NMR δ 2.05 (br s, 2H, 2 × OH), 3.21 (s, 6H, 2 × NCH_3), 3.69 (m, 4H, 2 × OCH_2CH_2OH,), 3.73 (s, 6H, 2 × OCH_3), 4.03 (t, 4H, 2 × OCH_2-CH_2OH, J = 4.5), 5.34 (br s, 4H, 2 × $PhCH_2$), 6.79 (d, 4H, 2 × Ar-H-3, Ar-H-5, J = 8.6), 7.14 (d, 4H, 2 × Ar-H-2, Ar-H-6, J = 8.6); MS (EI) 550 (M^+).

2,6-Di-(3′-hydroxypropoxy)-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (81). Compound **81** was prepared according to method V from NaH (0.14 g, 6 mmol), 1,3-propanediol (0.45 mL, 6 mmol), and **18** (0.13 g, 0.3 mmol). ^{1}H NMR δ 1.79 (m, 4H, 2 × $OCH_2CH_2CH_2OH$), 3.27 (s, 6H, 2 × NCH_3), 3.62 (t, 4H, 2 × CH_2OH), 4.04 (t, 4H, 2 ×

OCH_2), 5.51 (s, 4H, 2 × CH_2Ar), 7.28 (m, 10H, 10 × Ar-H); MS (EI) 518 (M^+).

2,6-Di-(2′-hydroxypropoxy)-4,8-di-(*N*-benzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (82). Compound **82** was prepared from NaH (0.30 g, 12.5 mmol), 2-triisopropylsilyloxypropan-1-ol (2.64 g, 11.4 mmol), **18** (1.00 g, 2.28 mmol), and TBAF (5.0 mL, 5.0 mmol) in accordance with method VI. IR 3426, 3029, 2973, 2930, 1530 cm^{-1}; ^{1}H NMR δ 1.06 (dd, 6H, 2 × CH_3, J = 14.8, 3.9), 3.35 (br s, 6H, 2 × NCH_3), 3.43 (s, 2H, 2 × $CH_3CHOHCH$), 3.82 (m, 3H, OH and 2 × CH_3-$CHOHCH$), 4.85 (m, 3H, 2 × $CH_3CHOHCH_2$ and OH), 5.55 (br s, 4H, 2 × PhCH_2), 7.41 (m, 10H, 10 × Ar-H); MS (EI) 518 (M^+).

2,6-Di-(3′-hydroxypropoxy)-4,8-di-(*N*-4′-methoxybenzyl-*N*-methylamino)pyrimido[5,4-*d*]pyrimidine (83). Reaction of **19** (0.87 g, 1.8 mmol) with NaH (0.18 g, 7.7 mmol), 3-triisopropylsilyloxypropan-1-ol (**3**) (1.87 g, 7.0 mmol), and TBAF (10.3 mL, 10.3 mmol), following method VI, yielded compound **83**. IR 3398, 3027, 2931, 1530 cm^{-1}; ^{1}H NMR δ 1.81 (m, 4H, 2 × $OCH_2CH_2CH_2$), 3.35 (s, 6H, 2 × NCH_3), 3.48 (m, 4H, 2 × $OCH_2CH_2CH_2OH$), 3.81 (s, 6H, 2 × OCH_3),4.13 (t, 4H, 2 × $OCH_2CH_2CH_2OH$), 4.58 (t, 2H, 2 × OH, J = 5.1), 5.50 (br s, 4H, 2 × ArCH_2), 6.98 (d, 4H, Ar-3-*H*, Ar-5-*H*, J = 8.6), 7.29 (d, 4H, Ar-2-*H*, Ar-6-*H*, J = 8.6); MS (EI) 578 (M^+).

A sample of **83**, identical (^{1}H NMR, MS, and mp) to that synthesized above, was also prepared in low yield directly from NaH (0.1 g, 4 mmol), 1,3-propanediol (0.3 mL, 4 mmol), and **19** by method V.

2,6-Di-(3′-hydroxypropoxy)-4,8-bis[(*N*-3′,4′-dimethoxybenzyl)-*N*-methylamino]pyrimido[5,4-*d*]pyrimidine (84). Compound **84** was prepared by method V from NaH (0.08 g, 3.2 mmol), 1,3-propanediol (0.24 mL, 3.2 mmol), and **20** (0.22 g, 0.4 mmol). IR 1650 cm^{-1}; ^{1}H NMR δ 1.87 (p, 4H, 2 × $OCH_2CH_2CH_2OH$), 2.08 (t, 2H, 2 × OH), 3.30 (s, 6H, 2 × NCH_3), 3.66 (t, 4H, 2 × OCH_2), 5.38 (s, 4H, 2 × CH_2Ar), 6.82 (s, 4H, 4 × Ar-H), 6.90 (s, 2H, 2 × Ar-H); MS (EI) 638 (M^+).

2,6-Di-(2′-methoxyethoxy)-4,8-di-[*N*-benzyl-*N*-methylamino]pyrimido[5,4-*d*]pyrimidine (85). Treatment of **18** (0.22 g, 0.50 mmol) with NaH (0.12 g, 5.0 mmol) and 2-methoxyethanol (0.38 g, 5.0 mmol), according to method V, furnished compound **85**. ^{1}H NMR δ 3.29 (s, 6H, 2 × NCH_3), 3.29 (br s, 6H, 2 × OCH_3), 3.48 (t, 4H, 2 × CH_2OCH_3), 4.04 (t, 4H, 2 × OCH_2), 5.49 (br s, 4H, 2 × NCH_2Ar), 7.24 (m, 10H, 10 × Ar-H); MS (EI) 518 (M^+).

2,6-Di-(2′-hydroxyethoxy)-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (86). Compound **86** was prepared from **15** (0.24 g, 0.50 mmol), sodium (0.24 g, 10 mmol), and ethane-1,2-diol (0.62 g, 10 mmol) in accordance with method IV. ^{1}H NMR δ 2.61 (br s, 2H, 2 × O*H*), 3.73 (s, 6H, 2 × ArOCH_3), 3.89 (t, 4H, 2 × CH_2OH), 4.40 (t, 4H, 2 × OCH_2), 4.62 (d, 4H, 2 × CH_2Ar), 6.79 (d, 4H, 4 × Ar-H), 6.97 (br s, 2H, 2 × NH), 7.21 (d, 4H, 4 × Ar-H); MS (EI) 522 (M^+).

2,6-Di-(3′-hydroxypropoxy)-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (87). Reaction of **15** (0.35 g, 0.75 mmol) with NaH (0.36 g, 15 mmol) and propane-1,3-diol (1.14 g, 15 mmol) according to method V gave compound **87**. ^{1}H NMR δ 1.97 (t, 4H, 2 × $OCH_2CH_2CH_2OH$), 2.30 (br s, 2H, 2 × OH), 3.74 (s, 6H, 2 × ArOCH_3), 3.78 (t, 4H, 2 × CH_2OH), 4.41 (t, 4H, 2 × OCH_2), 4.63 (d, 4H, 2 × CH_2Ar), 6.81 (d, 4H, 4 × Ar-H), 7.01 (t, 2H, 2 × NH), 7.26 (d, 4H, 4 × Ar-H); MS (EI) 550 (M^+).

2,6-Di-(2′-(*S*)-hydroxypropoxy)-4,8-di-(4′-methoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (88). Reaction of **15** (0.47 g, 1.0 mmol) with NaH (0.48 g, 20 mmol) and (*S*)-propane-1,2-diol (1.46 mL, 20 mmol) according to method V gave **88**. ^{1}H NMR δ 1.18 (d, 6H, 2 × CH_3), 2.84 (br s, 2H, 2 × OH), 3.76 (s, 6H, 2 × ArOCH_3), 4.16 (m, 4H, 2 × OCH_2), 4.16 (m, 2H, 2 × C*H*OH), 4.62 (d, 4H, 2 × CH_2Ar), 6.80 (d, 4H, 4 × Ar-H), 6.96 (t, 2H, 2 × NH), 7.21 (d, 4H, 4 × Ar-H); MS (EI) 550 (M^+).

2,6-Di-(2′-methoxyethoxy)-4,8-bis(dibenzylamino)pyrimido[5,4-*d*]pyrimidine (89). Compound **89** was prepared from **21** (0.35 g, 0.60 mmol), NaH (0.14 g, 6 mmol), and 2-methoxyethanol (0.47 mL, 6 mmol) by method V. ^{1}H NMR δ 3.25 (s, 6H, 2 × OCH_3). 3.38 (t, 4H, 2 × CH_2OCH_3), 3.87 (t, 4H, 2 × O$CH_2CH_2OCH_3$), 5.28 (br s, 8H, 4 × CH_2Ar), 7.28 (m, 20H, 20 × Ar-H); MS (EI) 669 ($M^+ - 1$).

2,6-Di-(2′-methoxyethoxy)-4,8-di-(benzylamino)pyrimido[5,4-*d*]pyrimidine (90). Acetyl chloride (0.06 g, 0.75 mmol) was added to dry MeOH (8 mL), the solution was stirred for 15 min, and **89** (0.15 g, 0.22 mmol) was added. To the resulting solution was added 10% Pd on carbon (40 mg), and the mixture was stirred vigorously under H_2 for 5 h at 25 °C. After filtration and removal of solvents in vacuo, the product was purified by chromatography on silica to afford **90** as a cream solid. IR 3450 cm^{-1}; ^{1}H NMR δ 3.39 (s, 6H, 2 × OCH_3), 3.72 (t, 4H, 2 × $CH_3OCH_2CH_2$), 4.46 (t, 4H, 2 × CH_3OCH_2), 4.77 (d, 4H, 2 × ArCH_2N), 7.02 (br s, 2H, 2 × NH), 7.33 (m, 10H, 2 × Ph); MS (EI) 490 (M^+).

2,6-Di-(2′-triisopropylsilyloxyethoxy)-4,8-bis(di-*N*,*N*-(3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (91). The reaction of **23** (1.00 g, 0.84 mmol) with NaH (0.07 g, 2.76 mmol) and 2-triisopropylsilyloxyethanol (0.55 g, 2.51 mmol), in accordance with method V, gave compound **91**. IR 2936, 2865, 1512, 1264, 1137 cm^{-1}; ^{1}H NMR δ 0.92 (m, 42H, 42 × ^{i}Pr-H), 3.72 (m, 16H, 4 × OCH_3 and 2 × SiOCH_2CH_2O), 3.80 (s, 12H, 4 × OCH_3), 3.97 (t, 4H, 2 × $SiOCH_2CH_2O$, J = 5.4), 5.16 (br s, 8H, 4 × PhCH_2), 6.76 (m, 12H, 12 × Ar-H); MS (EI) 1194 (M^+).

2,6-Di-(3′-triisopropylsilyloxypropoxy)-4,8-bis[di-*N*,*N*-(3′,4′-dimethoxybenzyl)amino]pyrimido[5,4-*d*]pyrimidine (92). Compound **92** was prepared from NaH (0.07 g, 2.8 mmol), 3-triisopropylsilyloxypropan-1-ol (0.74 g, 2.5 mmol), and **23** (1.00 g, 0.84 mmol) following method V. IR 2943, 2866, 2836, 1514 cm^{-1}; ^{1}H NMR δ 1.73 (m, 4H, 2 × $SiOCH_2CH_2$-CH_2O), 3.57 (t, 4H, 2 × $SiOCH_2CH_2CH_2O$, J = 6.2), 3.74 (s, 12H, 4 × OCH_3), 3.80 (s, 12H, 4 × OCH_3), 3.99 (t, 4H, 2 × $SiOCH_2CH_2CH_2O$, J = 6.4), 5.16 (br s, 8H, 4 × ArCH_2), 6.78 (m, 12H, 12 × Ar-H); MS (EI) 1222 (M^+).

2,6-Di-(2′-hydroxyethoxy)-4,8-bis[di-*N*,*N*-(3′,4′-dimethoxybenzyl)amino]pyrimido[5,4-*d*]pyrimidine (93). To a solution of **91** (0.40 g, 0.34 mmol) in THF (15 mL) was added TBAF (1.36 mL, 1.36 mmol), and the mixture was stirred at 25 °C for 12 h. Solvents were removed in vacuo, the residue was triturated with H_2O (30 mL), and the solid was collected to afford compound **93**. IR 3435, 2997, 2954, 2937 cm^{-1}; ^{1}H NMR δ 3.54 (m, 4H, 2 × $HOCH_2CH_2O$), 3.76 (s, 12H, 4 × OCH_3), 3.81 (s, 12H, 4 × OCH_3), 3.94 (t, 4H, 2 × $HOCH_2CH_2O$), 4.84 (t, 2H, 2 × OH), 5.30 (br s, 8H, 4 × ArCH_2), 6.96 (m, 12H, 12 × Ar-H); MS (EI) 882 (M^+).

2,6-Di-(3′-hydroxypropoxy)-4,8-bis[di-*N*,*N*-(3′,4′-dimethoxybenzyl)amino]pyrimido[5,4-*d*]pyrimidine (94). TBAF (2.12 mL, 2.12 mmol) was added to a solution of **92** (0.65 g, 0.53 mmol) in dry THF (15 mL) under nitrogen, and the mixture was stirred for 12 h at 25 °C. Evaporation of solvents in vacuo gave a yellow oil, which was triturated with H_2O (25 mL) to yield compound **94** as a white solid. IR 3555, 2936, 2838 cm^{-1}; ^{1}H NMR δ 1.73 (m, 4H, $HOCH_2CH_2CH_2O$), 3.45 (m, 4H, $HOCH_2CH_2CH_2O$), 3.79 (s, 12H, 4 × OCH_3), 3.83 (s, 12H, 4 × OCH_3), 4.00 (t, 4H, $HOCH_2CH_2CH_2O$), 4.57 (br s, 2H, 2 × OH), 5.20 (br s, 8H, 4 × ArCH_2), 6.98 (m, 12H, 12 × Ar-H); MS (EI) 911 (M^+).

2,6-Di-(2′-hydroxyethoxy)-4,8,-bis(3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (95). A solution of **93** (0.13 g, 0.15 mmol) in TFA (2.5 mL) was stirred at 25 °C for 3 h. Excess TFA was removed in vacuo to leave a yellow residue, which was triturated with saturated aqueous $NaHCO_3$ solution (20 mL). The yellow solid that was deposited was collected and purified by chromatography on silica to give **95** as a pale-yellow solid. IR 3538, 3290, 2955, 2932, 2836, 1570 cm^{-1}; ^{1}H NMR δ 3.80 (m, 6H, 2 × OCH_3), 3.82 (s, 6H, 2 × OCH_3), 4.41 (m, 4H, 2 × $HOCH_2CH_2O$), 4.69 (m, 8H, 2 × $HOCH_2CH_2O$ and 2 × ArCH_2), 4.92 (t, 2H, 2 × OH, J = 5.3), 7.05 (m, 6H, 6 × Ar-H), 8.51 (t, 2H, 2 × NH); MS (EI) 610 (M^+).

2,6-Di-(3′-hydroxypropoxy)-4,8-bis(3′,4′-dimethoxybenzylamino)pyrimido[5,4-*d*]pyrimidine (96). A solution of **94** (0.40 g, 0.44 mmol) in TFA (7 mL) was stirred at 25 °C for 2

h, excess TFA was removed in vacuo, and the residual red syrup was triturated with saturated $NaHCO_3$ solution (30 mL). The resultant pale-yellow solid was purified by chromatography on silica to afford **96**. IR 3390, 3377, 2998, 2935, 1567 cm^{-1}; ^1H NMR δ 1.93 (m, 4H, 2 × $HOCH_2CH_2CH_2O$), 3.63 (m, 4H, 2 × $HOCH_2CH_2CH_2O$), 3.80 (s, 6H, 2 × OCH_3), 3.82 (s, 6H, 2 × OCH_3), 4.45 (t, 4H, 2 × $HOCH_2CH_2CH_2O$, J = 6.1), 4.65 (m, 6H, 2 × $ArCH_2$ and 2 × OH), 7.05 (m, 6H, 6 × Ar-H), 8.47 (t, 2H, 2 × NH); MS (EI) 610 (M^+).

Biological Experimental Section. The inhibition of ^3H-thymidine uptake into L1210 leukaemia cells by DP and analogues was measured using a modified rapid mixing technique, combined with an inhibitor-stop method, as previously described.[1,24] The L1210 cells used in these studies were available in the authors' laboratory, pemetrexed was a generous gift from Eli Lilly Co. (Indianapolis, IN), and all other reagents were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.).

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

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