Tyrosine Kinase Inhibitors. 10. Isomeric 4-[(3-Bromophenyl)amino]pyrido[*d*]-pyrimidines Are Potent ATP Binding Site Inhibitors of the Tyrosine Kinase Function of the Epidermal Growth Factor Receptor

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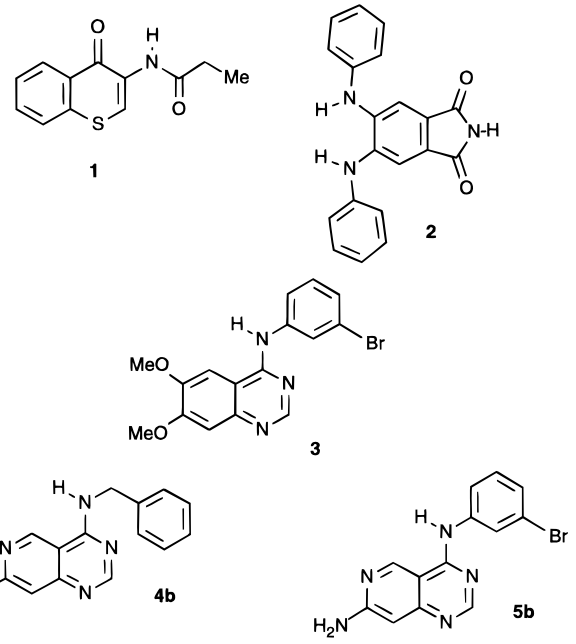
Following the discovery of the very high inhibitory ability of the 4-[(3-bromophenyl)amino]-quinazolines against the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) (e.g., **3**, IC_{50} 0.029 nM), four series of related pyrido[*d*]pyrimidines bearing electron-donating groups at the 6- or 7-positions have been synthesized and evaluated. The compounds were prepared by nucleophilic substitution of the corresponding 6- and 7-fluoro analogues. While members of all series showed potent inhibitory activity against isolated EGFR, there were important differences between the different isomeric pyrido[*d*]pyrimidines and the parent quinazolines. Overall, the [3,4-*d*] and [4,3-*d*] series were the most potent, followed by the [3,2-*d*] compounds, with the [2,3-*d*] analogues being least active. Whereas in the parent quinazoline series the addition of steric bulk to a 6- or 7-NH_2 substituent (i.e., NHMe and NMe_2 groups) dramatically decreased potency, no such trend was discernable in the [3,2-*d*] series. Furthermore, in the 7-substituted pyrido[4,3-*d*]- and 6-substituted pyrido[3,4-*d*]pyrimidine series, and to a limited extent in the 7-substituted pyrido[2,3-*d*] series, such substitution *increased* potency dramatically, to the extent that the 7-(methylamino)pyrido[4,3-*d*]pyrimidine (**5f**) (IC_{50} 0.13 nM) and 6-(methylamino)pyrido[3,4-*d*]pyrimidine (**7f**) (IC_{50} 0.008 nM) constitute important new leads. Selected compounds were evaluated for their ability to inhibit EGFR autophosphorylation in A431 cells, and a positive quantitative correlation was found between this activity and inhibitory activity against the isolated enzyme.

There is great current interest in compounds capable of modifying the cellular activities of receptor tyrosine kinase enzymes which constitute the starting points for many signal transduction pathways in cells.[1] In particular, the epidermal growth factor receptor (EGFR), a member of the *erbB2* oncogene family, is known to be overexpressed in a large number of human cancers, including mammary,[2,3] ovarian,[4] esophageal,[5] and squamous cell head and neck carcinomas.[6] Most of the initial work on such compounds focused on inhibitors of the substrate binding site,[7,8] because of concerns that inhibitors of the highly-conserved ATP binding site would exhibit little selectivity between different kinases.[9] However, a number of classes of compound have recently been shown to inhibit activity by competitive binding at the ATP site with considerable interenzyme selectivity. Examples include the aminoflavones,[10] benzothiopyrans (e.g., **1**),[11] the dianilinophthalimides (e.g., **2**),[12] and particularly the 4-(phenylamino)quinazolines (e.g., **3**), which have been shown independently by both Zeneca scientists[13] and ourselves[14−16] to be very potent inhibitors. For example, we have reported[14] that **3** has an IC_{50} for inhibition of phosphorylation of a PLCγ-derived substrate by the isolated EGFR enzyme of 29 pM and extraordinary selectivity for receptor tyrosine kinases of the *erbB2* family (particularly EGFR).

We have recently shown[17] that the related 4,7-diaminopyrido[4,3-*d*]pyrimidines (e.g., **4b** and **5b**) are also potent inhibitors of EGFR and have carried out structure−activity relationship (SAR) studies which suggest that a 3-(bromophenyl)amino side chain at the 4-position is highly favored in both the quinazoline and pyrido[4,3-*d*]pyrimidine series.[15,17] We now extend these studies by reporting the synthesis and EGFR tyrosine kinase inhibitory activities of four series of isomeric 4-[(3-bromophenyl)amino]pyrido[*d*]pyrimidines, each bearing a range of electron-donating substituents at positions adjacent to the nitrogen on the pyrido ring.



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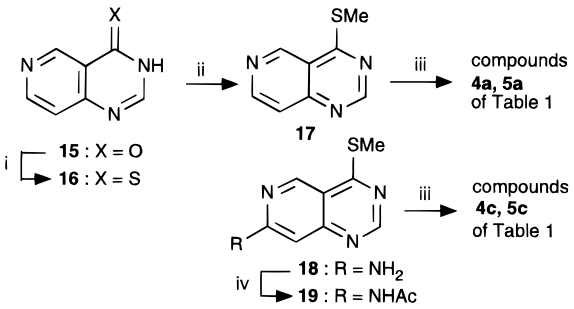
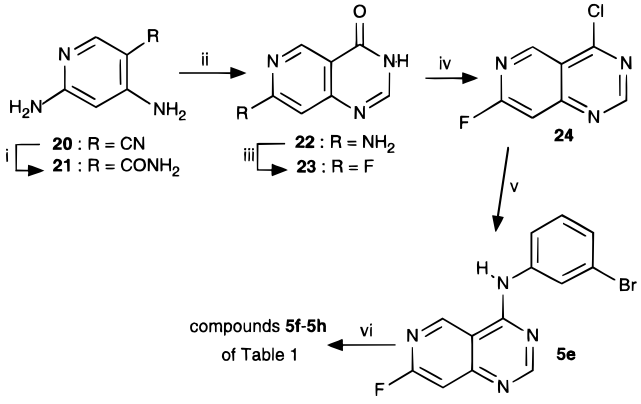
Scheme 1[a]



[a] (i) P_2S_5/pyridine/reflux/5 h; (ii) MeI/Et_3N/DMSO/25 °C/12 h; (iii) 3-bromoaniline/155 °C or benzylamine/140 °C; (iv) MeCOCl/Et_3N/THF/20 °C/4 h.

Scheme 2[a]



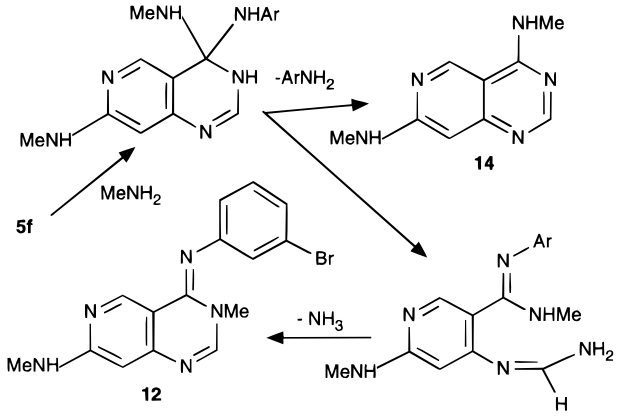
[a] (i) 90% H_2SO_4/60−70 °C/3 h; (ii) $(EtO)_3CH$/170 °C/1.5 d, then 2 N NaOH/90 °C/5 min; (iii) $NaNO_2$/50% HBF_4/0 °C/6 h; (iv) $SOCl_2$/reflux/8 h; (v) 3-bromoaniline/CH_2Cl_2/2-propanol/20 °C/16 h; (vi) $MeNH_2$·HCl or Me_2NH·HCl/Et_3N/2-propanol/95−100 °C (pressure vessel)/4−5 h, or 1 M MeONa/MeOH/reflux/42 h.

In addition, since the previous work had suggested that a 4-benzylamino side chain was also acceptable, this side chain was evaluated in each of the positional isomers studied here.

Chemistry

In the pyrido[4,3-*d*]pyrimidine series, the unsubstituted compounds **4a** and **5a** were prepared from the known[18] pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**15**), which was converted to the known[18] thione **16** and then methylated with MeI to give the 4-thiomethyl compound **17** (Scheme 1). Reaction of this with benzylamine or 3-bromoaniline gave **4a** and **5a**, respectively. The acetamido derivatives **4c** and **5c** were prepared by acetylation of the known[17] 7-amino-4-(methylthio)-pyrido[4,3-*d*]pyrimidine (**18**), followed by similar reaction of the resulting acetamide (**19**) with benzylamine or 3-bromoaniline (Scheme 1). The remainder of the 7-substituted compounds were prepared from the 7-fluoro derivative (**5e**). This was synthesized (Scheme 2) from the known[19] 3-cyano-4,6-diaminopyridine (**20**) by conversion to the carboxamide **21** (90% H_2SO_4) and ring closure in neat triethyl orthoformate to give 7-aminopyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**22**). Diazotization of **22** in 50% HBF_4 with solid sodium nitrite[20] gave the 7-fluoro derivative (**23**). This was treated with $SOCl_2$ to give the unstable 4-chloro-7-fluoropyrido[4,3-*d*]pyrimidine (**24**), which was coupled directly with 3-bromoaniline to give **5e**. Displacement of the fluorine in **5e** with appropriate nucleophiles ($MeNH_2$, Me_2NH, MeO^-) gave the desired 7-substituted derivatives **5f**−**h**. Reaction of **5e** with $MeNH_2$ at 100 °C for 14 h in a pressure vessel gave two significant byproducts, **12** (21% yield) and **14** (27% yield), in addition to the desired **5f** (29% yield) (Scheme 3). These byproducts were not observed when the reaction was conducted at 95 °C for a shorter time (5 h). The structure of **12** was deduced from its distinctive ^1H and ^{13}C NMR spectra, which were assigned by the use of HMQC and HMBC experiments. In particular, long-range $^1H-^{13}C$ correlations in the HMBC experiment between the NMe protons and both C-2 and C-4, together with a correlation between H-2 and the NMe carbon resonance implied structure **12**. Further support for **12** was obtained from its distinctive UV spectrum, which differs from that of **5f** and related pyridopyrimidines in that a broad band at λ_{max} 330 nm is missing. Compound **12** presumably arose from **5f** by nucleophilic attack of $MeNH_2$ at C-4, ring opening of the pyrimidine, and then ring closure with elimination of ammonia (Scheme 3). Similarly, **14** was formed from **5f** by nucleophilic attack of $MeNH_2$ at C-4, followed by elimination of 3-bromoaniline.

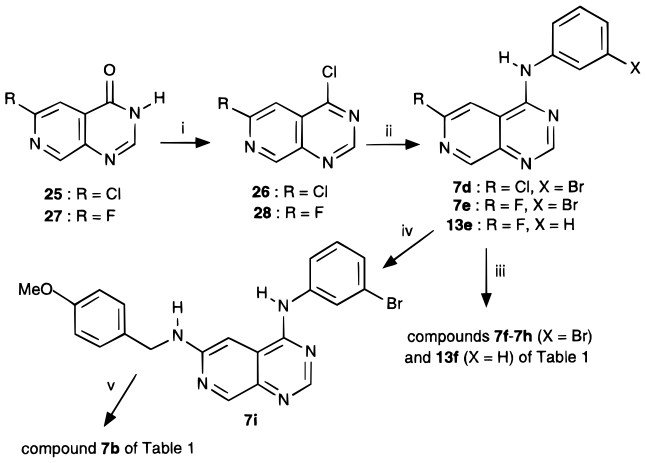
Scheme 3. Proposed Formation of Rearrangement Compound **12**



In the pyrido[3,4-*d*]pyrimidine series, the unsubstituted compounds (**6a** and **7a**) were prepared as above from the known[21] pyrido[3,4-*d*]pyrimidin-4(3*H*)-one, and the 6-chloro and 6-fluoro analogues (**7d** and **7e**) from the corresponding known[22] 6-substituted pyrido[3,4-*d*]-pyrimidin-4(3*H*)-ones **25** and **27** via the corresponding 4-chloro compounds **26** and **28** (Scheme 4). While the chlorine atom in **7d** could not easily be displaced by nucleophiles, the 6-fluoro derivative **7e** reacted successfully with methylamine, dimethylamine, and sodium methoxide to give **7f**−**h**, respectively. However, even **7e** did not react appreciably with ammonia to give **7b**, which was instead prepared by initial reaction of **7e** with 4-methoxybenzylamine, followed by cleavage of the resulting product **7i** with TFA (Scheme 4). Similar reaction of **28** with aniline, followed by methylamine, gave **13f**.

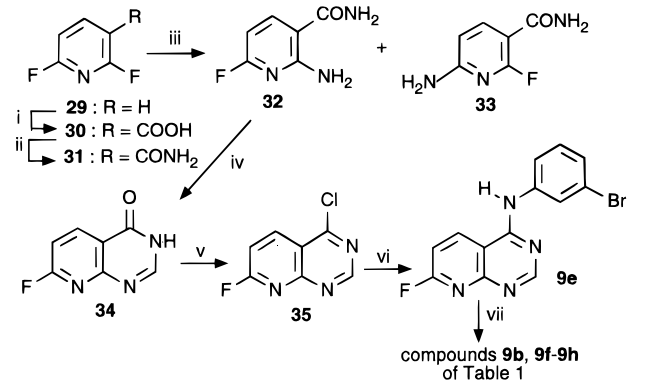
In the pyrido[2,3-*d*]pyrimidine series, the unsubstituted compounds **8a**[23] and **9a** were prepared as usual from the known[24] pyrido[2,3-*d*]pyrimidin-4(3*H*)-one. The 7-substituted pyrido[2,3-*d*]pyrimidines (**9b**,**f**−**h**) were synthesized by substitution of the the 6-fluoro derivative **9e** as described above. The latter compound was prepared from 2,6-difluoropyridine (**29**) by the method outlined in Scheme 5. Metalation of **29** with LDA at −78 °C,[25] followed by quenching with CO_2, gave the

Scheme 4[a]



[a] (i) $POCl_3$/reflux/2.5 h or $SOCl_2$/reflux/1 h; (ii) 3-bromoaniline or aniline/2-propanol/reflux/15 min; (iii) 40% aqueous $MeNH_2$ (or Me_2NH)/EtOH)/100 °C (pressure vessel)/18 h; or MeONa/MeOH/100 °C (pressure vessel)/48 h; (iv) 4-methoxybenzylamine/EtOH/100 °C (pressure vessel)/5 d; (v) TFA/reflux/1 h.

Scheme 5[a]

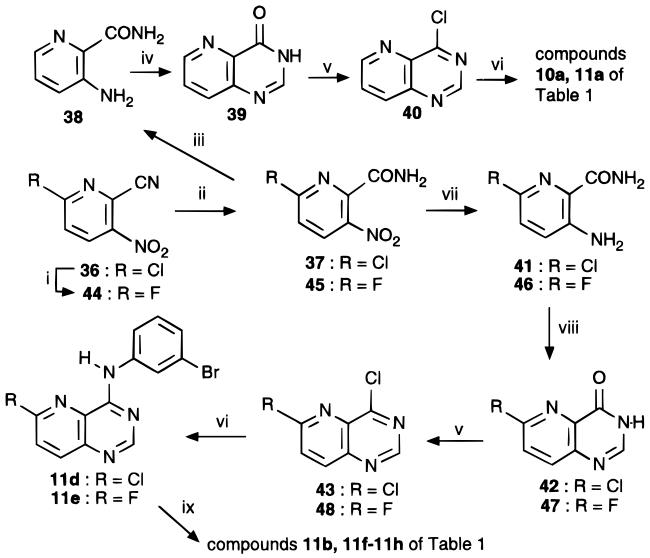


[a] (i) LDA/N_2/THF/−78 °C/2 h, then CO_2; (ii) $SOCl_2$/dichloroethane/reflux/4 h, then NH_4OH/0 °C; (iii) $HCONH_2$ saturated with NH_3/20 °C/24 h; (iv) $(EtO)_3CH$/reflux/8 h; (v) $POCl_3$/reflux/2 h; (vi) 3-bromoaniline/THF/2-propanol/20 °C/1 h; (vii) NH_3/EtOH/100 °C/30 h, or $MeNH_2$/EtOH, $NHMe_2$/EtOH or NaOMe/MeOH/100 °C (pressure vessel)/18 h.

difluoropyridine-3-carboxylic acid (**30**) in near quantitative yield. The derived carboxamide **31** was reacted with anhydrous ammonia in formamide[26] at room temperature to give a 1:2 mixture of the aminopyridines (**32** and **33**), in a combined yield of 66%. Separation of these and reaction of the minor isomer[26] (**32**) with neat triethyl orthoformate at reflux gave the pyrimidinone (**34**), which was converted into the chloropyrimidine (**35**) by treatment with $POCl_3$. Reaction of **35** with 3-bromoaniline afforded 4-[(3-bromophenyl)amino]-7-fluoropyrido[2,3-*d*]pyrimidine (**9e**), from which the 7-substituted analogues (**9b**,**f**−**h**) were obtained by reaction with appropriate nucleophiles at 100−120 °C, in a pressure vessel. In this case, the fluoro compound was sufficiently reactive to undergo direct replacement by ammonia to give **9b** in 90% yield.

Finally, the 6-substituted pyrido[3,2-*d*]pyrimidines were prepared (Scheme 6) from 6-chloro-2-cyano-3-nitropyridine[27] (**36**). To obtain the key 6-fluoro derivative (**11e**), **36** was converted into the fluoronitrile (**44**) by reaction with anhydrous KF in refluxing acetonitrile. Partial hydrolysis of the nitrile to the carboxamide (**45**), followed by reduction of the nitro group, gave 3-aminopyridine-2-carboxamide (**46**), which was reacted with triethyl orthoformate to give 6-fluoropyrido[3,2-*d*]pyrimidin-4(3*H*)-one (**47**). Conversion to the 4-chloro derivative (**48**) with $POCl_3$, followed by reaction with 3-bromoaniline, gave the 6-fluoro derivative (**11e**), from which the desired 6-substituted derivatives (**11b**,**f**−**h**) were obtained by displacement of fluoride with appropriate nucleophiles as above. The unsubstituted compounds of this series (**10a** and **11a**) were synthesized from 4-chloropyrido[3,2-*d*]pyrimidine[28] (**40**), which was prepared by partial hydrolysis of the chloronitrile (**36**) to 6-chloro-3-nitropyridine-2-carboxamide (**37**). Prolonged catalytic hydrogenation of this resulted in concomitant reduction of the nitro group and hydrogenolysis of the chlorine, and the resulting 3-aminopyridine-2-carboxamide (**38**) was elaborated to the known[29] pyrimidinone (**39**) and thence to **40**. Hydrogenation of **37** for a shorter time gave 3-amino-6-chloropyridine-2-carboxamide (**41**), from which 4,6-dichloropyrido[3,2-*d*]-pyrimidine (**43**) (for preparation of **11d**) was obtained via the pyrimidinone (**42**).

Scheme 6[a]

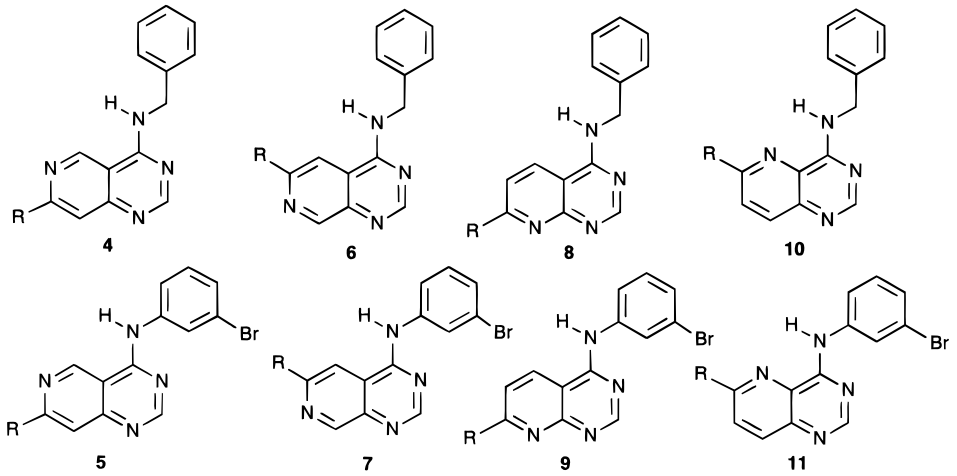


[a] (i) KF/MeCN/reflux/18 h; (ii) 90% H_2SO_4/70 °C/1.5−3.5 h; (iii) H_2/Pd/C/EtOAc/MeOH/20 °C/6 d; (iv) $(EtO)_3CH$/reflux/42 h; (v) $POCl_3$/reflux/3−4 h; (vi) 3-bromoaniline or benzylamine/2-propanol/50−80 °C/30 min; (vii) H_2/Pd/C/EtOAc/20 °C/20 min to 2 h; (viii) $(EtO)_3CH$/reflux/18 h; (ix) NH_3/EtOH, $MeNH_2$·HCl or Me_2NH·HCl/Et_3N/EtOH or NaOMe/MeOH/100 °C (pressure vessel)/3−18 h.

Results and Discussion

The structures, physicochemical properties, and EGFR inhibitory potencies (IC_{50}s in nanomolar) of the compounds prepared are given in Table 1. The IC_{50} values are the average of at least two independent determinations of full dose−response curves. The assay used a purified full-length EGFR, stimulated by EGF, phosphorylating a tyrosine-containing peptide based upon the major EGFR phosphorylation site on PLCγ1.[14] Similar results were seen with representative compounds when the phosphopeptide substrate was substituted by a Glu-Tyr copolymer. As noted in detail elsewhere,[14] the conditions under which this assay was used are critical, requiring highly pure enzyme preparations. Because previous work with both quinazolines[15,16] and pyrido[4,3-*d*]pyrimidines[17] had suggested

Table 1. Physicochemical and Tyrosine Kinase Inhibitory Properties of Isomeric Pyrido[*d*]pyrimidine Analogues



no.	R	mp, °C	formula	anal.	IC_{50}[a] (nM)
			Pyrido[4,3-*d*]pyrimidines		
4a	H	175	$C_{14}H_{12}N_4$	C,H	1460
4b	NH_2		ref 17		578
4c	NHAc	253−255	$C_{16}H_{15}N_5O$	C,H,N	100
5a	H	245	$C_{13}H_9BrN_4$		35
5b	NH_2		ref 17		10
5c	NHAc	307−309	$C_{15}H_{12}BrN_5O$	C,H	29
5e	F	219−221.5	$C_{13}H_8BrFN_4$	C,H,N	13
5f	NHMe	288−290	$C_{14}H_{12}BrN_5$	C,H,N	0.13
5g	NMe_2	240−241.5	$C_{15}H_{14}BrN_5$	C,H,N	0.091
5h	OMe	276−279	$C_{14}H_{11}BrN_4O$	C,H,N	39
12[b]		263−264	$C_{15}H_{14}BrN_5 \cdot 0.5H_2O$	C,H,N	3.1
			Pyrido[3,4-*d*]pyrimidines		
6a	H		ref 31		2030
7a	H	209	$C_{13}H_9BrN_4 \cdot 0.25H_2O$	C,H,N	51
7b	NH_2	241.5−242	$C_{13}H_{10}BrN_5$	C,H,N	0.13
7d	Cl	201−202	$C_{13}H_8BrClN_4$	C,H,N	40
7e	F	219.5−221	$C_{13}H_8BrFN_4$	C,H,N,F	124
7f	NHMe	172−173	$C_{14}H_{12}BrN_5$	C,H,N	0.008
7g	NMe_2	244.5−245.5	$C_{15}H_{14}BrN_5$	C,H,N	0.006
7h	OMe	275−276	$C_{14}H_{11}BrN_4O$	C,H,N	2.6
7i	$NHCH_2PhOMe$	178−179.5	$C_{21}H_{18}BrN_5O$	C,H,N	2.3
13f[c]	NHMe[c]	212−212.5	$C_{14}H_{13}N_5$	C,H,N	9.0
			Pyrido[2,3-*d*]pyrimidines		
8a	H		ref 23		$>10^4$
9a	H	221−224	$C_{13}N_9BrN_4$	C,H,N	688
9b	NH_2	297−300	$C_{13}H_{10}BrN_5 \cdot H_2O$	C,H,N	940
9e	F	211−213	$C_{13}H_8BrFN_4 \cdot H_2O$	C,H,N	684
9f	NHMe	245−247	$C_{14}H_{12}BrN_5$	C,H,N	52
9g	NMe_2	287−289	$C_{15}H_{14}BrN_5$	C,H,N	324
9h	OMe	260−261	$C_{14}H_{11}BrN_4O$	C,H,N	263
			Pyrido[3,2-*d*]pyrimidines		
10a	H	83	$C_{14}H_{12}N_4$	C,H,N	603
11a	H	176−178	$C_{13}H_9BrN_4$	C,H,N,Br	34
11b	NH_2	225−227	$C_{13}H_{10}BrN_5$	C,H,N	7.6
11d	Cl	167−169	$C_{13}H_8BrClN_4$	C,H,N,Cl	18
11e	F	162	$C_{13}H_8BrFN_4$	C,H,N	44
11f	NHMe	206−208	$C_{14}H_{12}BrN_5$	C,H,N	3.1
11g	NMe_2	158	$C_{15}H_{14}BrN_5$	C,H,N	9.6
11h	OMe	142	$C_{14}H_{11}BrN_4O$	C,H,N	4.3

[a] IC_{50}, concentration of drug (nM) to inhibit the phosphorylation of a 14-residue fragment of phospholipase C-γ1 by EGFR (prepared from human A431 carcinoma cell vesicles by immunoaffinity chromatography). See Experimental Section for details. Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [b] See Scheme 3 for structure. [c] Debromo derivative.

that a 4-benzylamino side chain was also useful, we evaluated this for each of the positional isomers studied here. However, in each case this resulted in less potent compounds (**4a**, **6a**, **8a**, **10a**) than did the 3-(bromophenyl)amino side chain (cf. **5a**, **7a**, **9a**, **11a**). Also, in the [4,3-*d*] series, the 7-amino-substituted 4-benzylamino compound (**4b**) was much less active (IC_{50} 578 nM) than the corresponding 4-[(3-bromophenyl)amino] derivative (**5b**) (IC_{50} 10 nM),[17] and the same was true in the present case for the two 7-acetylamino derivatives (**4c** and **5c**).

The bulk of the work in the isomeric series, and the discussions below, therefore focused on the 4-[(3-bromophenyl)amino] derivatives. We have previously shown[15] with the conformationally similar quinazolines that substitution in the 6- and/or 7-positions (off the long

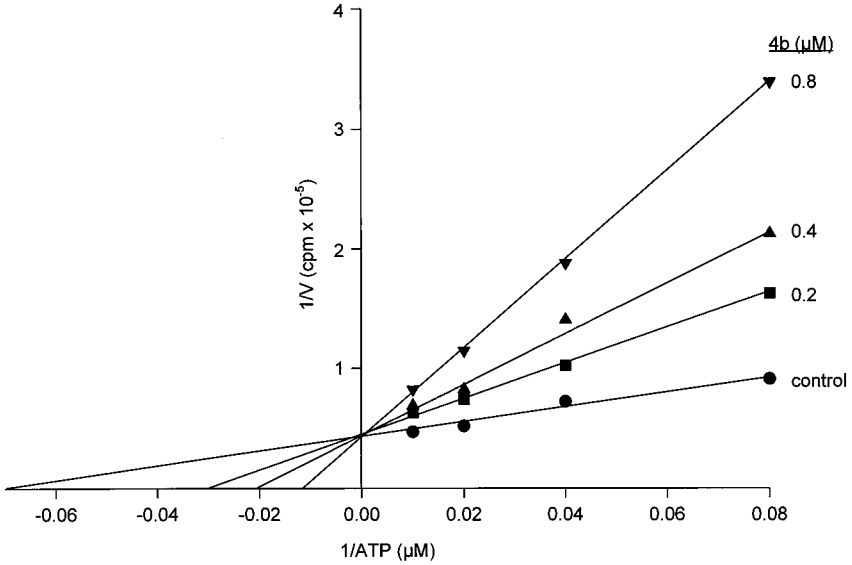


Figure 1. Double reciprocal plot for inhibition of EGFR tyrosine kinase by compound **4b**. Enzyme activity was determined as described in the Experimental Section, using the indicated concentrations of **4b** and ATP. Lines were determined by least squares analysis.

Table 2. Comparison of Inhibitory Potencies (IC_{50}s in nM against Isolated EGFR) of 6- and 7-Substituted 4-[(3-Bromophenyl)amino]quinazolines and Related Isomeric Pyrido[*d*]pyrimidines (pypy)

		substituent				
chromophore	position	H	NH_2	NHMe	NMe_2	OMe
quinazoline[a]	6	27	0.79	7	120	30
[3,2-*d*]pypy[b]	6	34 (**11a**)	7.6 (**11b**)	3.1 (**11f**)	9.6 (**11g**)	4.3 (**11h**)
[3,4-*d*]pypy[b]	6	51 (**7a**)	0.13 (**7b**)	0.008 (**7f**)	0.006 (**7g**)	2.6 (**7h**)
quinazoline[a]	7	27	0.10	4	11	10
[4,3-*d*]pypy[b]	7	35 (**5a**)	10 (**5b**)	0.13 (**5f**)	0.09 (**5g**)	39 (**5h**)
[2,3-*d*]pypy[b]	7	688 (**9a**)	940 (**9b**)	52 (**9f**)	324 (**9g**)	263 (**9h**)

[a] Data from refs 15 and 16. [b] Data from Table 1 (bold numbers in parentheses refer to compound numbers in Table 1).

axis of the quinazoline) was much superior to substitution at the 5- or 8-positions and also that electron-donating groups were preferred. The present study was therefore restricted primarily to electron-donating groups (NR_1R_2, OMe) at the 6- or 7-positions, together with the corresponding halogenated derivatives used as synthetic precursors. Figure 1 shows a double-reciprocal plot for inhibition of EGFR by **4b** with respect to ATP concentration. The converging lines on the *Y* axis indicate that it behaves as a pure competitive inhibitor at the ATP site, as shown previously[16] for related 4-(phenylamino)-quinazolines. Because very tight binding ligands do not conform to the equations derived for steady-state enzyme kinetics, these analyses could not be performed for the more potent inhibtors (e.g., **5g**, **7g**).

Table 1 shows that in the 7-substituted [4,3-*d*] series the mildly electron-withdrawing NHAc and fluoro derivatives (**5c** and **5e**) retained high potency in the isolated enzyme assay (IC_{50}s < 30 nM) and in fact proved more active than the OMe compound (**5h**). However, the most interesting results were with the *N*-methylated analogues of the 7-amino derivative **5b**, where addition of one or two methyl groups increased potency nearly 100-fold (**5f**, IC_{50} 0.13 nM; **5g**, IC_{50} 0.091 nM). A broadly similar pattern is seen in the 6-substituted [3,4-*d*] series except that, with the exception of the 6-F derivative **7e**, these compounds are more potent. This is particularly so for the 7-NHMe and 7-NMe_2 analogues (**7f**,**g**), which have IC_{50}s of 0.006−0.008 nM (Table 1). In contrast, both the 6-substituted [3,2-*d*] and 7-substituted [2,3-*d*] compounds were less effective overall (the latter particularly so, with the 7-NHMe derivative **9f** having an IC_{50} of 52 nM, compared with a value of 0.13 nM for the corresponding [4,3-*d*] derivative **5f)**.

Because the pyridopyrimidines can be considered as aza derivatives of quinazolines, which were the initial class of compounds studied,[13−16] it was instructive to compare the data for the isomeric pyridopyrimidines with results for the corresponding 6- or 7-substituted quinazolines generated in the same assay.[15,16] In Table 2, the effects of differing electron-donating substituents at the 6- and 7-positions on the various 4-[(3-bromophenyl)amino]pyrido[*d*]pyrimidine chromophores are compared, both within the isomer series and with the corresponding "parent" quinazolines. A comparison of IC_{50} values between the parent (unsubstituted chromophore) compounds of the four series of pyrido[*d*]-pyrimidines and previously-reported[15] quinazolines show that an aza atom in ring B is neutral or moderately detrimental. Compared with the quinazoline (IC_{50} 27 nM),[15] a 5- or 6-aza atom has little effect, while a 7-aza atom lowers the IC_{50} by only 2-fold (**7a**, IC_{50} 51 nM), corresponding to a loss in binding energy of ca. 0.4 kcal/mol (by use of the Boltzmann equation $\Delta G = -RT \ln K$ and assuming that enzyme inhibitory potencies for this structurally similar group of compounds are directly proportional to their binding energies to the enzyme).[16] An 8-aza substituent causes an even bigger loss (25-fold, ca. 1.9 kcal/mol) (**9a**, IC_{50} 688 nM).

For the NH_2 and OMe derivatives, the results are broadly similar to those seen with the unsubstituted

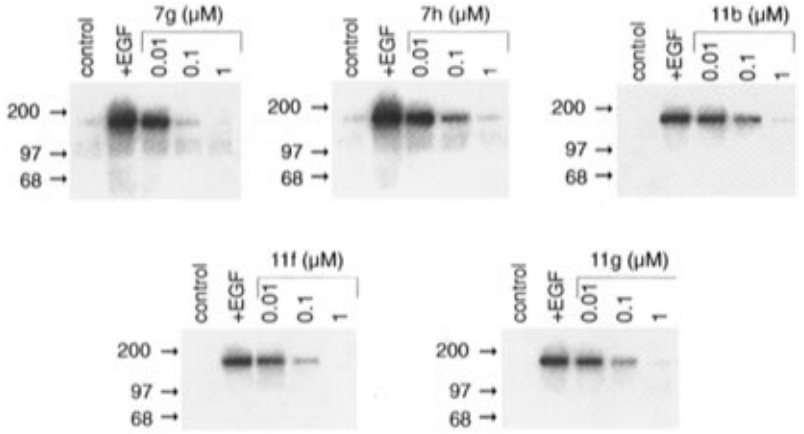


Figure 2. Representative western blots for inhibition of EGFR autophosphorylation in human epidermoid carcinoma cells. Cells were treated with various concentrations of the indicated compounds for 2 h and then exposed to EGF (100 ng/mL) for 5 min. Receptor autophosphorylation was quantitated by western blotting as described in the Experimental Section.

chromophores, with the [3,4-*d*] analogues (**7b**,**h**) showing slightly increased potencies in comparison to the corresponding quinazoline, with the [4,3-*d*], [2,3-*d*], and [3,2-*d*] analogues (**5b**,**h**, **9b**,**h**, and **11b**,**h**) being less effective except for **11h**. The major surprise comes with the alkylamino substituents NHMe and NMe_2. In the quinazoline series,[16] replacement of either a 6- or 7-NH_2 group with the corresponding NHMe or NMe_2 groups lowered potency significantly (by 9−150-fold). In contrast, very little change is seen for substitution of a 6-NH_2 in the [3,2-*d*] series, while large (15−110-fold) *increases* in potency were seen in the [3,4-*d*] and [4,3-*d*] series, and lesser but significant (3−18-fold) increases in the [2,3-*d*] series (Table 2). Energetically, dimethylation of the 6- or 7-amine in the [3,4-*d*]- and [4,3-*d*]-pyridopyrimidines respectively resulted in an increase in binding energy of 1.8−2.8 kcal/mol, compared with a comparable *loss* of 2.8−3.0 kcal/mol for the corresponding quinazolines.

Although it is possible that the quinazolines and pyridopyrimidines bind quite differently to the enzyme, so that comparisons are invalid, this does not seem likely. The compounds are structurally very similar, and both quinazolines[16] and pyridopyrimidines[17] show clearly reversible ATP-competitive kinetics of inhibition. Additionally, both demonstrate the phenomenon of enormous "supra-additive" enhancements in activity with certain combinations of 3′-phenyl and 6/7-chromophore substituents. This has been reported previously for the quinazolines[16] and is demonstrated here for the [3,4-*d*]pyridopyrimidines by the large difference in IC_{50}s between **7f** and its corresponding debromo and des(methylamino) derivatives **13f** and **7a** (1125-fold and 6375-fold respectively, corresponding to 4.18 and 5.21 kcal/mol increases in binding energy by addition of the respective second substituent). Two other possible explanations can be offered. Because the pyridopyrimidines are more electron deficient than the quinazolines, it is possible that the slightly higher electron-donating ability of the NHMe and NMe_2 derivatives have greater effects in the former series. It is also possible that there are some steric constraints at these positions, with the pyridopyrimidines providing less out-of-plane conformations of the bulky methylamino and dimethylamino groups due to resonance interactions between substituent and adjacent ring nitrogen atoms. However, both of these arguments should apply as well to the [2,3-*d*] and [3,2-*d*] series, where the peak potency is in fact with the methylamino derivatives, suggesting the effect is too complex to be accounted for by these factors alone.

Table 3. Inhibition by Selected Pyridopyrimidines of EGFR Autophosphorylation in A431 Human Epidermoid Carcinoma Cells

no.	structure	IC_{50} (nM)[a]	no.	structure	IC_{50} (nM)[a]
4b	7-NH_2-[4,3-*d*] (benzyl)	2085	**7h**	6-OMe-[3,4-*d*]	12
5b	7-NH_2-[4,3-*d*]	110	**9f**	7-NHMe-[2,3-*d*]	552
5f	7-NHMe-[4,3-*d*]	16	**9g**	7-NMe_2-[2,3-*d*]	2300
5g	7-NMe_2-[4,3-*d*]	14	**11b**	6-NH_2-[3,2-*d*]	53
7b	6-NH_2-[3,4-*d*]	16	**11f**	6-NHMe-[3,2-*d*]	20
7f	6-NHMe-[3,4-*d*]	15	**11g**	6-NMe_2-[3,2-*d*]	32
7g	6-NMe_2[3,4-*d*]	21			

[a] IC_{50}s for inhibition of phosphorylation of EGFR in A431 cells in culture. Values were determined from data similar to that shown in Figure 2 and are the average of two experiments. See Experimental Section for details.

Selected compounds were evaluated for their ability to inhibit autophosphorylation of EGFR in A431 human epidermoid carcinoma cells,[14] quantitating the response by western blotting (Figure 2). The results are summarized in Table 3 and show that some compounds in this series efficiently inhibit receptor autophosphorylation in viable cells, with IC_{50}s in the low nanomolar range. For the less effective compounds (IC_{50}s for enzyme inhibition >1 nM) there was a very good direct correlation between enzyme inhibition and inhibition of receptor autophosphorylation (eq 1).

$$\log (IC_{50}[\text{autophos}]) = 0.98(\pm 0.09) \log (IC_{50}[\text{enzyme}]) + 0.81(\pm 0.14) \quad (1)$$

$$n = 8 \qquad r = 0.98 \qquad F_{2,6} = 127 \qquad p < 0.001$$

While this relationship does not hold for the very potent compounds, which are less effective than predicted in the the cellular assay, this is probably a limitation of the assay. For these very lipophilic compounds, nonspecific binding to other cellular components probably limits their availability at low concentrations. Furthermore, given that the assay contains a calculated 6 pmol of receptor per well in a 2 mL volume, the absolute minimum IC_{50} the assay could measure, even for stoichiometric inhibition, would be ca. 3 nM [6 pmol = $(3 \times 10^{-9}$ mol/L$)(2 \times 10^{-3}$ L)].

Conclusions

The above results show that, while all four isomeric series of 4-[(3-bromophenyl)amino]pyrido[*d*]pyrimidines are effective inhibitors of the tyrosine kinase activity of EGFR, there are significant differences both between them and the parent 4-[(3-bromophenyl)amino]quinazolines and between the different pyridopyrimidine series. Generally, the [3,4-*d*] and [4,3-*d*] series are the most potent, followed by the [3,2-*d*] compounds, with the [2,3-*d*] analogues being least active. Within each series the SAR also differ. In the quinazoline series, addition of bulk (methyl groups) to either 6- or 7-amino substituents dramatically decreased potency in the isolated enzyme assay. In the [3,2-*d*] series, no such trend is discernable, but in the 7-substituted pyrido[4,3-*d*]- and 6-substituted pyrido[3,4-*d*]pyrimidine series, and to a lesser extent in the 7-substituted pyrido[2,3-*d*] series, such substitution *increases* potency equally dramatically, to the extent that the 7-(methylamino)pyrido[4,3-*d*]- and 6-(methylamino)pyrido[3,4-*d*]pyrimidines (**5f** and **7f)** constitute important new leads with low picomolar potencies, comparable to the best quinazoline inhibitors.[16] The reasons for the different SAR are not clear, but may partially relate to steric constraints, with the pyridopyrimidines providing nearer to planar conformations than quinazoline for bulky substituents. The positive quantitative correlation (for the compounds as a whole) between their abilities to inhibit phosphorylation of substrate in isolated enzyme assays and to inhibit autophosphorylation in EGFR-expressing cells suggest similar SAR exist for both activities.

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ, or by Parke-Davis Pharmaceutical Research Analytical Department. Melting points were determined using an Electrothermal Model 9200 or Gallenkamp digital melting point apparatus, and are as read. NMR spectra were measured on Bruker AC-200 or AM-400 or Varian Unity 400 MHz spectrometers and referenced to Me_4Si. Mass spectra were recorded either on a Varian VG 7070 spectrometer at nominal 5000 resolution or a Finnigan MAT 900Q spectrometer.

Pyrido[4,3-*d*]pyrimidines: 4-[(Phenylmethyl)amino]-pyrido[4,3-*d*]pyrimidine (4a) and 4-[(3-Bromophenyl)-amino]pyrido[4,3-*d*]pyrimidine (5a) (Scheme 1). A solution of pyrido[4,3-*d*]pyrimidin-4(3*H*)-one[18] (**15**) (780 mg, 5.3 mmol) in pyridine (5 mL) was treated with P_2S_5 (2.59 g, 5.83 mmol), and the mixture was heated under reflux for 5 h and then cooled thoroughly. The resulting precipitate was collected, suspended in water (20 mL), and filtered to yield pyrido-[4,3-*d*]pyrimidine-4(3*H*)-thione (**16**) (676 mg, 78%) as a black solid, which was used directly (lit.[18] mp 323−325 °C): ^{1}H NMR [$(CD_3)_2SO$] δ 14.53 (br s, 1 H, NH), 9.65 (s, 1 H, H-5), 8.84 (d, J = 6.0 Hz, 1 H, H-7), 8.32 (s, 1 H, H-2), 7.64 (d, J = 6.0 Hz, 1 H, H-8).

A mixture of **16** (676 mg, 4.14 mmol), Et_3N (1.4 mL, 10.31 mmol), DMSO (4 mL), and MeI (0.48 mL, 7.72 mmol) was stirred for 12 h under N_2 at 25 °C, then poured into water, and extracted with EtOAc. The organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give crude 4-(methylthio)pyrido[4,3-*d*]pyrimidine (**17**) as a brown solid, which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 9.52 (s, 1 H, H-5), 9.16 (s, 1 H, H-2), 8.95 (d, J = 6.0 Hz, 1 H, H-7), 7.86 (d, J = 6.0 Hz, 1 H, H-8), 2.75 (s, 3 H, SCH_3).

A mixture of crude **17** (171 mg, 0.96 mmol) and 3-bromoaniline (1 mL) was heated to 100 °C for 2 h and then cooled. The resulting precipitate was collected and crystallized from EtOH to yield 4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidine (**5a**) (30 mg, 10%): mp 245 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.33 (s, 1 H, NH), 9.86 (s, 1 H, H-5), 8.84 (d, J = 5.8 Hz, 1 H, H-7), 8.79 (s, 1 H, H-2), 8.22 (br s, 1 H, H-2′), 7.89 (d, J = 8.0 Hz, 1 H, H-6′), 7.69 (d, J = 5.8 Hz, 1 H, H-8), 7.40 (dt, J = 8.0, 1.5 Hz, 2 H, H-4′,5′). Anal. ($C_{13}H_9BrN_4$) C, H, N.

Similar reaction of crude **17** with benzylamine gave 4-[(3-phenylmethyl)amino]pyrido[4,3-*d*]pyrimidine (**4a**) (17%): mp 175 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.60 (s, 1 H, H-5), 9.37 (t, J = 5.8 Hz, 1 H, NH), 8.72 (d, J = 5.8 Hz, 1 H, H-7), 8.57 (s, 1 H, H-2), 7.54 (d, J = 5.8 Hz, 1 H, H-8), 7.37 (d, J = 7.0 Hz, 2 H, ArH), 7.33 (d, J = 7.3 Hz, 2 H, ArH), 7.25 (t, J = 7.2 Hz, 1 H, ArH), 4.81 (d, J = 5.8 Hz, 2 H, CH_2). Anal. ($C_{14}H_{12}N_4$) C, H.

7-Acetamido-4-[(phenylmethyl)amino]pyrido[4,3-*d*]pyrimidine (4c) (Scheme 1). Acetyl chloride (0.70 mL, 9.84 mmol) was added to a solution of 7-amino-4-(methylthio)-pyrido[4,3-*d*]pyrimidine[17] (**18**) (0.20 g, 1.04 mmol) and Et_3N (1.51 mL, 10.8 mmol) in THF at 0 °C, and then the mixture was stirred at 20 °C for 4 h. Water (50 mL) was added, and the solution was extracted with EtOAc (3 × 50 mL). Evaporation and chromatography of the residue on alumina, eluting with $CHCl_3$/EtOH (99:1), gave 7-acetamido-4-(methylthio)-pyrido[4,3-*d*]pyrimidine (**19**) (0.12 g, 49%): mp (CH_2Cl_2/petroleum ether) 229−232.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.05 (s, 1 H, NH), 9.30 (s, 1 H, H-5), 9.02 (s, 1 H, H-2), 8.38 (s, 1 H, H-8), 2.71 (s, 3 H, SCH_3), 2.18 (s, 3 H, $COCH_3$); ^{13}C NMR δ 171.96 (s, C-4), 169.95 (s, CONH), 156.92 (d, C-2), 153.76, 152.48 (2s, C-7,8a), 148.18 (d, C-5), 116.04 (s, C-4a), 106.17 (d, C-8), 23.97 (q, $COCH_3$), 11.73 (q, SCH_3); HREIMS m/z calcd for $C_{10}H_{10}N_4OS$ 234.0575 (M^+), found 234.0576. Anal. ($C_{10}H_{10}N_4OS$) C, H.

A mixture of **19** (0.40 g, 1.71 mmol) and benzylamine (1.0 mL, 9.15 mmol) was stirred under N_2 at 140 °C for 1 h, and the resulting product was chromatographed on silica gel (EtOAc) to give 7-acetamido-4-[(phenylmethyl)amino]pyrido-[4,3-*d*]pyrimidine (**4c**) (0.31 g, 62%): mp (CH_2Cl_2/petroleum ether) 253−255 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.79 (s, 1 H, NH), 9.42 (s, 1 H, H-5), 9.23 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.49 (s, 1 H, H-2), 8.18 (s, 1 H, H-8), 7.39 (dt, J = 6.9, 1.7 Hz, 1 H, ArH), 7.34 (tt, J = 7.3, 1.7 Hz, 1 H, ArH), 7.25 (tt, J = 7.1, 1.7 Hz, 1 H, ArH), 4.80 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$), 2.15 (s, 3 H, $COCH_3$); ^{13}C NMR δ 169.60 (s, CONH), 159.13 (s + d, 2 C, C-2,4), 154.81, 153.14 (2 s, C-7,8a), 147.23 (d, C-5), 138.80 (s, C-1′), 128.31, 127.31 (2 d, 2 × 2 C, C-2′,3′,5′,6′), 126.89 (d, C-4′), 107.99 (s, C-4a), 106.15 (d, C-8), 43.44 (t, NCH_2), 23.96 (q, CH_3). Anal. ($C_{16}H_{15}N_5O$) C, H, N.

7-Acetamido-4-[(3-bromophenyl)amino]pyrido[4,3-*d*]-pyrimidine (5c). Similar reaction of **19** (118 mg, 0.50 mmol) with 3-bromoaniline (1.00 mL, 9.19 mmol) at 155 °C for 1 h, followed by chromatography of the resulting product on silica gel, eluting with 3−4% MeOH/CH_2Cl_2, gave 7-acetamido-4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidine (**5c**) (125 mg, 69%): mp (MeOH) 307−309 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.91 (s, 1 H, NH), 10.21 (s, 1 H, NH), 9.64 (s, 1 H, H-5), 8.69 (s, 1 H, H-2), 8.28 (s, 1 H, H-8), 8.21 (t, J = 1.6 Hz, 1 H, H-2′), 7.88 (br d, J = 7.6 Hz, 1 H, H-6′), 7.39 (t, J = 7.8 Hz, 1H, H-5′), 7.35 (dt, J = 8.0, 1.6 Hz, 1 H, H-4′), 2.17 (s, 3 H, CH_3). Anal. ($C_{15}H_{12}BrN_5O$) C, H.

4-[(3-Bromophenyl)amino]-7-fluoropyrido[4,3-*d*]pyrimidine (5e) (Scheme 2). 3-Cyano-4,6-diaminopyridine[19] (**20**) (4.30 g, 0.032 mol) was added to 90% H_2SO_4 (25 mL) and then stirred at 60−70 °C for 3 h. The resulting solution was added to cold concentrated NaOH (40%), precipitating a mixture of 4,6-diaminopyridine-3-carboxamide (**21**) and inorganic salts. An analytically pure sample of **21** was obtained by chromatography on alumina (10−50% MeOH/$CHCl_3$) as a pale yellow solid: mp (MeOH/$CHCl_3$) 278−280 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.15 (s, 1 H, H-2), 6.91 (br s, 2 H, 4-NH_2), 7.7−6.3 (br m, 2 H, $CONH_2$), 5.78 (br s, 2 H, 6-NH_2), 5.56 (s, 1 H, H-5); ^{13}C NMR δ 170.56 (s, $CONH_2$), 161.24, 156.04 (2 s, C-4,6), 150.36 (d, C-2), 103.19 (s, C-3), 89.18 (d, C-5). Anal. ($C_6H_8N_4O$) C, H, N.

Crude **21** (9.2 g) was heated in purified $(EtO)_3CH$ (60 mL, distilled from Na) at 170 °C for 1.5 d. After removing the solvent, the residue was dissolved in hot 2 M NaOH, heated at 90 °C for 5 min, filtered, neutralized (concentrated HCl), and cooled to give 7-aminopyrido[4,3-*d*]pyrimidin-4(3*H*)-one (**22**) (3.57 g, 69% from the nitrile): mp >350 °C; ^{1}H NMR

[$(CD_3)_2SO$] δ 11.79 (br s, 1 H, NH), 8.74 (s, 1 H, H-5), 7.97 (s, 1 H, H-2), 6.76 (s, 2 H, 7-NH_2), 6.38 (s, 1 H, H-8); ^{13}C NMR δ 163.71, 160.20, 154.78 (3 s, C-4,7,8a), 150.14, 149.09 (2 d, C-2,5), 108.95 (s, C-4a), 98.87 (d, C-8). Anal. ($C_7H_6N_4O$) C, H, N.

A solution of **22** (5.00 g, 30.9 mmol) in 50% HBF_4 (125 mL) at 0 °C was treated with solid $NaNO_2$ (4.25 g, 61.6 mmol, added in portions over 4 h), then stirred at 0 °C for a further 2.5 h, and kept at −20 °C for 16 h. The resulting mixture was neutralized with solid Na_2CO_3/ice, keeping the temperature below −10 °C, and extracted with EtOAc (6 × 200 mL). The combined extracts were washed with water and filtered through silica gel, eluting with EtOAc, to give 7-fluoropyrido-[4,3-*d*]pyrimidin-4(3*H*)-one (**23**) (2.91 g, 57%): mp (EtOAc) >258 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 12.69 (br s, 1 H, NH), 9.01 (s, 1 H, H-5), 8.31 (s, 1 H, H-2), 7.34 (s, 1 H, H-8); ^{13}C NMR δ 166.25 (d, J_{C-F} = 238 Hz, C-7), 159.66 (s, C-4), 158.31 (d, J_{C-F} = 12 Hz, C-8a), 151.14 (d, C-2), 149.44 (dd, J_{C-F} = 19 Hz, C-5), 117.63 (d, J_{C-F} = 3 Hz, C-4a), 104.27 (dd, J_{C-F} = 37 Hz, C-8). Anal. ($C_7H_4FN_3O$) C, H, N.

A suspension of **23** (2.91 g, 17.6 mmol) in $SOCl_2$ (100 mL) containing 2 drops of DMF was stirred under reflux for 8 h and then concentrated under reduced pressure to give crude 4-chloro-7-fluoropyrido[4,3-*d*]pyrimidine (**24**) as an oil. This was ice-cooled, and a solution of 3-bromoaniline (5 mL, 45.9 mmol) in CH_2Cl_2 (50 mL) was added, followed by dry 2-propanol (50 mL), and the mixture was stirred at 20 °C for 16 h. Addition of aqueous $NaHCO_3$ gave a precipitate which was collected and washed with water and CH_2Cl_2 to give 4-[(3-bromophenyl)amino]-7-fluoropyrido[4,3-*d*]pyrimidine (**5e**) (5.05 g, 90%): mp (2-propanol/water) 219−221.5 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.38 (br s, 1 H, NH), 9.59 (s, 1 H, H-5), 8.72 (s, 1 H, H-2), 8.17 (s, 1 H, H-2′), 7.85 (m, 1 H, H-6′), 7.38 (m, 3 H, H-4′,5′,8); ^{13}C NMR δ 164.76 (d, J_{C-F} = 237 Hz, C-7), 159.07 (d, C-2), 157.81 (s, C-4), 157.20 (d, J_{C-F} = 13 Hz, C-8a), 148.41 (dd, J_{C-F} = 19 Hz, C-5), 139.78 (s, C-1′), 130.44 (d, C-5′), 127.01, 124.86 (2 d, C-2′,4′), 121.30 (d, C-6′), 121.19 (s, C-3′), 110.51 (d, J_{C-F} = 3 Hz, C-4a), 103.00 (dd, J_{C-F} = 36 Hz, C-8). Anal. ($C_{13}H_8BrFN_4$) C, H, N.

4-[(3-Bromophenyl)amino]-7-(methylamino)pyrido[4,3-*d*]pyrimidine (5f). A mixture of **5e** (74 mg, 0.23 mmol), Et_3N (7 mL, 50 mmol), and methylamine hydrochloride (3.0 g, 44 mmol) in 2-propanol (30 mL) was stirred in a pressure vessel at 95 °C (oil bath) for 5 h. The resulting mixture was concentrated under reduced pressure, basified with aqueous Na_2CO_3, diluted with water, and extracted with EtOAc (3 × 100 mL). Chromatography of this extract on silica gel, eluting with MeOH/CH_2Cl_2 (3:97), gave 4-[(3-bromophenyl)amino]-7-(methylamino)pyrido[4,3-*d*]pyrimidine (**5f**) (50 mg, 65%): mp (MeOH/CH_2Cl_2/petroleum ether) 288−290 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.93 (s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.47 (s, 1 H, H-2), 8.18 (s, 1 H, H-2′), 7.84 (d, J = 7.8 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.30 (br d, J = 8.1 Hz, 1 H, H-4′), 7.19 (q, J = 4.7 Hz, 1 H, 7-N*H*CH_3), 6.35 (s, 1 H, H-8), 2.85 (d, J = 4.8 Hz, 3 H, 7-NHC*H*$_3$); ^{13}C NMR δ 161.55 (s, C-7), 157.81 (d, C-2), 157.68 (s, C-4), 154.58 (s, C-8a), 148.43 (d, C-5), 140.78 (s, C-1′), 130.37 (d, C-5′), 126.02, 124.29 (2 d, C-2′,4′), 121.15 (s, C-3′), 120.80 (d, C-6′), 103.61 (s, C-4a), 94.70 (br d, C-8), 28.40 (q, NCH_3). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

When the above reaction was carried out at 100 °C for 14 h, a mixture of products was obtained (Scheme 3). Chromatography on silica gel and elution with 1−2% MeOH/CH_2Cl_2 gave firstly N^4-(3-bromophenyl)-3-methyl-7-(methylamino)-pyrido[4,3-*d*]pyrimidin-4(3*H*)-imine (**12**) (23 mg, 21%): mp (MeOH/CH_2Cl_2/petroleum ether) 263−264 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.14 (s, 1 H, H-2), 7.79 (s, 1 H, H-5), 7.30 (J = 8.0 Hz, 1 H, ArH), 7.20 (ddd, J = 7.9, 1.8, 0.8 Hz, 1 H, ArH), 7.03 (br q, J = 4.9 Hz, 1 H, 7-N*H*CH_3), 7.01 (t, J = 1.9 Hz, 1 H, ArH), 6.82 (ddd, J = 7.8, 1.8, 0.9 Hz, 1 H, ArH), 6.25 (s, 1 H, H-8), 3.40 (s, 3 H, NCH_3), 2.73 (d, J = 4.9 Hz, 3 H, 7-NHC*H*$_3$); ^{13}C NMR δ 160.93 (s, C-7), 153.74 (s, C-8a), 153.16 (s, C-4a), 151.65 (d, C-2), 149.61 (d, C-5), 145.30 (s, C-4), 131.75 (d, C-5′), 124.38 (d, C-2′), 122.62 (s, C-3′), 121.98 (s, C-2′), 118.71 (d, C-6′), 105.06 (s, C-4a), 99.14 (br d, C-8), 36.20 (q, NCH_3), 27.89 (q, 7-$NHCH_3$). Anal. ($C_{15}H_{14}BrN_5$·0.5H_2O) C, H, N.

Further elution with 4% MeOH/CH_2Cl_2 gave **5f** (30 mg, 29%). Further elution with 8−15% MeOH/CH_2Cl_2 gave 4,7-bis(methylamino)pyrido[4,3-*d*]pyrimidine (**14**) (16 mg, 27%) as a brown solid: mp (MeOH/$CHCl_3$) >300 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 9.93 (br s, 1 H, 4-NH), 9.24 (s, 1 H, H-5), 8.59 (s, 1 H, H-2), 7.73 (br s, 1 H, 7-NH), 6.33 (br s, 1 H, H-8), 3.10 (d, J = 4.5 Hz, 3 H, 4-NHC*H*$_3$), 2.86 (d, J = 4.7 Hz, 3 H, 7-NHC*H*$_3$); HREIMS m/z calcd for $C_9H_{11}N_5$ 189.1014 (M^+), found 189.1011.

4-[(3-Bromophenyl)amino]-7-(dimethylamino)pyrido-[4,3-*d*]pyrimidine (5g). Similar reaction of **5e** (101 mg, 0.32 mmol), Et_3N (4.4 mL, 32 mmol), and dimethylamine hydrochloride (2.58 g, 32 mmol) in 2-propanol (30 mL) in a pressure vessel at 100 °C (oil bath) for 4 h gave 4-[(3-bromophenyl)-amino]-7-(dimethylamino)pyrido[4,3-*d*]pyrimidine (**5g**) (102 mg, 94%): mp (MeOH/$CHCl_3$) 240−241.5 °C; ^1H NMR [$(CD_3)_2$-SO] δ 9.93 (s, 1 H, NH), 9.42 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 8.19 (s, 1 H, H-2′), 7.85 (d, J = 7.7 Hz, 1 H, H-6′), 7.35 (t, J = 7.9 Hz, 1 H, H-5′), 7.30 (br d, J = 7.8 Hz, 1 H, H-4′), 6.53 (s, 1 H, H-8), 3.16 (s, 6 H, 7-N(CH_3)$_2$); ^{13}C NMR δ 160.36 (s, C-7), 157.98 (d, C-2), 157.74 (s, C-4), 154.78 (s, C-8a), 147.75 (d, C-5), 140.73 (s, C-1′), 130.38 (d, C-5′), 126.04, 124.27 (2 d, C-2′,4′), 121.17 (s, C-3′), 120.79 (d, C-6′), 103.24 (s, C-4a), 95.75 (d, C-8), 37.66 (q, 2 C, N(CH_3)$_2$). Anal. ($C_{15}H_{14}BrN_5$) C, H, N.

4-[(3-Bromophenyl)amino]-7-methoxypyrido[4,3-*d*]pyrimidine (5h). A solution of **5e** (100 mg, 0.31 mmol) in 1 M NaOMe in MeOH (30 mL) was stirred under reflux for 42 h. The resulting mixture was concentrated under vacuum, diluted with water, and neutralized with dilute HCl to give 4-[(3-bromophenyl)amino]-7-methoxypyrido[4,3-*d*]pyrimidine (**5h**) (92 mg, 89%): mp (MeOH/H_2O) 276−279 °C; ^1H NMR [$(CD_3)_2$-SO] δ 10.22 (s, 1 H, NH), 9.57 (s, 1 H, H-5), 8.63 (s, 1 H, H-2), 8.19 (s, 1 H, H-2′), 7.86 (br d, J = 7.9 Hz, 1 H, H-6′), 7.39 (t, J = 7.9 Hz, 1 H, H-5′), 7.35 (br dd, J = 7.9, 1.5 Hz, 1 H, H-4′), 6.96 (s, 1 H, H-8), 4.00 (s, 3 H, 7-OCH_3); ^{13}C NMR δ 165.93 (s, C-7), 158.45 (d, C-2), 157.89 (s, C-4), 155.76 (s, C-8a), 147.79 (d, C-5), 140.21 (s, C-1′), 130.48 (d, C-5′), 126.68, 124.76 (2 d, C-2′,4′), 121.24 (s+d, 2 C, C-3′,6′), 107.56 (s, C-4a), 101.27 (d, C-8), 54.38 (q, OCH_3). Anal. ($C_{14}H_{11}BrN_4O$) C, H, N.

Pyrido[3,4-*d*]pyrimidines: 4-[(Phenylmethyl)amino]-pyrido[3,4-*d*]pyrimidine (6a) and 4-[(3-Bromophenyl)-amino]pyrido[3,4-*d*]pyrimidine (7a). A mixture of pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one[21] (366 mg, 2.49 mmol) and P_2S_5 (1.1 g, 2.5 mmol) in pyridine (4 mL) was heated under reflux for 4 h under N_2 to give a black tar, which was dissolved in water. The resultant solid was collected by filtration, washed with water, and dried in a vacuum oven to yield the known[18] pyrido-[3,4-*d*]pyrimidine-4(3*H*)-thione (370 mg, 91%) as a yellow solid which was used directly (lit.[18] mp 325 °C): ^1H NMR [$(CD_3)_2$-SO] δ 14.48 (br s, 1 H, NH), 9.13 (s, 1 H, H-8), 8.70 (d, J = 5.4 Hz, 1 H, H-6), 8.29 (s, 1 H, H-2), 8.27 (d, J = 5.4 Hz, 1 H, H-5). A mixture of this (370 mg, 2.26 mmol), Et_3N (0.6 mL, 4.5 mmol), DMSO (2 mL), and MeI (0.24 mL, 3.96 mmol) was stirred under N_2 at 25 °C for 12 h and then poured into water. The resulting solid was filtered and dried in a vacuum oven to yield the known[18] 4-(methylthio)pyrido[3,4-*d*]pyrimidine (222 mg, 55%) as a brown solid which was used directly (lit.[18] mp 132 °C): ^1H NMR [$(CD_3)_2SO$] δ 9.51 (s, 1 H, H-8), 9.18 (s, 1 H, H-2), 8.79 (d, J = 5.8 Hz, 1 H, H-6), 7.97 (d, J = 5.8 Hz, 1 H, H-5), 2.73 (s, 3 H, SCH_3). Reaction of the methylthio compound with benzylamine at 100 °C for 2 h, followed by purification of the mixture on preparative silica gel TLC, gave 4-[(phenylmethyl)amino]pyrido[3,4-*d*]pyrimidine (**6a**) (21 mg, 20%): mp 136−144 °C (lit.[31] mp 150−154 °C); ^1H NMR [$(CD_3)_2$-SO] δ 9.21 (t, J = 5.8 Hz, 1 H, NH), 9.19 (s, 1 H, H-8), 8.63 (d, J = 5.8 Hz, 1 H, H-6), 8.58 (s, 1 H, H-2), 8.20 (d, J = 5.8 Hz, 1 H, H-5), 7.41−7.30 (m, 4 H, H-2′,3′,5′,6′), 7.26 (t, J = 7.1 Hz, 1 H, H-4′), 4.81 (d, J = 5.8 Hz, 2 H, CH_2). Anal. ($C_{14}H_{12}N_4$·0.5 H_2O) C, H.

Similar reaction of the methylthio compound with 3-bromoaniline, and purification of the product by silica gel chromatography, eluting with a gradient of 0−5% MeOH in $CHCl_3$, gave 4-[(3-bromophenyl)amino]pyrido[3,4-*d*]pyrimidine (**7a**) (53% yield): mp (Et_2O) 209 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.15 (s, 1 H, NH), 9.21 (s, 1 H, H-8), 8.80 (s, 1 H, H-2), 8.76 (d, J = 5.8 Hz, 1 H, H-6), 8.44 (d, J = 5.6 Hz, 1 H, H-5), 8.25 (s, 1 H,

H-2′), 7.93 (d, J = 7.7 Hz, 1 H, H-6′), 7.45−7.37 (m, 2 H, H-4′,5′); EIMS m/z 299 (^{79}BrM − H), 301 (^{81}BrM − H). Anal. ($C_{13}H_9BrN_4 \cdot 0.25H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-6-chloropyrido[3,4-*d*]pyrimidine (7d) (Scheme 4). A stirred suspension of 6-chloropyrido[3,4-*d*]pyrimidin-4(3*H*)-one[22] (**25**) (1.82 g, 10 mmol) in $POCl_3$ (10 mL) was heated under reflux until dissolved (ca. 2 h) and for a further 30 min. Excess reagent was removed under reduced pressure, and the residue was treated with a mixture of CH_2Cl_2 and ice-cold aqueous Na_2CO_3. The resulting organic layer was dried (Na_2SO_4) and evaporated to give a quantitative yield of crude, unstable 4,6-dichloropyrido[3,4-*d*]-pyrimidine (**26**), which was used directly: ^{1}H NMR ($CDCl_3$) δ 9.38 (d, J = 0.5 Hz, 1 H, H-8), 9.19 (s, 1 H, H-2), 8.09 (d, J = 0.5 Hz, 1 H, H-5); ^{13}C NMR δ 161.6 (s), 149.1 (s), 144.0 (s), 129.0 (s), 155.1 (d), 154.2 (d), 117.0 (d).

Reaction of **26** and 3-bromoaniline as before gave 4-[(3-bromophenyl)amino]-6-chloropyrido[3,4-*d*]pyrimidine (**7d**) (38% yield): mp (MeOH) 201−202 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.12 (s, 1 H, NH), 9.03 (s, 1 H, H-8), 8.77 (s, 1 H, H-2), 8.63 (s, 1 H, H-5), 8.21 (s, 1 H, H-2′), 7.89 (d, J = 8.1 Hz, 1 H, H-6′), 7.43−7.32 (m, 2 H, H-4′,5′). Anal. ($C_{13}H_8BrClN_4$) C, H, N, Cl.

4-[(3-Bromophenyl)amino]-6-fluoropyrido[3,4-*d*]pyrimidine (7e) (Scheme 4). A suspension of 6-fluoropyrido[3,4-*d*]pyrimidin-4(3*H*)-one[22] (**27**) (1.65 g, 10 mmol) in $SOCl_2$ (50 mL) and 2 drops DMF was heated under reflux until a clear solution was obtained (20 min), and then for a further 30 min. The $SOCl_2$ was removed under reduced presssure, and the residue was dissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3. The solvent was dried and removed to give crude 4-chloro-6-fluoropyrido[3,4-*d*]pyrimidine (**28**) which was dissolved in 2-propanol (50 mL) containing 3-bromoaniline (2.1 g, 12 mmol). The mixture was heated under reflux for 15 min to give a precipitate, which was redissolved by the addition of Et_3N. After the addition of water the solution was concentrated and cooled to give 4-[(3-bromophenyl)amino]-6-fluoropyrido[3,4-*d*]pyrimidine (**7e**) (2.92 g, 91%): mp (MeOH) 219.5−221 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.12 (br s, 1 H, NH), 8.97 (s, 1 H, H-8), 8.76 (s, 1 H, H-2,), 8.28 (s, 1 H, H-5), 8.25 (br s, 1 H, H-2′), 7.90 (br d, J = 7.5 Hz, 1 H, H-6′), 7.41 (t, J = 7.8 Hz, 1 H, H-5′), 7.37 (d, J = 8.1 Hz, 1 H, H-4′); ^{13}C NMR δ 159.8 (d, J_{C-F} = 233 Hz, C-6), 156.6 (d, J_{C-F} = 5 Hz, C-8a), 154.9 (d, C-2), 150.7 (dd, J_{C-F} = 15 Hz, C-8), 142.8 (s), 140.0 (s), 130.5 (d), 126.7 (d), 124.1 (d), 123.2 (d, J_{C-F} = 9 Hz, C-4a), 121.2 (s), 120.6 (d), 99.6 (dd, J_{C-F} = 40 Hz, C-5); HREIMS m/z calcd for $C_{13}H_8BrFN_4$ 317.9916 and 319.9896 (M^+), found 317.9904 and 319.9898. Anal. ($C_{13}H_8BrFN_4$) C, H, N, F.

Preparation of 7f−h from 4-[(3-Bromophenyl)amino]-6-fluoropyrido[3,4-*d*]pyrimidine (7e). A mixture of **7e** (0.48 g, 1.5 mmol) and 40% aqueous methylamine (13 mL, 150 mmol) in EtOH (100 mL) was heated at 100 °C in a sealed pressure vessel for 18 h. The solvent was removed, and the residue was chromatographed on silica gel, eluting with EtOAc/CH_2Cl_2 (3:2) to give 4-[(3-bromophenyl)amino]-6-(methylamino)pyrido[3,4-*d*]pyrimidine (**7f**) (0.37 g, 71%): mp (aqueous MeOH) 172−173 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (br s, 1 H, NH), 8.77 (s, 1 H, H-8), 8.42 (s, 1 H, H-2), 8.23 (t, J = 1.8 Hz, 1 H, H-2′), 7.95 (br d, J = 8.4 Hz, 1 H, H-6′), 7.38 (t, J = 8.0 Hz, 1 H, H-5′), 7.32 (br d, J = 7.8 Hz, 1 H, H-4′), 7.07 (s, 1 H, H-5), 6.84 (q, J = 5.0 Hz, 1 H, NH), 2.90 (d, J = 5.0 Hz, 3 H, CH_3); ^{13}C NMR δ 157.7 (s), 155.4 (s), 151.1 (d), 150.1 (d), 140.8 (s), 137.2 (s), 130.3 (d), 126.0 (d), 124.0 (d), 122.2 (s) 121.1 (s), 120.5 (d), 89.6 (d), 29.2 (q, CH_3). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

Similar treatment of **7e** with dimethylamine in EtOH in a pressure vessel at 100 °C for 18 h gave 4-[(3-bromophenyl)-amino]-6-(dimethylamino)pyrido[3,4-*d*]pyrimidine (**7g**) (67%): mp (MeOH) 177−178.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.71 (s, 1 H, NH), 8.83 and 8.43 (2 s, 2 H, H-2,8), 8.21 (br s, 1 H, H-2′), 7.94 (br d, J = 7.5 Hz, 1 H, H-6′), 7.42−7.29 (m, 2 H, H-4′,5′), 7.26 (s, 1 H, H-5), 3.17 (s, 6 H, $N(CH_3)_2$). Anal. ($C_{15}H_{14}BrN_5$) C, H, N.

Reaction of **7e** with NaOMe in MeOH in a pressure vessel at 100 °C for 48 h gave 4-[(3-bromophenyl)amino]-6-methoxypyrido[3,4-*d*]pyrimidine (**7h**) (72%): mp (MeOH) 177−178.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.93 (s, 1 H, NH), 8.94 and 8.61 (2 s, 2 H, H-2,8), 8.26 (br s, 1 H, H-2′), 7.94 (br d, J = 7.6 Hz, 1 H, H-6′), 7.88 (s, 1 H, H-5), 7.43−7.32 (m, 2 H, H-4′,5′), 4.01 (s, 1 H, OCH_3). Anal. ($C_{14}H_{11}BrN_4O$) C, H, N.

6-(Methylamino)-4-(phenylamino)pyrido[3,4-*d*]pyrimidine (13f) (Scheme 4). Reaction of **28** with aniline as above gave 6-fluoro-4-(phenylamino)pyrido[3,4-*d*]pyrimidine (**13e**) (0.46 g, 63%): mp (aqueous MeOH) 224−225.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.04 (s, 1 H, NH), 8.92 (s, 1 H, H-8), 8.68 (s, 1 H, H-5), 8.28 (s, 1 H, H-2), 7.88 (d, J = 7.8 Hz, 2 H, H-2′,6′), 7.44 (t, J = 7.8 Hz, 2 H, H-3′,5′), 7.20 (t, J = 7.3 Hz, 1 H, H-4′); ^{13}C NMR δ 159.8 (d, J_{C-F} = 232.8 Hz, C-6), 156.8 (d, J_{C-F} = 4.7 Hz, C-8a), 155.2 (d, C-2), 150.6 (dd, J_{C-F} = 15.0 Hz, C-8), 142.9 (d, J_{C-F} = 2.8 Hz, C-4), 138.2 (s, C-1′), 128.5 (d), 124.4 (d), 123.3 (d, J_{C-F} = 8.7 Hz, C-4a), 122.3 (d), 99.6 (d, J_{C-F} = 40.2 Hz, C-5). Anal. ($C_{13}H_9FN_4$) C, H, N.

A mixture of **13e** (0.20 g, 0.83 mmol) and 40% aqueous methylamine (3.6 mL, 42 mmol) in EtOH (50 mL) was heated at 100 °C in a sealed pressure vessel for 18 h. The product was worked up in EtOAc and chromatographed on silica gel, eluting with EtOAc/CH_2Cl_2 (3:1), to give a yellow solid which was recrystallized from aqueous MeOH to give 6-(methylamino)-4-(phenylamino)pyrido[3,4-*d*]pyrimidine (**13f**) (0.17 g, 81%): mp 212−212.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.67 (s, 1 H, NH), 8.75 (s, 1 H, H-8), 8.36 (s, 1 H, H-2), 7.87 (d, J = 7.7 Hz, 2 H, H-2′,6′), 7.42 (t, J = 7.7 Hz, 2 H, H-3′,5′), 7.15 (t, J = 7.4 Hz, 1 H, H-4′), 7.11 (s, 1 H, H-5), 6.79 (q, J = 5.0 Hz, 1 H, NH), 2.91(d, J = 5.0 Hz, 3 H, CH_3); ^{13}C NMR δ 157.7 (s), 155.7 (s), 150.9 (d), 150.5 (d), 138.9 (s), 137.2 (s), 128.4 (d), 123.7 (d, C-4′), 122.3 (d), 122.2 (s) , 89.9 (d, C-5), 29.3 (q, CH_3). Anal. ($C_{14}H_{13}N_5$) C, H, N

6-Amino-4-[(3-bromophenyl)amino]pyrido[3,4-*d*]pyrimidine (7b) (Scheme 4). A mixture of **7e** (0.48 g, 1.5 mmol) and 4-methoxybenzylamine (10.3 g, 75 mmol) in EtOH (50 mL) was heated to 100 °C for 5 days. The resulting product was chromatographed on silica gel, eluting with CH_2Cl_2/EtOAc (3:1), to give 4-[(3-bromophenyl)amino]-6-[(4-methoxyphenyl)-methylamino]pyrido[3,4-*d*]pyrimidine (**7i**) (0.18 g, 28%): mp (aqueous MeOH) 178−179.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.70 (br s, 1 H, NH), 8.76 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.88 (br d, J = 7.9 Hz, 1 H, H-6′), 7.38−7.29 (m, 5 H, H-4′,5′,2″,6″ and NH), 7.19 (s, 1 H, H-5), 6.88 (d, J = 8.7 Hz, 2 H, H-3″,5″), 4.49 (d, J = 6.3 Hz, 2 H, CH_2), 3.71 (s, 3 H, OCH_3). Anal. ($C_{21}H_{18}BrN_5O$) C, H, N.

A solution of **7i** (0.10 g, 0.23 mmol) in TFA (5 mL) was heated under reflux for 1 h, and the mixture was evaporated to dryness. The residue was partitioned between EtOAc and aqueous ammonia, and the crude product was chromatographed on alumina, eluting with CH_2Cl_2/MeOH (97:3), to give 6-amino-4-[(3-bromophenyl)amino]pyrido[3,4-*d*]pyrimidine (**7b**) (0.04 g, 55%): mp (CH_2Cl_2) 241.5−242 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.76 (br s, 1 H, NH), 8.71 (s, 1 H, H-8), 8.40 (s, 1 H, H-2), 8.25 (br s, 1 H, H-2′), 7.90 (br d, J = 8.2 Hz, 1 H, H-6′), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 6.31 (br s, 2 H, NH_2). Anal. ($C_{13}H_{10}BrN_3$) C, H, N.

Pyrido[2,3-*d*]pyrimidines: 4-[(Phenylmethyl)amino]-pyrido[2,3-*d*]pyrimidine (8a) and 4-[(3-bromophenyl)-amino]pyrido[2,3-*d*]pyrimidine (9a). Reaction of pyrido-[2,3-*d*]pyrimidin-4(3*H*)-one[24] with P_2S_5 in pyridine under reflux for 3 h gave pyrido[2,3-*d*]pyrimidine-4(3*H*)-thione (1.72 g, 78%) as a solid which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 9.06 (dd, J = 4.3, 1.9 Hz, 1 H, H-7), 8.90 (dd, J = 8.2, 1.9 Hz, 1 H, H-5), 8.36 (s, 1 H, H-2), 7.65 (dd, J = 8.2, 4.3 Hz, 1 H, H-6). Treatment of the thione (100 mg, 0.76 mmol) in DMSO (2 mL) with Et_3N (154 mg, 1.52 mmol) and MeI (161 mg, 1.14 mmol) for 12 h at 25 °C gave crude 4-(methylthio)pyrido[2,3-*d*]-pyrimidine (134 mg, 100%) as a solid which was used directly: ^{1}H NMR [$(CD_3)_2SO$] δ 9.25 (dd, J = 4.2, 1.8 Hz, 1 H, H-7), 9.17 (s, 1 H, H-2), 8.59 (dd, J = 8.2, 1.9 Hz, 1 H, H-5), 7.75 (dd, J = 8.2, 4.3 Hz, 1 H, H-6), 2.73 (s, 3 H, SCH_3). Reaction of the crude thiomethyl compound and benzylamine as above gave the previously-reported[23] 4-[(phenylmethyl)-amino]pyrido[2,3-*d*]pyrimidine (**8a**): mp 255−257 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.14 (br t, J = 5.7 Hz, 1 H, NH), 8.99 (dd, J = 4.3, 1.8 Hz, 1 H, H-7), 8.76 (dd, J = 8.3, 1.9 Hz, 1 H, H-5), 8.59 (s, 1 H, H-2), 7.56 (dd, J = 8.1, 4.4 Hz, 1 H, H-6), 7.36 (d,

J = 6.9 Hz, 2 H, H-2′,6′), 7.32 (t, J = 7.0 Hz, 2 H, H-3′,5′), 7.24 (t, J = 7.1 Hz, 1 H, H-4′), 4.80 (d, J = 5.8 Hz, 2 H, PhCH_2).

Similar reaction of the crude thiomethyl compound with 3-bromoaniline at 100 °C for 2 h gave 4-[(3-bromophenyl)-amino]pyrido[2,3-*d*]pyrimidine (**9a**) (55 mg, 20%): mp 221−224 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.13 (s, 1 H, NH), 9.11 (dd, J = 4.3, 1.7 Hz, 1 H, H-7), 9.01 (dd, J = 8.2, 1.7 Hz, 1 H, H-5), 8.81 (s, 1 H, H-2), 8.22 (s, 1 H, H-2′), 7.90 (d, J = 7.7 Hz, 1 H, H-6′), 7.71 (dd, J = 8.0, 4.3 Hz, 1 H, H-6), 7.40 (m, 2 H, H-4′,5′). Anal. ($C_{13}H_9BrN_4$) C, H, N.

4-[(3-Bromophenyl)amino]-7-fluoropyrido[2,3-*d*]pyrimidine (9e) (Scheme 5). 2,6-Difluoropyridine (**29**) (7.89 mL, 0.087 mmol) was added dropwise under N_2 at −78 °C to a stirred solution of lithium diisopropylamide (59.0 mL of a 1.5 N solution in cyclohexane, 0.089 mmol) in THF (250 mL). After 2 h at −78 °C, a stream of dry CO_2 was passed through the solution and the mixture was diluted with water and washed with EtOAc. The aqueous portion was neutralized with 3 N HCl, extracted with EtOAc, and worked up to give 2,6-difluoropyridine-3-carboxylic acid (**30**) (13.4 g, 97%): mp (EtOAc/petroleum ether) 167−170 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.59 (dd, J = 9.2, 8.2 Hz, 1 H, H-4), 7.30 (dd, J = 8.2, 2.1 Hz, 1 H, H-5), 4.03 (br s, 1 H, COOH); ^{13}C NMR δ 163.06 (d, J_{C-F} = 7 Hz, COOH), 162.29 (dd, J_{C-F} = 250, 15 Hz), 159.54 (dd, J_{C-F} = 255, 15 Hz), 148.99 (dd, J_{C-F} = 9 Hz), 111.86 (dd, J_{C-F} = 21, 5 Hz), 107.44 (ddd, J_{C-F} = 34, 5 Hz). Anal. ($C_6H_3F_2NO_2$) C, H, N, F.

A solution of **30** (7.4 g, 0.046 mmol) and $SOCl_2$ (20 mL) in 1,2-dichloroethane (60 mL) containing DMF (1 drop) was heated under reflux for 4 h and then concentrated to dryness under reduced pressure. The residue was dissolved in Et_2O (100 mL), cooled to 0 °C, and treated dropwise with concentrated ammonia (10.0 mL, 0.17 mmol). After 10 min the solution was washed with aqueous $NaHCO_3$ and worked up to give 2,6-difluoropyridine-3-carboxamide (**31**) (5.61 g, 76%): mp (EtOAc/petroleum ether) 107−108 °C; ^{1}H NMR ($CDCl_3$) δ 8.70 (dd, J = 9.6, 8.3 Hz, 1 H, H-4), 7.00 (ddd, J = 8.3, 2.9, 1.1 Hz, 1 H, H-5), 6.71, 6.55 (2 br s, 2 H, $CONH_2$); ^{13}C NMR δ 162.83 (dd, J_{C-F} = 254, 16 Hz), 162.40 (d, J_{C-F} = 6 Hz, $CONH_2$), 158.56 (dd, J_{C-F} = 245, 15 Hz), 148.43 (dd, J_{C-F} = 9 Hz), 112.40 (dd, J_{C-F} = 24, 5 Hz), 107.65 (ddd, J_{C-F} = 34, 5 Hz). Anal. ($C_6H_4F_2N_2O$) C, H, N, F.

A solution of **31** (4.68 g, 0.029 mmol) in dry formamide (30 mL) was saturated with ammonia and allowed to stand at room temperature for 24 h. Water (50 mL) was added, and the resultant precipitate was filtered off and washed well with water to give 6-amino-2-fluoropyridine-3-carboxamide (**33**) (1.41 g, 31%): mp 236−237 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.89 (dd, J = 10.4, 8.4 Hz, 1 H, H-4), 7.31, 7.16 (2 br s, $CONH_2$), 6.93 (br s, 2 H, NH_2), 6.36 (dd, J = 8.4, 2.4 Hz, 1 H, H-5); ^{13}C NMR δ 164.25 (d, J_{C-F} = 6 Hz, $CONH_2$), 160.76 (d, J_{C-F} = 20 Hz), 160.19 (d, J_{C-F} = 236 Hz), 142.55 (dd, J_{C-F} = 3 Hz), 104.92 (dd, J_{C-F} = 2 Hz), 101.69 (d, J_{C-F} = 28 Hz). Anal. ($C_6H_6FN_3O$) C, H, N, F.

The filtrate and washings were combined and extracted exhaustively with EtOAc, and the extract was chromatographed on silica gel. EtOAc/petroleum ether (1:1) eluted a forerun, while elution with EtOAc/petroleum ether (2:1) and with EtOAc gave 2-amino-6-fluoropyridine-3-carboxamide (**32**) (1.57 g, 35%): mp (EtOAc/petroleum ether) 199−200 °C (lit.[26] mp 198−200 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 8.13 (t, J = 8.4 Hz, 1 H, H-4), 7.90, 7.30 (2 br s, 2 H, $CONH_2$), 7.65 (br s, 2 H, NH_2), 6.23 (dd, J = 8.4, 2.6 Hz, 1 H, H-5); ^{13}C NMR δ 168.90 (s, $CONH_2$), 163.38 (d, J_{C-F} = 238 Hz), 159.20 (d, J_{C-F} = 20 Hz), 142.93 (dd, J_{C-F} = 10 Hz), 106.02 (d, J_{C-F} = 5 Hz), 94.35 (dd, J_{C-F} = 38 Hz).

A suspension of the 2-amino-6-fluoro isomer **32** (0.74 g, 4.77 mmol) in triethyl orthoformate (25 mL) was heated at reflux for 8 h. The mixture was cooled to room temperature, and the precipitate was filtered off and washed well with petroleum ether to give 7-fluoropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (**34**) (0.76 g, 96%): mp >370 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 12.75 (br s, 1 H, NH), 8.66 (t, J = 8.4 Hz, 1 H, H-5), 8.38 (s, 1 H, H-2), 7.33 (dd, J = 8.4, 2.6 Hz, 1 H, H-6); ^{13}C NMR δ 165.01 (d, J_{C-F} = 244 Hz), 160.46 (s), 158.41 (d, J_{C-F} = 20 Hz), 150.38 (d), 141.98 (dd, J_{C-F} = 10 Hz), 116.61 (s), 109.45 (dd, J_{C-F} = 39 Hz). Anal. ($C_7H_4FN_3O$) C, H, N.

A suspension of **34** (0.20 g, 1.21 mmol) in $POCl_3$ (10 mL) was heated under reflux for 2 h. Reagent was then removed under reduced pressure, and the residue was partitioned between aqueous $NaHCO_3$ and EtOAc. The organic extract was worked up to give crude 4-chloro-7-fluoropyrido[2,3-*d*]-pyrimidine (**35**), which was used directly. A solution of this product (0.20 g, 1.09 mmol) and 3-bromoaniline (0.23 mL, 2.18 mmol) in 2-propanol (1.0 mL) and THF (10 mL) containing a trace of concentrated HCl was stirred at 20 °C for 1 h and then concentrated to dryness. The residue was dissolved in EtOAc, washed with aqueous $NaHCO_3$, and worked up to give an oil which was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:5) gave 3-bromoaniline, while EtOAc/petroleum ether (1:1) eluted 4-[(3-bromophenyl)amino]-7-fluoropyrido[2,3-*d*]pyrimidine (**9e**) (0.18 g, 47%): mp (MeOH) 211−213 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.18 (br s, 1 H, NH), 9.17 (t, J = 8.6 Hz, 1 H, H-5), 8.80 (s, 1 H, H-2), 8.17 (t, J = 1.8 Hz, 1 H, H-2′), 7.85 (dt, J = 7.6, 1.8 Hz, 1 H, H-6′), 7.53 (dd, J = 8.6, 2.7 Hz, 1 H, H-6), 7.41−7.34 (m, 2 H, H-4′,5′); ^{13}C NMR δ 164.72 (d, J = 245 Hz), 158.86 (d), 157.97 (s), 157.86 (d, J_{C-F} = 21 Hz), 140.30 (s), 139.61 (dd, J_{C-F} = 11 Hz), 130.47 (d), 126.63 (d), 124.45 (d), 121.18 (s), 120.94 (d), 109.88 (dd, J_{C-F} = 41 Hz), 108.77 (s). Anal. ($C_{13}H_8BrFN_4 \cdot H_2O$) C, H, N.

Preparation of 9b,f−h from 4-[(3-Bromophenyl)amino]-7-fluoropyrido[2,3-*d*]pyrimidine (9e). A solution of **9e** (0.20 g, 0.63 mmol) in EtOH (20 mL) was saturated with ammonia and warmed at 100 °C in a pressure vessel for 30 h. The solvent was removed under reduced pressure to give 7-amino-4-[(3-bromophenyl)amino]pyrido[2,3-*d*]pyrimidine (**9b**) (0.18 g, 90%): mp (MeOH) 297−300 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.97 (br s, 1 H, NH), 8.59 (s, 1 H, H-2), 8.51 (d, J = 9.0 Hz, 1 H, H-5), 8.11 (br s, 1 H, H-2′), 7.77 (d, J = 7.5 Hz, 1 H, H-6′), 7.44 (br s, 2 H, NH_2), 7.37−7.30 (m, 2 H, H-4′,5′), 6.81 (d, J = 9.0 Hz, 1 H, H-6); ^{13}C NMR δ 162.58 (s), 157.76 (s), 155.64 (d), 146.22 (s), 140.55 (s), 132.99 (d), 130.37 (d), 126.24 (d), 124.39 (d), 121.12 (s), 120.89 (d), 111.87 (d), 101.31 (s). Anal. ($C_{13}H_{10}BrN_5 \cdot H_2O$) C, H, N.

Similar reaction of **9e** with methylamine in a pressure vessel at 100 °C for 18 h gave 4-[(3-bromophenyl)amino]-7-(methylamino)pyrido[2,3-*d*]pyrimidine (**9f**) (77%): mp (MeOH) 245−247 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.53 (s, 1 H, NH), 8.54 (s, 1 H, H-2), 8.41 (d, J = 8.1 Hz, 1 H, H-5), 8.17 (t, J = 1.8 Hz, 1 H, H-2′), 7.83 (dt, J = 8.0, 1.9 Hz, 1 H, H-6′), 7.66 (br s, 1 H, NH), 7.32 (t, J = 8.0 Hz, 1 H, H-5′), 7.24 (dt, J = 8.0, 1.8 Hz, 1 H, H-4′), 6.77 (d, J = 8.1 Hz, 1 H, H-6), 2.92 (d, J = 4.8 Hz, 3 H, NCH_3); ^{13}C NMR δ 161.49 (s), 160.13 (s), 157.49 (s), 157.10 (d), 141.47 (s), 131.44 (d), 130.30 (d), 125.26 (d), 123.49 (d), 121.17 (s), 120.04 (d), 112.23 (d), 101.73 (s), 27.49 (q, NCH_3). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

Similar reaction of **9e** with dimethylamine in a pressure vessel at 100 °C for 18 h gave 4-[(3-bromophenyl)amino]-7-(dimethylamino)pyrido[2,3-*d*]pyrimidine (**9g**) (84%): mp (MeOH) 287−289 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.58 (s, 1 H, NH), 8.56 (d, J = 9.3 Hz, 1 H, H-5), 8.54 (s, 1 H, H-2), 8.18 (t, J = 1.9 Hz, 1 H, H-2′), 7.84 (dt, J = 8.0, 1.9 Hz, 1 H, H-6′), 7.33 (dd, J = 8.1, 8.0 Hz, 1 H, H-5′) 7.25 (dt, J = 8.1, 1.9 Hz, 1 H, H-4′), 7.10 (d, J = 9.3 Hz, 1 H, H-6), 3.18 (s, 6 H, NMe_2); ^{13}C NMR δ 160.69 (s), 159.26 (s), 157.59 (s), 157.45 (d), 141.40 (s), 132.76 (d), 130.32 (d), 125.33 (d), 123.52 (d), 121.17 (s), 120.06 (d), 108.63 (d), 101.40 (d), 37.55 (q, 2 C, NMe_2). Anal. ($C_{15}H_{14}BrN_5$) C, H, N.

Similar reaction of **9e** with NaOMe in MeOH in a pressure vessel at 90 °C for 18 h gave 4-[(3-bromophenyl)amino]-7-methoxypyrido[2,3-*d*]pyrimidine (**9h**) (86%): mp (MeOH) 260−261 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.88 (s, 1 H, NH), 8.82 (d, J = 8.9 Hz, 1 H, H-5), 8.71 (s, 1 H, H-2), 8.18 (t, J = 1.9 Hz, 1 H, H-6′), 7.36 (dd, J = 8.1, 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.1, 1.9 Hz, 1 H, H-4′), 7.15 (d, J = 8.9 Hz, 1 H, H-6), 4.01 (s, 3 H, OCH_3); ^{13}C NMR δ 166.51 (s), 158.74 (s), 158.16 (s), 157.84 (d), 140.83 (s), 135.25 (d), 130.44 (d), 126.08 (d), 124.07 (d), 121.22 (s), 120.59 (d), 112.45 (d), 105.33 (s), 53.85 (q, OCH_3). Anal. ($C_{14}H_{11}BrN_4O$) C, H, N.

Pyrido[3,2-*d*]pyrimidines: 4-[(Phenylmethyl)amino]-pyrido[3,2-*d*]pyrimidine (10a) and 4-[(3-Bromophenyl)-

amino]pyrido[3,2-*d*]pyrimidine (11a) (Scheme 6). A solution of 6-chloro-3-nitropyridine-2-carboxamide (**37**) (see below) (2.00 g, 9.91 mmol) in EtOAc/MeOH (1:1, 100 mL) was hydrogenated over 5% Pd/C (0.40 g) at 60 psi for 6 days, with additions of fresh catalyst after 2 and 4 days. After removal of the catalyst by filtration, the solution was concentrated to dryness to give 3-aminopyridine-2-carboxamide (**38**) as an orange oil, which was used directly. The crude product was stirred under reflux with triethyl orthoformate (50 mL) for 42 h, during which time a tan precipitate formed. After cooling, the solid was filtered off, washed well with petroleum ether, and dried under vacuum to give pyrido[3,2-*d*]pyrimidin-4(3*H*)-one (**39**) (1.27 g, 87%): mp 343−345 °C (lit.[29] mp 346−347 °C); ^{1}H NMR [$(CD_3)_2SO$] δ 12.56 (br s, 1 H, NH), 8.80 (dd, J = 4.3, 1.5 Hz, 1 H, H-6), 8.16 (s, 1 H , H-2), 8.09 (dd, J = 8.3, 1.5 Hz, 1 H, H-8), 7.82 (dd, J = 8.3, 4.3 Hz, 1 H, H-7); ^{13}C NMR δ 159.44 (s), 149.09 (d), 146.11 (d), 145.69 (s), 139.28 (s), 135.48 (d), 128.65 (d).

A suspension of **39** (1.00 g, 6.80 mmol) in $POCl_3$ (30 mL) was heated under reflux for 4 h and then concentrated to dryness under reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution, and the organic layer was worked up to give 4-chloropyrido[3,2-*d*]-pyrimidine[28] (**40**) (0.97 g, 86%) as a tan solid which was used directly. A solution of freshly prepared **40** (0.10 g, 0.60 mmol) and benzylamine (0.13 mL, 1.20 mmol) in 2-propanol (15 mL) containing a trace of concentrated HCl was warmed at 50 °C for 30 min and then concentrated to dryness. The residue was partitioned between water and EtOAc, and the organic layer was worked up and chromatographed on silica gel. EtOAc eluted foreruns, while MeOH/EtOAc (1:9) eluted 4-[(phenylmethyl)amino]pyrido[3,2-*d*]pyrimidine (**10a**) (0.11 g, 77%): mp (EtOAc/petroleum ether) 83 °C; ^{1}H NMR ($CDCl_3$) δ 8.67 (s, 1 H, H-2), 8.65 (dd, J = 4.3, 1.5 Hz, 1 H, H-6), 8.10 (dd, J = 8.5, 1.5 Hz, 1 H, H-8), 7.63 (dd, J = 8.8, 4.3 Hz, 1 H, H-7), 7.55 (m, 1 H, NH), 7.41−7.29 (m, 5 H, ArH), 4.86 (d, J = 5.9 Hz, 2 H, NHCH_2); ^{13}C NMR δ 159.57 (s), 156.25 (d), 148.12 (d), 144.12 (s), 137.70 (s), 135.69 (d), 131.88 (s), 128.67 (d), 127.72 (d), 127.60 (d), 127.55 (d), 44.56 (t, CH_2). Anal. ($C_{14}H_{12}N_4$) C, H, N.

Similar reaction of **40** with 3-bromoaniline gave 4-[(3-bromophenyl)amino]pyrido[3,2-*d*]pyrimidine (**11a**) (87% yield): mp (MeOH) 176−178 °C; ^{1}H NMR ($CDCl_3$) δ 9.19 (br s, 1 H, NH), 8.83 (s, 1 H, H-2), 8.80 (dd, J = 4.3, 1.5 Hz, 1 H, H-6), 8.29 (br s, 1 H, H-2′), 8.19 (dd, J = 8.5, 1.5 Hz, 1 H, H-8), 7.83 (m, 1 H, H-6′), 7.76 (dd, J = 8.5, 4.3 Hz, 1 H, H-7), 7.29−7.27 (m, 2 H, H-4′,5′); ^{13}C NMR δ 157.14 (s), 155.81 (d), 148.82 (d), 144.56 (s), 139.45 (s), 136.35 (d), 131.65 (s), 130.30 (d), 128.04 (d), 126.86 (d), 123.14 (d), 122.75 (s), 118.75 (d). Anal. ($C_{13}H_9BrN_4$) C, H, N, Br.

4-[(3-Bromophenyl)amino]-6-chloropyrido[3,2-*d*]pyrimidine (11d) (Scheme 6). A solution of 6-chloro-2-cyano-3-nitropyridine[28] (**36**) (1.00 g, 5.45 mmol) in 90% H_2SO_4 (15 mL) was warmed at 70 °C for 3.5 h and then poured into ice−water. The mixture was extracted four times with EtOAc, and the combined extracts were worked up to give 6-chloro-3-nitropyridine-2-carboxamide (**37**) (0.80 g, 73%): mp (aqueous MeOH) 160−161 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.55 (d, J = 8.5 Hz, 1 H, H-4), 8.31, 8.04 (2 br s, 2 H, $CONH_2$), 7.93 (d, J = 8.5 Hz, 1 H, H-5); ^{13}C NMR δ 164.12 (s, $CONH_2$), 152.23 (s), 147.87 (s), 143.53 (s), 136.24 (d), 126.87 (d); HREIMS m/z calcd for $C_6H_4ClN_3O_3$ 202.9912, 200.9941 (M^+), found 202.9901, 200.9932.

A solution of **37** (0.30 g, 1.49 mmol) in EtOAc (30 mL) was hydrogenated at 60 psi over 5% Pd−C (0.10 g) for 20 min. After removal of the catalyst by filtration the solution was concentrated to dryness to give 3-amino-6-chloropyridine-2-carboxamide (**41**) as a yellow oil, which was used directly. This was dissolved in triethyl orthoformate (30 mL), and the mixture was heated under reflux for 18 h. Petroleum ether (30 mL) was added to the cooled solution, and the resulting precipitate of crude 6-chloropyrido[3,2-*d*]pyrimidin-4(3*H*)-one (**42**) (0.27 g, 99%) was filtered off, dried under vacuum, and used without further purification: ^{1}H NMR δ [$(CD_3)_2SO$] 14.50 (br s, 1 H, NH), 8.20 (s, 1 H, H-2), 8.15 (d, J = 8.5 Hz, 1 H, H-8), 7.89 (d, J = 8.5 Hz, 1 H, H-7).

A suspension of **42** (0.20 g, 1.10 mmol) in $POCl_3$ (30 mL) was heated under reflux for 3 h and then concentrated to dryness under reduced pressure. The residue was partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution, and the organic portion was worked up to give 4,6-dichloropyrido[3,2-*d*]pyrimidine **43** (0.16 g, 73%) as a tan solid, which was used directly. A solution of **43** (0.16 g, 0.80 mmol) and 3-bromoaniline (0.17 mL, 1.60 mmol) in 2-propanol (25 mL) containing a trace of concentrated HCl was warmed at 50 °C for 30 min. The cooled mixture was poured into saturated $NaHCO_3$ and extracted with EtOAc, and the extract was worked up and chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:4) gave 3-bromoaniline, while EtOAc/petroleum ether (1:1) eluted 4-[(3-bromophenyl)amino]-6-chloropyrido[3,2-*d*]-pyrimidine (**11d**) (0.17 g, 63%): mp (MeOH) 167−169 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (br s, 1 H, NH), 8.84 (s, 1 H, H-2), 8.30 (t, J = 2.0 Hz, 1 H, H-2′), 8.17 (d, J = 8.8 Hz, 1 H, H-8), 7.82−7.78 (m, 1 H, H-6′), 7.73 (d, J = 8.8 Hz, 1 H, H-7), 7.32−7.29 (m, 2 H, H-4′,5′); ^{13}C NMR δ 156.08 (d), 149.35 (s), 144.06 (s), 139.41 (d), 139.07 (s), 131.23 (s), 130.34 (d), 129.65 (d), 127.26 (d), 123.43 (d), 122.77 (s), 119.05 (d). Anal. ($C_{13}H_8BrClN_4$) C, H, N, Cl.

4-[(3-Bromophenyl)amino]-6-fluoropyrido[3,2-*d*]pyrimidine (11e) (Scheme 6). A mixture of **36** (10.0 g, 0.054 mol) and KF (9.48 g, 0.163 mol) in MeCN (200 mL) was heated under reflux with stirring for 18 h, then poured into water, and extracted with EtOAc. The extract was washed with water and worked up, and the residue was chromatographed on silica gel, EtOAc/petroleum ether (3:7) eluting 2-cyano-6-fluoro-3-nitropyridine (**44**) (7.2 g, 79%): mp (benzene/petroleum ether) 51 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (dd, J = 9.0, 6.0 Hz, 1 H, H-4), 7.48 (dd, J = 9.0, 3.0 Hz, 1 H, H-5); ^{13}C NMR δ 164.16 (d, J_{C-F} = 256 Hz, C-6), 144.81 (s, CN), 139.08 (dd, J_{C-F} = 10.1 Hz, C-4), 126.92 (d, J_{C-F} = 17.6 Hz, C-3), 115.28 (dd, J_{C-F} = 38.1 Hz, C-5), 112.59 (s, C-2). Anal. ($C_6H_2FN_3O_2$) C, H, N, F.

A solution of **44** (1.40 g, 8.39 mmol) in 90% H_2SO_4 (30 mL) was warmed at 70 °C for 90 min, then cooled, poured onto ice, and basified with concentrated ammonia. Extraction with EtOAc and workup gave 6-fluoro-3-nitropyridine-2-carboxamide (**45**) (0.94 g, 61%): mp (EtOAc/petroleum ether) 189 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.70 (dd, J = 8.9, 6.5 Hz, H-4), 8.30, 8.03 (2 br s, 2 H, $CONH_2$), 7.62 (dd, J = 8.9, 2.9 Hz, H-5); ^{13}C NMR δ 163.92 (s, $CONH_2$), 162.16 (d, J_{C-F} = 246 Hz, C-6), 146.53 (d, J_{C-F} = 15.1 Hz, C-3), 142.56 (s, C-2), 139.36 (dd, J_{C-F} = 10.0 Hz, C-4), 112.60 (dd, J_{C-F} = 39.2 Hz, C-5). Anal. ($C_6H_4FN_3O_3$) C, H, N.

A solution of **45** (1.50 g, 8.10 mmol) in EtOAc (80 mL) was hydrogenated over 5% Pd−C (0.30 g) at 60 psi for 2 h. After removal of the catalyst by filtration, the solvent was removed under reduced pressure to give a residue of crude 3-amino-6-fluoropyridine-2-carboxamide (**46**), which was used directly. Triethyl orthoformate (60 mL) was added, and the mixture was heated under reflux with vigorous stirring for 18 h. The cooled mixture was diluted with an equal volume of petroleum ether, and the resulting precipitate was collected by filtration and washed well with petroleum ether to give 6-fluoropyrido-[3,2-*d*]pyrimidin-4(3*H*)-one (**47**) (1.26 g, 84%): mp >300 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 12.72 (br s, 1 H, NH), 8.31 (dd, J = 8.6, 7.7 Hz, 1 H, H-8), 8.20 (s, 1 H, H-2), 7.66 (dd, J = 8.6, 3.0 Hz, H-7); ^{13}C NMR δ 160.16 (d, J_{C-F} = 239 Hz, C-6), 158.66 (s, CO), 146.14 (d, C-2), 144.81 (s), 142.48 (dd, J_{C-F} = 10.1 Hz), 136.33 (d, J_{C-F} = 15.1 Hz), 116.96 (dd, J_{C-F} = 41.2 Hz). Anal. ($C_7H_4FN_3O$) C, H, N, F.

A suspension of **47** (0.20 g, 1.21 mmol) in $POCl_3$ (30 mL) was heated under reflux with stirring until homogeneous (2 h), and then for a further 1 h. Excess $POCl_3$ was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Workup of the organic portion gave crude 4-chloro-6-fluoropyrido[3,2-*d*]-pyrimidine (**48**) (0.22 g, 100%) as an unstable solid which was used directly. A solution of **48** (0.20 g, 10.9 mmol) and 3-bromoaniline (0.12 mL, 2.18 mmol) in 2-propanol (20 mL) containing concentrated HCl (1 drop) was heated under reflux for 15 min, then cooled, poured into water, and extracted with EtOAc. Chromatography of the residue on silica gel, eluting

with EtOAc/petroleum ether (1:2), gave 4-[(3-bromophenyl)-amino]-6-fluoropyrido[3,2-*d*]pyrimidine (**11e**) (0.18 g, 52%), mp (MeOH) 162 °C; ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1 H, H-2), 8.65 (br s, 1 H, NH), 8.31 (dd, J = 8.6, 7.4 Hz, 1 H, H-8), 8.27 (br s, 1 H, H-2′), 7.77 (m, 1 H, H-6′), 7.41 (dd, J = 8.9, 2.2 Hz, 1 H, H-7), 7.29 (m, 2 H, H-4′,5′); ^{13}C NMR δ 160.03 (d, J_{C-F} = 293 Hz, C-6), 156.30 (s), 155.40 (d), 143.71 (s), 142.74 (dd, J_{C-F} = 10.1 Hz), 139.15 (s), 130.31 (d), 127.10 (d), 123.24 (d), 122.75 (s), 118.84 (d), 116.95 (dd, J_{C-F} = 40.7 Hz). Anal. ($C_{13}H_8FBrN_4$) C, H, N.

Preparation of 11b,f−h from 4-[(3-Bromophenyl)-amino]-6-fluoropyrido[3,2-*d*]pyrimidine (11e). A mixture of **11e** (0.15 g, 0.47 mmol), dimethylamine hydrochloride (0.11 g, 1.41 mmol), and Et_3N (0.23 mL, 1.64 mmol) in EtOH (15 mL) was heated in a pressure vessel at 100 °C for 18 h. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and water. The organic portion was worked up, and the residue was chromatographed on silica gel. Elution with EtOAc/petroleum ether (1:1) gave foreruns, while EtOAc eluted 4-[(3-bromophenyl)amino]-6-(dimethylamino)pyrido[3,2-*d*]pyrimidine (**11g**) (0.14 g, 86%): mp (MeOH) 157−158 °C; ^{1}H NMR ($CDCl_3$) δ 8.72 (br s, 1 H, NH), 8.56 (s, 1 H, H-2), 8.17 (t, J = 1.9 Hz, 1 H, H-2′), 7.85 (d, J = 9.3 Hz, 1 H, H-8), 7.77 (dt, J = 7.5, 1.9 Hz, 1 H, H-6′), 7.27−7.18 (m, 2 H, H-4′,5′), 7.08 (d, J = 9.3 Hz, 1 H, H-7), 3.21 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR δ 156.39 (s), 154.91 (s), 150.80 (d), 140.18 (s), 138.34 (s), 137.14 (d), 130.19 (d), 129.83 (s), 125.80 (d), 122.60 (s), 122.36 (d), 118.11 (d), 114.91 (d), 38.25 (q, $N(CH_3)_2$). Anal. ($C_{15}H_{14}BrN_5$) C, H, N.

Similar reaction of **11e** with ammonia in a pressure vessel at 100 °C for 30 h gave 6-amino-4-[(3-bromophenyl)amino]-pyrido[3,2-*d*]pyrimidine (**11b**) (90%): mp (MeOH) 225−227 °C; ^{1}H NMR ($CDCl_3$) δ 8.76 (br s, 1 H, NH), 8.64 (s, 1 H, H-2), 8.23 (br s, 1 H, H-2′), 7.93 (d, J = 9.0 Hz, 1 H, H-8), 7.81 (dt, J = 7.7, 1.8 Hz, 1 H, H-6′), 7.28−7.22 (m, 2 H, H-4′,5′), 7.00 (d, J = 9.0 Hz, 1 H, H-7), 4.90 (br s, 2 H, NH_2); ^{13}C NMR δ 156.63 (s), 155.26 (s), 152.02 (d), 140.09 (s), 138.30 (d), 130.27 (d), 129.90 (s), 126.15 (d), 122.65 (d), 118.31 (d), 117.62 (d) (two singlets not observed). Anal. ($C_{13}H_{10}BrN_5$) C, H, N.

Similar reaction of **11e** with methylamine in a pressure vessel at 100 °C for 18 h gave 4-[(3-bromophenyl)amino]-6-(methylamino)pyrido[3,2-*d*]pyrimidine (**11f**) (77%): mp (MeOH) 206−208 °C; ^{1}H NMR ($CDCl_3$) δ 8.81 (br s, 1 H, NH), 8.61 (s, 1 H, H-2), 8.19 (t, J = 1.8 Hz, 1 H, H-2′), 7.86 (d, J = 9.1 Hz, 1 H, H-8), 7.83 (dt, J = 7.7, 1.8 Hz, 1 H, H-6′), 7.28−7.21 (m, 2 H, H-4′,5′), 6.92 (d, J = 9.1 Hz, 1 H, H-7), 4.97 (q, J = 5.0 Hz, 1 H, N*H*CH_3), 3.13 (d, J = 5.0 Hz, 3 H, NHC*H*$_3$); ^{13}C NMR δ 156.64 (s), 155.22 (s), 151.30 (d), 140.19 (s), 139.61 (s), 137.24 (d), 130.28 (d), 129.91 (s), 126.03 (d), 122.61 (d), 118.33 (d), 117.79 (s), 28.73 (q, $NHCH_3$). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

Similar reaction of **11e** with NaOMe in a pressure vessel at 90 °C for 3 h gave 4-[(3-bromophenyl)amino]-6-methoxypyrido[3,2-*d*]pyrimidine (**11h**) (82%): mp (MeOH) 142 °C; ^{1}H NMR ($CDCl_3$) δ 8.73 (s, 1 H, H-2), 8.66 (br s, 1 H, NH), 8.18 (m, 1 H, H-2′), 8.05 (d, J = 8.9 Hz, 1 H, H-8), 7.83−7.80 (m, 1 H, H-6′), 7.30−7.24 (m, 2 H, H-4′,5′), 7.23 (d, J = 8.9 Hz, 1 H, H-7), 4.12 (s, 3 H, OCH_3); ^{13}C NMR δ 161.91 (s), 155.81 (s), 153.28 (d), 141.96 (s), 139.71 (s), 139.41 (d), 130.31 (d), 128.23 (s), 126.53 (d), 122.94 (d), 122.71 (s), 119.81 (d), 118.61 (d), 54.08 (q, OCH_3). Anal. ($C_{14}H_{11}BrN_4O$) C, H, N.

Enzyme Assay. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles by immunoaffinity chromatography as previously described,[30] and the assays were carried out as reported previously.[14] The substrate used was based on a portion of phospholipase Cγ1, having the sequence Lys-His-Lys-Lys-Leu-Ala-Glu-Gly-Ser-Ala-Tyr^{472}-Glu-Glu-Val. The reaction was allowed to proceed for 10 min at room temperature and then was stopped by the addition of 2 mL of 75 mM phosphoric acid. The solution was then passed through a 2.5 cm phosphocellulose disk which bound the peptide. This filter was washed with 75 mM phosphoric acid (5×), and incorporated label was assessed by scintillation counting in an aqueous fluor. Control activity (no drug) gave a count of approximately 100 000 cpm. At least two independent dose−response curves were done and the IC_{50} values computed. The reported values are averages; variation was generally ±15%.

EGFR Autophosphorylation in A431 Human Epidermoid Carcinoma Cells. Cells were grown to confluence in 6-well plates (35 mm diameter) and exposed to serum-free medium for 18 h. The cells were treated with compound for 2 h and then with 100 ng/mL of EGF for 5 min. The monolayers were lysed in 0.2 mL of boiling Laemmli buffer (2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, and 50 mM Tris, pH 6.8), and the lysates were heated to 100 °C for 5 min. Proteins in the lysate were separated by polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. The membrane was washed once in a mixture of 10 mM Tris, pH 7.2, 150 mM NaCl, 0.01% azide (TNA), and blocked overnight in TNA containing 5% bovine serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody (UBI, 1 μg/mL in blocking buffer) and then washed twice in TNA, once in TNA containing 0.05% Tween-20 and 0.05% nonidet P-40, and twice in TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 μCi/mL of [125]I-protein A and then washed again as above. After the blots were dry they were loaded into a film cassette and exposed to X-AR X-ray film for 1−7 days. Band intensities were determined with a Molecular Dynamics laser densitometer.

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