Tyrosine Kinase Inhibitors. 7. 7-Amino-4-(phenylamino)- and 7-Amino-4-[(phenylmethyl)amino]pyrido[4,3-*d*]pyrimidines: A New Class of Inhibitors of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor

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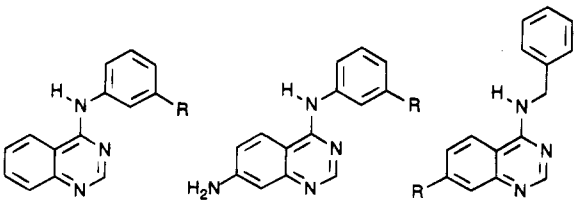
The synthesis of 7-aminopyrido[4,3-*d*]pyrimidines bearing aromatic side chains at the 4-position is reported. These compounds are shown to be a new class of inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR). Structure–activity relationships (SARs) for substitution in both 4-(phenylamino)- and 4-[(phenylmethyl)amino] side chains were determined, using a series of substituents (NO_2, Br, CF_3, OMe, NH_2, and NMe_2) selected primarily for their wide range of electronic properties. In the phenylamino series, 3-substituted derivatives were more potent than the corresponding 2- and 4-substituted analogues. For the 3-substituted compounds, activity was favored by electron withdrawal, in a relationship which could be quantified, with the 3-Br being the most potent compound (IC_{50} = 0.01 μM compared with IC_{50} = 0.34 μM for the unsubstituted side chain derivative). No such correlation was apparent for the 2- or 4-substituent, although Br was still the best substituent. In contrast, in the 4-[(phenylmethyl)amino] series, substitution of the side chain was not beneficial. For the 4-(phenylamino) series, the substituent SARs are broadly similar to that found previously for 4-(phenylamino)quinazolines. These results suggest that side chain SARs may be broadly invariant over a range of different chromophores, with the side chain of choice for optimization of EGFR inhibitory activity being 4-[(3-bromophenyl)amino].

Introduction

The epidermal growth factor receptor (EGFR) is one of the key transmembrane receptors at the beginning of the cell signal transduction pathway by which extracellular growth factors exercise control over cell growth and division.[1] Transformation or overexpression of the EGFR is thought to play a major role in malignant transformation, with studies linking mammary,[2,3] ovarian,[4] esophageal,[5] and squamous cell head and neck carcinomas[6] with overproduction of the EGFR and/or the closely homologous product of the *c-erbB2* oncogene. Because of this association, there is great interest[1,7,8] in the development of compounds capable of potent and/or selective inhibition of the ability of these enzymes to phosphorylate tyrosines in their substrates (the primary mechanism of signal transduction in both normal and transformed cells[9]).

The EGFR is one of the best-characterized tyrosine kinase signal transduction enzymes,[10] containing neighboring binding domains for both the tyrosine-containing substrate and the ATP cofactor in the catalytic site. While most known inhibitors of this enzyme are considered to bind to the tyrosine site,[7,8,11,12] we and others have recently reported on a series of 4-(phenylamino)-quinazolines which appear to act at the ATP site.[13–16] Preliminary structure–activity relationship (SAR) studies for this class showed an absolute requirement for the pyrimidine ring of the quinazoline chromophore and indicate a requirement for small lipophilic electron-withdrawing groups at the 3-position on the aniline, as indicated by a large increase in inhibitory potencies between **1** and **2** (IC_{50}s = 0.34 and 0.027 μM, respectively). There was also a requirement for electron-donating groups at the 6- and 7-positions of the quinazoline, with the 7-amino analogue **3** proving to be the most active monosubstituted derivative, with a large further increase in potency (IC_{50} = 0.0001 μM).[14] The parent 4-[(phenylmethyl)amino]quinazoline derivative **4** had equivalent potency to **1**, with an IC_{50} of 0.32 μM, but modifications in this series were generally less effective inhibitors; thus the 7-amino compound **5** had an IC_{50} of 1.25 μM.[14]



1: R = H
2: R = Br

3: R = Br
6: R = H

4: R = H
5: R = NH_2

We now report that the related aza derivatives of **3** and **5**, the 7-aminopyrido[4,3-*d*]pyrimidines **7** and **8**, show significant activity as inhibitors of the EGFR, also by competitive binding at the ATP site. Because the previous side chain SARs for the quinazolines were somewhat limited in scope, we report here a more detailed SAR study for side chain substituent effects in both the 4-(phenylamino)- (**7**) and 4-[(phenylmethyl)-amino]- (**8**) pyrido[4,3-*d*]pyrimidines.

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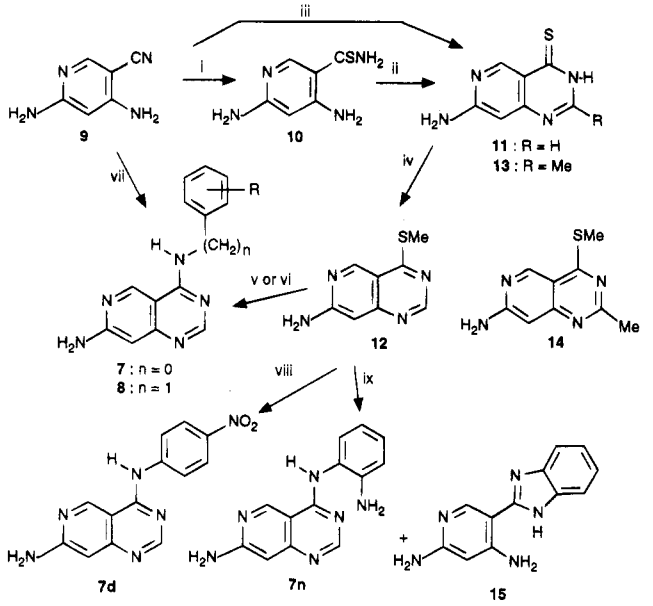
Scheme 1[a]



[a] (i) H_2S/Et_3N/pyridine/20 °C; (ii) $(EtO)_3CH$/110 °C; (iii) $(EtO)_3CH/Ac_2O$/reflux and then NaSH/EtOH/reflux; (iv) MeI/KOH/MeOH/water/20 °C; (v) neat amine/120–200 °C or amine/2-propanol/120–130 °C; (vi) $RPhNH_2$/HCl/160–180 °C; (vii) $RPhCH_2NH_2$/HCOOH/200 °C; (viii) 4-NO_2PhNH_2/NaH/DMF/120 °C; (ix) 2-NH_2PhNH_2/115 °C.

Chemistry

The 7-amino-4-(phenylamino)pyrido[4,3-*d*]pyrimidines **7** and the 7-amino-4-[(phenylmethyl)amino]pyrido[4,3-*d*]pyrimidines **8** of Table 1 were prepared by the general synthetic methods outlined in Scheme 1, from the key intermediate **12**. Reaction of 4,6-diamino-3-cyanopyridine (**9**)[17] with H_2S/Et_3N in pyridine[18] gave the thiocarboxamide **10** in good yield. However, ring closure of this in triethyl orthoformate gave the desired 7-aminopyrido-[4,3-*d*]pyrimidine-4(3*H*)-thione (**11**) as a mixture with byproducts from which it was not readily isolable. A preferable, alternative synthesis of **11** was developed, involving treatment of **9** with triethyl orthoformate/acetic anhydride followed by ethanolic sodium hydrosulfide, as described by Taylor.[18] S-Methylation of crude **11** then gave the key intermediate 7-amino-4-(methylthio)pyrido[4,3-*d*]pyrimidine (**12**). Contamination of some batches of **12** with small amounts (<10%) of the 2-methyl derivative **14** occurred. This was not resolvable by chromatography and necessitated careful chromatography and/or crystallization of the final products **7** and **8**. Production of **14** was attributed to competing acetylation of the starting diaminopyridine by the acetic anhydride in the previous step (this was found necessary for good yields), resulting in **11** contaminated with **13**.

A variety of methods were used to attach the side chains. In most cases, heating **12** with an excess of the appropriate aniline or benzylamine (in the absence of solvent) gave adequate yields, with the anilines generally requiring higher temperatures. Some reactions with substituted benzylamines were mediated by 2-propanol, but this often slowed the reactions and occasionally competed as a nucleophile to give an isopropyl ether derivative. Both 2- and 4-aminobenzylamines reacted preferentially via the aliphatic amine to give the desired products **8n,p**, although in the latter case difficulties in purification led to a low yield. The 3-amino derivatives **7o** and **8o** were obtained in moderate yields by hydrogenation of the poorly soluble 3-nitro analogues **7c** and **8c**. Base hydrolysis of the 4-acetamido derivative **7aa** gave the desired 4-amino compound **7p** in only moderate yield due to competing cleavage of the side chain, and a similar reaction of the 3-acetamido derivative **7z** gave none of the desired amine product **7o**. Treatment of **12** with 1,2-phenylenediamine at the usual temperature (150–180 °C) gave only the benzimidazole **15**, formed by nucleophilic attack of the 2′-amino group of the desired product **7n** at the C-4 position, opening of the pyrimidine ring, and elimination of HCN (Scheme 1). Conducting the reaction at a lower temperature (115 °C) in the absence of solvent gave a mixture of **7n** and **15**, which was separated by preparative reversed-phase HPLC. Treatment of **12** with 3-aminobenzonitrile gave the benzylamine derivative **8o** as the only isolable product, evidently involving reduction of the nitrile group by displaced thiomethoxide.

The above general method gave low yields for the less nucleophilic 2- and 4-nitrobenzylamines and failed completely for the analogous 2- and 4-nitro- and (trifluoromethyl)anilines. However, addition of at least 1 equiv of anhydrous HCl to the reaction mixture provided good to excellent yields of the desired products. In the case of 4-nitroaniline, conversion to the anion with NaH in DMF gave successful coupling to **12**, but this method failed for the 2-nitroaniline isomer. Certain benzylamine derivatives (**8a,m**) could be prepared directly in superior overall yield from the acetate salt of **9** by treatment with the appropriate benzylamines in the presence of formic acid at 200 °C. However, this method was completely ineffective with other benzylamines (R = NO_2, Br, NMe_2) and for aniline side chains.

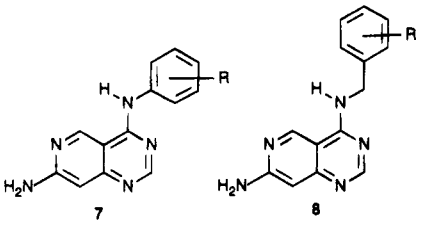
The majority of the required substituted anilines and benzylamines required in Scheme 1 were commercially available. Other benzylamines were prepared from the appropriate benzonitriles or benzamides by reduction with borane–DMS.[19] 2-(Dimethylamino)benzonitrile was prepared from 2-chlorobenzonitrile in HMPA.[20]

Results and Discussion

The structures and physicochemical properties of the compounds prepared are given in Table 1. All the analogues were evaluated for their ability to inhibit tyrosine phosphorylation of a polypeptide (a portion of phospholipase C-γ1) by full-length EGFR enzyme isolated from A431 cells.[13] Full dose–response curves were determined for each compound, and the resulting IC_{50}s listed in Table 1 are the average of at least two such determinations. For determination of SAR for side chain substituents in both the 4-[(phenylmethyl)amino]- and 4-(phenylamino) series, six substituents (NO_2, Br, CF_3, OMe, NH_2, and NMe_2) were used, at each of the three available aromatic positions. These substituents were selected primarily for their wide range of electronic properties (from σ +0.78 to −0.82),[21] but they also possessed significant variations in lipophilic and hydrogen-bonding effects.

In the phenylamino series, the parent compound **7a** was only slightly less potent than the corresponding 7-aminoquinazoline **6** (IC_{50}s = 0.25 and 0.1 μM, respectively).[22] For the phenylamino-substituted compounds **7b–s**, several overall conclusions can be drawn. In all cases, the 3-substituted derivatives were the most

Table 1. Tyrosine Kinase Inhibitory Properties of 7-Aminopyrido[4,3-*d*]pyrimidine Analogues



7 8

no.	R	IC_{50}[a] (μM)	no.	R	IC_{50}[a] (μM)
7a	H	0.25	**7x**	3′-Me	0.04
7b	2′-NO_2	1.25	**7y**	3′-aza	>10
7c	3′-NO_2	0.04	**7z**	3′-NHAc	>10
7d	4′-NO_2	65	**7aa**	4′-NHAc	>10
7e	2′-Br	0.24	**8a**	H	0.58
7f	3′-Br	0.01	**8b**	2′-NO_2	4.25
7g	4′-Br	0.09	**8c**	3′-NO_2	10.1
7h	2′-CF_3	10.0	**8d**	4′-NO_2	>10
7i	3′-CF_3	0.015	**8e**	2′-Br	1.67
7j	4′-CF_3	4.70	**8f**	3′-Br	1.00
7k	2′-OMe	3.71	**8g**	4′-Br	2.46
7l	3′-OMe	0.13	**8h**	2′-CF_3	10.0
7m	4′-OMe	0.67	**8i**	3′-CF_3	1.95
7n	2′-NH_2	5.25	**8j**	4′-CF_3	8.0
7o	3′-NH_2	1.55	**8k**	2′-OMe	100
7p	4′-NH_2	5.9	**8l**	3′-OMe	2.87
7q	2′-NMe_2	69	**8m**	4′-OMe	>100
7r	3′-NMe_2	1.79	**8n**	2′-NH_2	0.23
7s	4′-NMe_2	4.86	**8o**	3′-NH_2	>10
7t	3′-F	0.84	**8p**	4′-NH_2	>10
7u	3′-Cl	0.12	**8q**	2′-NMe_2	>100
7v	3′-I	0.26	**8r**	3′-NMe_2	10.0
7w	3′-OH	0.07	**8s**	4′-NMe_2	>100

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

potent, with no clear pattern emerging for the 2- and 4-substituents. The magnitude of this positional effect was variable but quite large in some cases (ratio of potencies for 4-substituted versus 3-substituted compounds: 300-fold for the CF_3 derivatives **7i,j**, 9-fold for the Br compounds **7f,g**, and a massive 1600-fold for the NO_2 compounds **7c,d**). For **7a** and the 3-substituted compounds **7c,f,i,l,o,r**, a similar pattern to that seen previously[14] for the 4-(phenylamino)quinazolines could be discerned, with electron-withdrawing groups favoring activity (eq 1) and the 3-Br being the most potent compound (IC_{50} = 0.01 μM). No such correlation was apparent for the 2- or 4-substituent, although Br was still the best substituent.

$$\log(1/IC_{50}) = 1.32(\pm 0.34)\sigma_m + 0.93(\pm 0.19) \quad (1)$$

$$n = 6, r = 0.87, s = 0.50$$

The validity of this relationship was tested by the evaluation of a further set of 3-substituted derivatives (**7t–z**). While the 3-aza and 3-NHAc compounds were inactive, the IC_{50} values of the other compounds (with the exception of the Me derivative **7x**) were accommodated reasonably well (eq 2).

$$\log(1/IC_{50}) = 1.93(\pm 0.67)\sigma_m + 0.38(\pm 0.58) \quad (2)$$

$$n = 11, r = 0.68, s = 0.58$$

No improvement in the correlation could be achieved by the use of lipophilicity or steric parameters. The unexpectedly good activity of the 3-Me derivative **7x** is unexplained.

In the 4-[(phenylmethyl)amino] series, the parent compound **8a** was 2 times as potent as the corresponding 7-aminoquinazoline **5** (IC_{50}s = 0.58 and 1.25 μM, respectively).[14] In contrast to the phenylamino series, substitution of the side chain was not beneficial, with only the 2-NH_2 derivative **8n** proving more potent. Despite the overall lowering of activity, the positional trend was similar to that seen in the phenylamino series, with the 3-substituted compounds being generally the most potent (except for the NH_2 and NO_2 cases), although the differentials were not as great. As in the phenylamino series, there was a broad trend in the 3-substituted compounds favoring electron withdrawal (although no statistically significant equation could be obtained), and the 3-Br was again the most potent 3-substituted compound.

Representative compounds were studied for their effects in A431 human epidermoid carcinoma cells, which overexpress EGFR, and significant inhibitory activity was seen (**7f**, IC_{50} = 0.11 μM; **7c**, IC_{50} = 0.25 μM; **8a**, IC_{50} = 2.1 μM), in the same rank order as their activities against the isolated enzyme. These compounds also rapidly and specifically inhibit EGFR autophosphorylation in A431 cells (unpublished data).

Conclusions

The above substituent SAR study for both 4-[(phenylmethyl)amino] and 4-(phenylamino) side chains in the 7-aminopyrido[4,3-*d*]pyrimidines is more comprehensive than that carried out by us previously[14] for the corresponding series of 4-(arylamino)quinazolines, but the results are broadly similar. Thus, the parent (unsubstituted side chain) compounds in each series have roughly similar potencies: IC_{50}s of 0.34 and 0.32 μM, respectively, for **1** and **4** in the quinazoline series and IC_{50}s of 0.25 and 0.58 μM, respectively, for **7a** and **8a** in the 7-aminopyrido[4,3-*d*]pyrimidine series. In each series, activity was substantially improved by electron-withdrawing substituents at the 3-position of the phenylamino side chain, with the 3-Br providing the largest increase in potency (12-fold from **1** to **2** in the quinazoline series and 25-fold from **7a** to **7f** in the 7-aminopyrido[4,3-*d*]pyrimidine series). In the latter series, this relationship was able to be quantified (eqs 1 and 2). In contrast, substitutions in the 4-[(phenylmethyl)amino] side chain were much less useful in each series. In the 7-aminopyrido[4,3-*d*]pyrimidine series studied here, no significant increases in potency over the parent compound **8a** were seen, even when a large and varied range of substituents were employed.

These results suggest that side chain SAR may be broadly invariant over a range of different chromophores, with the side chain of choice for optimization of EGFR inhibitory activity being 4-[(3-bromophenyl)-amino].

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined using an Electrothermal Model 9200 digital melting point apparatus and are as read. NMR spectra were measured on Bruker AM-400 or AC-200 spectrometers and are referenced to Me_4Si; ^{13}C NMR spectra were recorded in the

same solvent stated for the ^1H NMR spectra. Mass spectra were recorded on a Varian VG 7070 spectrometer at nominal 5000 resolution.

Preparation of 7-Amino-4-(methylthio)pyrido[4,3-*d*]pyrimidine (12). Method A. A mixture of 3-cyano-4,6-diaminopyridine (**9**)[17] (7.66 g, 0.057 mol), $(EtO)_3CH$ (75 mL, distilled from Na), and Ac_2O (75 mL, distilled) was stirred under reflux for 4 h, and solvents were then removed under reduced pressure. The liquid residue was stirred with a freshly prepared solution of NaSH in dry EtOH (1.5 M, 250 mL) at 100 °C for 16 h. After removal of solvent, the residue was dissolved in hot water, neutralized with concentrated HCl, and cooled to give crude 7-aminopyrido[4,3-*d*]pyrimidine-4(3*H*)-thione (**11**) (10.8 g) as a brown solid, which was used without purification: ^1H NMR [$(CD_3)_2SO$] δ 13.10 (br s, 1 H, NH), 9.18 (s, 1 H, H-5), 7.97 (s, 1 H, H-2), 7.10 (s, 2 H, 7-NH_2), 6.35 (s, 1 H, H-8).

A mixture of crude **11** (10.8 g, ca. 0.05 mol) and KOH (4.0 g, 0.061 mol) in MeOH/water (1:1, 200 mL) was stirred at 20 °C for 5 min. MeI (4.3 mL, 0.07 mol) was then added in portions over 10 min, and the mixture was stirred at 20 °C for a further 3 h. Removal of the MeOH and then cooling gave crude 7-amino-4-(methylthio)pyrido[4,3-*d*]pyrimidine (**12**) (9.60 g, 87% overall yield from **9**) as a brown solid. Chromatography of a sample on alumina, eluting with $CHCl_3$/EtOH (99:1), gave a pale yellow solid: mp (EtOH/$CHCl_3$/light petroleum) 229.5–231 °C; ^1H NMR [$(CD_3)_2SO$] δ 8.99 (d, J = 0.5 Hz, 1 H, H-5), 8.71 (s, 1 H, H-2), 6.93 (s, 2 H, 7-NH_2), 6.51 (br s, 1 H, H-8), 2.64 (s, 3 H, SCH_3). ^{13}C NMR δ 170.88 (s, C-4), 162.38 (s, C-7), 156.35 (d, C-2), 152.24 (s, C-8a), 149.11 (d, C-5), 112.29 (s, C-4a), 96.36 (d, C-8), 11.38 (q, SCH_3); HREIMS calcd for $C_8H_8N_4S$ m/z (M^+) 192.0470, found 192.0463. Anal. ($C_8H_8N_4S$) C, H, N, S.

Method B. H_2S gas was bubbled through a stirred solution of **9** (2.28 g, 17.0 mmol) in pyridine (10 mL) and Et_3N (2.40 mL, 17.2 mmol) at 20 °C for 1 h, and the solution was then stirred at 20 °C for 3 h. This procedure was repeated, and the resulting solution was diluted with water (150 mL) and extracted with EtOAc (5 × 150 mL). Removal of the solvent and chromatography of the residue on neutral alumina, eluting with a gradient of 7–10% MeOH/CH_2Cl_2, gave 4,6-diamino-3-pyridinethiocarboxamide (**10**) (1.92 g, 67%): mp (MeOH/CH_2Cl_2/light petroleum) 164 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 9.05, 9.00 (2 s, 2 H, $CSNH_2$), 7.94 (s, 1 H, H-2), 7.09 (s, 2 H, 4-NH_2), 5.91 (s, 2 H, 6-NH_2), 5.61 (s, 1 H, H-5); ^{13}C NMR δ 196.47 (s, $CSNH_2$), 160.70, 154.85 (2 s, C-4,6), 147.89 (d, C-2), 111.29 (s, C-3), 89.52 (d, C-5). Anal. ($C_6H_8N_4S$) C, H, N, S.

Reaction of **10** with neat triethyl orthoformate under a variety of conditions gave complex mixtures containing **11** which were difficult to purify.

7-Amino-4-[[(2-aminophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (8n): Example of 2-Propanol Method. A mixture of **12** (136 mg, 0.71 mmol) and 2-aminobenzylamine (1.70 g, 13.8 mmol) in iPrOH (5 mL) was stirred at 130 °C for 1 h. The resulting product was chromatographed on silica gel (eluting with a gradient of 7–20% EtOH/EtOAc) and alumina (eluting with a gradient of 6–10% EtOH in $CHCl_3$) to give 7-amino-4-[[(2-aminophenyl)methyl]amino]pyrido[4,3-*d*]-pyrimidine (**8n**) (89 mg, 47%): mp (EtOH/$CHCl_3$/light petroleum) 221 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 9.08 (s, 1 H, H-5), 8.68 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 7.05 (d, J = 7.4 Hz, 1 H, ArH), 6.96 (t, J = 7.6 Hz, 1 H, ArH), 6.63 (d, J = 7.9 Hz, 1 H, ArH), 6.51 (t, J = 7.4 Hz, 1 H, ArH), 6.46 (s, 2 H, 7-NH_2), 6.35 (s, 1 H, H-8), 5.20 (br s, 2 H, 2′-NH_2), 4.56 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.79, 159.25 (2 s, C-4,7), 158.34 (d, C-2), 154.40 (s, C-8a), 147.86 (d, C-5), 146.31 (s, C-2′), 129.02, 127.77 (2 d, C-4′,6′), 121.85 (s, C-1′), 115.83, 114.71 (2 d, C-3′,5′), 103.77 (s, C-4a), 97.07 (d, C-8), 40.40 (t, NCH_2). Anal. ($C_{14}H_{14}N_6$·0.25H_2O) C, H, N.

Similar reaction of a stirred solution of **12** (252 mg, 1.31 mmol) and 2-methoxybenzylamine (0.64 mL, 4.90 mmol) in 2-propanol (5 mL) under N_2 at 120 °C for 4 h, followed by evaporation of the solvent and chromatography on silica gel (2–10% EtOH/EtOAc), gave 7-amino-4-[[(2-methoxyphenyl)-methyl]amino]pyrido[4,3-*d*]pyrimidine (**8k**) (235 mg, 64%): mp (EtOAc/light petroleum) 211.5–213 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.14 (s, 1 H, H-5), 8.67 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.22 (s, 1 H, H-2), 7.23 (t, J = 8.3 Hz, 1 H, ArH), 7.17 (d, J = 7.9 Hz, 1 H, ArH), 7.00 (d, J = 8.2 Hz, 1 H, ArH), 6.87 (t, J = 7.4 Hz, 1 H, ArH), 6.44 (s, 2 H, 7-NH_2), 6.36 (s, 1 H, H-8), 4.69 (d, J = 5.7 Hz, 2 H, NHC*H*$_2$), 3.84 (s, 3 H, OCH_3); ^{13}C NMR δ 161.64, 159.46 (2 s, C-4,7), 158.44 (d, C-2), 156.58 (s, C-2′), 154.35 (s, C-8a), 147.86 (d, C-5), 127.82, 127.14 (2 d, C-4′,6′), 126.50 (s, C-1′), 120.01 (d, C-5′), 110.35 (d, C-3′), 103.83 (s, C-4a), 96.94 (d, C-8), 55.23 (q, OCH_3), 38.42 (t, NCH_2). Anal. ($C_{15}H_{15}N_5O$·0.5 EtOAc, solvent detected by NMR) C, H, N.

Similar reaction of a stirred solution of **12** (136 mg, 0.71 mmol) and 3-methoxybenzylamine (1.37 g, 10.0 mmol) in 2-propanol (3 mL) under N_2 at 130 °C for 3 h, followed by evaporation of the solvent and chromatography on silica gel (5–10% EtOH/EtOAc), gave 7-amino-4-[[(3-methoxyphenyl)-methyl]amino]pyrido[4,3-*d*]pyrimidine (**8l**) (153 mg, 77%): mp (EtOH/$CHCl_3$/light petroleum) 212–213.5 °C; ^1H NMR [$(CD_3)_2$-SO] δ 9.11 (s, 1 H, H-5), 8.83 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 7.24 (td, J = 8.1, 0.8 Hz, 1 H, ArH), 6.92 (m, 2 H, ArH), 6.81 (dt, J = 8.2, 1.2 Hz, 1 H, ArH), 6.46 (s, 2 H, 7-NH_2), 6.37 (s, 1 H, H-8), 4.71 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$), 3.73 (s, 3 H, OCH_3); ^{13}C NMR δ 161.74, 159.36, 159.28 (3 s, C-4,7,3′), 158.49 (d, C-2), 154.41 (s, C-8a), 147.87 (d, C-5), 141.03 (s, C-1′), 129.37 (d, C-5′), 119.38 (d, C-6′), 113.02, 112.03 (2 d, C-2′,4′), 103.82 (s, C-4a), 97.00 (d, C-8), 54.95 (q, OCH_3), 43.15 (t, NCH_2). Anal. ($C_{15}H_{15}N_5O$) C, H, N.

Similar reaction of a stirred solution of **12** (136 mg, 0.71 mmol) and 4-methoxybenzylamine (1.37 g, 10.0 mmol) in 2-propanol (3 mL) under N_2 at 130 °C for 2 h, followed by evaporation of the solvent and purification of the product on preparative TLC, gave 7-amino-4-[[(4-methoxyphenyl)methyl]-amino]pyrido[4,3-*d*]pyrimidine (**8m**), isolated as the dimesylate salt: mp 119–120 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.46 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 9.22 (s, 1 H, H-5), 8.70 (s, 1 H, H-2), 7.32 (d, J = 8.7 Hz, 2 H, ArH), 6.92 (d, J = 8.7 Hz, 2 H, ArH), 6.36 (s, 1 H, H-8), 4.83 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$), 3.74 (s, 3 H, OCH_3), 2.34 (s, 6 H, 2 CH_3). Anal. ($C_{15}H_{15}N_5O$·2CH_3SO_3H·H_2O) C, H, N.

Similar reaction of a stirred solution of **12** (377 mg, 1.96 mmol) and 4-aminobenzylamine (1.88 g, 15.4 mmol) in 2-propanol (20 mL) at 120 °C for 20 h, followed by chromatography of the resulting product on silica gel (10–15% EtOH/EtOAc) and then on alumina (3–10% EtOH/$CHCl_3$), gave a crude oily product (0.45 g, ca. 60% pure). Crystallization gave pure 7-amino-4-[[(4-aminophenyl)methyl]amino]pyrido[4,3-*d*]-pyrimidine (**8p**) (70 mg, 13%): mp (EtOH/$CHCl_3$) 210–212 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.07 (s, 1 H, H-5), 8.65 (t, J = 5.5 Hz, 1 H, N*H*CH_2), 8.25 (s, 1 H, H-2), 7.02, 6.51 (2 d, J = 8.3 Hz, 2 × 2 H, ArH), 6.40 (s, 2 H, 7-NH_2), 6.34 (s, 1 H, H-8), 4.95 (br s, 2 H, 4′-NH_2), 4.55 (d, J = 5.4 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.66, 159.18 (2 s, C-4,7), 158.58 (d, C-2), 154.45 (s, C-8a), 147.86 (d, C-5), 147.60 (s, C-4′), 128.40 (d, 2 C, C-2′,6′), 126.12 (s, C-1′), 113.69 (d, 2 C, C-3′,5′), 103.89 (s, C-4a), 97.00 (d, C-8), 43.01 (t, NCH_2); HREIMS calcd for $C_{14}H_{14}N_6$ m/z (M^+) 266.1280, found 266.1280. Anal. ($C_{14}H_{14}N_6$·0.25H_2O) C, H.

Similar reaction of a stirred solution of **12** (137 mg, 0.71 mmol) and 2-(dimethylamino)benzylamine (1.01 g, 6.73 mmol) in 2-propanol (3 mL) under N_2 at 130 °C for 1 h, followed by chromatography of the product on silica gel (10-15% EtOH/EtOAc) and alumina (1% EtOH/$CHCl_3$), gave 7-amino-4-[[[2-(dimethylamino)phenyl]methyl]amino]pyrido[4,3-*d*]pyrimidine (**8q**) (152 mg, 73%): mp (EtOH/$CHCl_3$/light petroleum) 167.5–169 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.13 (s, 1 H, H-5), 8.83 (t, J = 5.5 Hz, 1 H, N*H*CH_2), 8.27 (s, 1 H, H-2), 7.21 (m, 2 H, ArH), 7.14 (d, J = 7.8 Hz, 1 H, ArH), 6.98 (d, J = 7.4 Hz, 1 H, ArH), 6.49 (s, 2 H, 7-NH_2), 6.37 (s, 1 H, H-8), 4.83 (d, J = 5.6 Hz, 2 H, NHC*H*$_2$), 2.68 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR δ 161.79, 159.63 (2 s, C-4,7), 158.37 (d, C-2), 153.94 (s, C-8a), 151.83 (s, C-2′), 148.01 (d, C-5), 132.73 (s, C-1′), 127.39 (d, 2 C, C-4′,6′), 122.80 (d, C-3′), 118.79 (d, C-5′), 103.70 (s, C-4a), 96.64 (d, C-8), 44.55 (q, 2 C, $N(CH_3)_2$), 39.21 (t, NCH_2). Anal. ($C_{16}H_{18}N_6$·0.25H_2O) C, H, N.

Similar reaction of a stirred solution of **12** (236 mg, 1.23 mmol) and 3-(dimethylamino)benzylamine (1.36 g, 9.07 mmol) in 2-propanol (5 mL) under N_2 at 120–130 °C for 1 h, and chromatography of the resulting product on silica gel (10–15%

EtOH/EtOAc) and then on alumina (1% EtOH/$CHCl_3$), gave 7-amino-4-[[[3-(dimethylamino)phenyl]methyl]amino]pyrido-[4,3-*d*]pyrimidine (**8r**) (145 mg, 40%): mp (EtOH/$CHCl_3$/light petroleum) 175–179 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.11 (s, 1 H, H-5), 8.79 (t, J = 5.9 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 7.11 (dd, J = 8.0, 7.7 Hz, 1 H, ArH), 6.73 (br s, 1 H, ArH), 6.63 (d, J = 7.6 Hz, 1 H, ArH), 6.60 (dd, J = 8.1, 2.2 Hz, 1 H, ArH), 6.44 (s, 2 H, 7-NH_2), 6.35 (s, 1 H, H-8), 4.67 (d, J = 5.8 Hz, 2 H, NHCH_2), 2.86 (s, 6 H, N(CH_3)$_2$); ^{13}C NMR δ 161.71, 159.35 (2 s, C-4,7), 158.48 (d, C-2), 154.35 (s, C-8a), 150.50 (s, C-3′), 147.89 (d, C-5), 139.92 (s, C-1′), 128.82 (d, C-5′), 115.21 (d, C-4′), 111.44, 111.07 (2 d, C-2′,6′), 103.84 (s, C-4a), 96.93 (d, C-8), 43.68 (t, NCH_2), 40.12 (q, 2 C, N(CH_3)$_2$). Anal. ($C_{16}H_{18}N_6$·0.25H_2O) C, H, N.

Similar reaction of a stirred solution of **12** (227 mg, 1.18 mmole) and 4-(dimethylamino)benzylamine (1.34 g, 8.93 mmol) in 2-propanol (5 mL) under N_2 at 120–130 °C for 45 min, followed by chromatography of the product on silica gel (10–20% EtOH/EtOAc) and then on alumina (1% EtOH/$CHCl_3$), gave 7-amino-4-[[[4-(dimethylamino)phenyl]methyl]amino]-pyrido[4,3-*d*]pyrimidine (**8s**) (156 mg, 45%): mp (EtOH/$CHCl_3$/light petroleum) 215–218.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.07 (s, 1 H, H-5), 8.70 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.25 (s, 1 H, H-2), 7.18 (d, J = 8.6 Hz, 2 H, ArH), 6.67 (d, J = 8.6 Hz, 2 H, ArH), 6.40 (s, 2 H, 7-NH_2), 6.34 (s, 1 H, H-8), 4.60 (d, J = 5.7 Hz, 2 H, NHCH_2), 2.84 (s, 6 H, N(CH_3)$_2$); ^{13}C NMR δ 161.59, 159.12 (2 s, C-4,7), 158.48 (d, C-2), 154.37 (s, C-8a), 149.63 (s, C-4′), 147.76 (d, C-5), 128.31 (d, 2 C, C-2′,6′), 126.70 (s, C-1′), 112.31 (d, 2 C, C-3′,5′), 103.82 (s, C-4a), 96.93 (d, C-8), 42.76 (t, NCH_2), 40.20 (q, 2 C, N(CH_3)$_2$). Anal. ($C_{16}H_{18}N_6$) C, H, N.

7-Amino-4-[[(2-bromophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (8e): Example of Neat Amine Method. A mixture of **12** (225 mg, 1.17 mmol) and 2-bromobenzylamine (0.84 g, 4.52 mmol) was stirred under N_2 at 140 °C for 1 h. The resulting product was chromatographed on silica gel, eluting with a gradient of 1–5% EtOH in EtOAc, to give 7-amino-4-[[(2-bromophenyl)methyl]amino]pyrido[4,3-*d*]-pyrimidine (**8e**) (175 mg, 45%): mp (EtOAc/light petroleum) 221–221.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.16 (s, 1 H, H-5), 8.85 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.24 (s, 1 H, H-2), 7.64 (d, J = 7.8 Hz, 1 H, ArH), 7.34 (dd, J = 7.7, 7.1 Hz, 1 H, ArH), 7.31 (dd, J = 7.7, 2.4 Hz, 1 H, ArH), 7.21 (ddd, J = 7.8, 6.9, 2.4 Hz; 1 H, ArH), 6.50 (s, 2 H, 7-NH_2), 6.39 (s, 1 H, H-8), 4.74 (d, J = 5.7 Hz, 2 H, NHCH_2); ^{13}C NMR δ 161.74, 159.32 (2 s, C-4,7), 158.35 (d, C-2), 154.31 (s, C-8a), 147.90 (d, C-5), 137.56 (s, C-1′), 132.29, 128.75, 128.38, 127.63 (4 d, C-3′,4′,5′,6′), 122.14 (s, C-2′), 103.70 (s, C-4a), 96.92 (d, C-8), 43.75 (t, NCH_2). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

Similar reaction of **12** (0.136 g, 0.7 mmol) and aniline (0.5 mL, 5.5 mmol) under N_2 at 180 °C for 1 h gave, on cooling, 7-amino-4-(phenylamino)pyrido[4,3-*d*]pyrimidine (**7a**) (84 mg, 51%): mp (iPrOH) 283–284 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 9.82 (s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.37 (s, 1 H, H-2), 7.80 (d, J = 7.5 Hz, 2 H, ArH), 7.38 (t, J = 7.5 Hz, 2 H, ArH), 7.12 (t, J = 7.5 Hz, 1 H, ArH), 6.61 (br s, 2 H, 7-NH_2), 6.43 (s, 1 H, H-8). Anal. ($C_{13}H_{11}N_5$·1.5H_2O) C, H, N.

Similar reaction of **12** (127 mg, 0.66 mmol) and 3-nitroaniline (1.70 g, 12.3 mmol) under N_2 at 200 °C for 1.5 h, followed by chromatography of the product on alumina (5–20% EtOH/$CHCl_3$), gave 7-amino-4-[(3-nitrophenyl)amino]-pyrido[4,3-*d*]pyrimidine (**7c**) (81 mg, 39%): mp (MeOH/$CHCl_3$/light petroleum) 287–289.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.17 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.87 (br s, 1 H, ArH), 8.48 (s, 1 H, H-2), 8.33 (br d, J = 7.5 Hz, 1 H, ArH), 7.95 (ddd, J = 8.2, 2.1, 1.0 Hz, 1 H, ArH), 7.67 (t, J = 8.2 Hz, 1 H, ArH), 6.70 (s, 2 H, 7-NH_2), 6.47 (s, 1 H, H-8); ^{13}C NMR δ 162.06 (s, C-7), 157.69 (sd, C-2,4), 154.67 (s, C-8a), 148.65 (d, C-5), 147.84 (s, C-3′), 140.50 (s, C-1′), 129.80 (d, C-5′), 127.75 (d, C-6′), 117.71, 115.88 (2 d, C-2′,4′), 103.83 (s, C-4a), 96.99 (d, C-8). Anal. ($C_{13}H_{10}N_6O_2$·CH_3OH·0.5H_2O) C, H, N.

Similar reaction of **12** (198 mg, 1.03 mmol) and 2-bromoaniline (1.00 mL, 9.18 mmol) under N_2 at 180 °C for 2.5 h, and chromatography of the product on alumina (1% EtOH/$CHCl_3$), gave 7-amino-4-[(2-bromophenyl)amino]pyrido[4,3-*d*]-pyrimidine (**7e**) (108 mg, 33%): mp (MeOH/water) 235.5–237.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.91 (br s, 1 H, NH), 9.27 (s, 1 H, H-5), 8.20 (s, 1 H, H-2), 7.73 (d, J = 7.9 Hz, 1 H, ArH), 7.50 (br m, 1 H, ArH), 7.44 (t, J = 6.9 Hz, 1 H, ArH), 7.25 (br m, 1 H, ArH), 6.59 (s, 2 H, 7-NH_2), 6.42 (s, 1 H, H-8); ^{13}C NMR δ 161.85 (s, C-7), 158.79 (br s, C-4), 158.17 (br d, C-2), 154.56 (br s, C-8a), 148.41 (d, C-5), 137.16 (br s, C-1′), 132.67 (d, C-3′), 130.09 (br d, C-5′), 128.09 (br d, 2 C, C-4′,6′), 121.95 (s, C-2′), 103.61 (s, C-4a), 96.69 (br d, C-8). Anal. ($C_{13}H_{10}BrN_5$) C, H, N.

Similar reaction of **12** (0.136 g, 0.7 mmol) and 3-bromoaniline (1.00 mL, 9.18 mmol), followed by purification of the product as the dimesylate salt, gave 7-amino-4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidine dimesylate salt (**7f**): mp 204–205 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 14.0 (v br s, 1 H, NH), 11.40 (br s, 1 H, NH), 9.43 (s, 1 H, H-5), 8.77 (s, 1 H, H-2), 7.96 (t, J = 1.9 Hz, 1 H, ArH), 7.65 (dd, J = 7.0, 1.7 Hz, 1 H, ArH), 7.53 (br d, J = 7.9 Hz, 1 H, ArH), 7.46 (t, J = 8.0 Hz, 1 H, ArH), 7.10 (t, J = 5.1 Hz, 1 H, H-8), 2.34 (s, 6 H, 2 CH_3). Anal. ($C_{13}H_{10}BrN_5$·2CH_3SO_3H·H_2O) C, H, N.

Similar reaction of **12** (261 mg, 1.36 mmol) and 4-bromoaniline (1.00 g, 5.81 mmol) under N_2 at 200 °C for 15 min, and chromatography on silica gel (10–15% EtOH/EtOAc), gave 7-amino-4-[(4-bromophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7g**) (200 mg, 46%): mp (EtOAc/light petroleum) 289.5–290.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.88 (s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.40 (s, 1 H, H-2), 7.83 (d, J = 8.8 Hz, 2 H, ArH), 7.55 (d, J = 8.8 Hz, 2 H, ArH), 6.64 (s, 2 H, 7-NH_2), 6.44 (s, 1 H, H-8); ^{13}C NMR δ 161.83 (s, C-7), 157.78 (d, C-2), 157.54 (s, C-4), 154.58 (s, C-8a), 148.38 (d, C-5), 138.40 (s, C-1′), 131.11 (d, 2 C, C-3′,5′), 123.95 (d, 2 C, C-2′,6′), 115.17 (s, C-4′), 103.78 (s, C-4a), 96.88 (d, C-8). Anal. ($C_{13}H_{10}BrN_5$) C, H, N.

Similar reaction of **12** (234 mg, 1.22 mmol) and 3-aminobenzotrifluoride (2.00 mL, 16.0 mmol) under N_2 at 190–200 °C for 2 h, followed by chromatography on silica gel (5–10% EtOH/EtOAc) and then alumina (5–7% EtOH/$CHCl_3$), gave 7-amino-4-[[3-(trifluoromethyl)phenyl]amino]pyrido[4,3-*d*]-pyrimidine (**7i**) (157 mg, 42%): mp (EtOH/$CHCl_3$/light petroleum) 296–298 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.04 (s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.46 (s, 1 H, H-2), 8.31 (s, 1 H, ArH), 8.19 (d, J = 8.2 Hz, 1 H, ArH), 7.62 (t, J = 8.0 Hz, 1 H, ArH), 7.45 (d, J = 7.7 Hz, 1 H, ArH), 6.69 (s, 2 H, 7-NH_2), 6.47 (s, 1 H, H-8); ^{13}C NMR δ 161.99 (s, C-7), 157.80 (d, C-2), 157.71 (s, C-4), 154.66 (s, C-8a), 148.53 (d, C-5), 140.00 (s, C-1′), 129.60 (d, C-6′), 129.19 (q, J_{CF} = 31 Hz, C-3′), 125.47 (d, C-5′), 124.20 (q, J_{CF} = 272 Hz, 3′-CF_3), 119.60, 117.98 (2dq, J_{CF} = 3.9 Hz, C-2′,4′), 103.82 (s, C-4a), 96.96 (d, C-8). Anal. ($C_{14}H_{10}F_3N_5$) C, H, N.

Similar reaction of **12** (227 mg, 1.18 mmol) and *o*-anisidine (1.00 mL, 8.87 mmol) under N_2 at 180 °C for 2.5 h, and purification of the resulting product by chromatography on silica gel (5% EtOH/EtOAc), gave 7-amino-4-[(2-methoxyphenyl)amino]pyrido[4,3-*d*]pyrimidine (**7k**) (147 mg, 47%): mp (EtOH/$CHCl_3$/light petroleum) 229–232 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 9.44 (br s, 1 H, NH), 9.25 (s, 1 H, H-5), 8.22 (s, 1 H, H-2), 7.54 (dd, J = 7.7, 1.4 Hz, 1 H, ArH), 7.24 (ddd, J = 8.1, 7.4, 1.5 Hz, 1 H, ArH), 7.10 (dd, J = 8.2, 1.2 Hz, 1 H, ArH), 6.98 (td, J = 7.5, 1.3 Hz, 1 H, ArH), 6.52 (s, 2 H, 7-NH_2), 6.41 (s, 1 H, H-8), 3.79 (s, 3 H, OCH_3); ^{13}C NMR δ 161.70 (s, C-7), 158.77 (br s, C-4), 158.25 (d, C-2), 154.59 (s, C-8a), 153.48 (s, C-2′), 148.27 (d, C-5), 127.48 (d, C-4′), 126.68 (s, C-1′), 126.65 (d, C-5′), 120.06 (d, C-6′), 111.69 (d, C-3′), 103.91 (s, C-4a), 96.88 (d, C-8), 55.57 (q, OCH_3). Anal. ($C_{14}H_{13}N_5O$) C, H, N.

Similar reaction of **12** (226 mg, 1.18 mmol) and *m*-anisidine (1.00 mL, 8.90 mmol) under N_2 at 190 °C for 1.5 h, and chromatography of the product on silica gel (5–7% EtOH/EtOAc), gave 7-amino-4-[(3-methoxyphenyl)amino]pyrido[4,3-*d*]pyrimidine (**7l**) (136 mg, 43%): mp (EtOH/$CHCl_3$/light petroleum) 259–260.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.78 (br s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.40 (s, 1 H, H-2), 7.50 (br s, 1 H, ArH), 7.44 (d, J = 8.0 Hz, 1 H, ArH), 7.28 (t, J = 8.2 Hz, 1 H, ArH), 6.71 (dd, J = 8.2, 2.3 Hz, 1 H, ArH), 6.61 (s, 2 H, 7-NH_2), 6.45 (s, 1 H, H-8), 3.77 (s, 3 H, OCH_3); ^{13}C NMR δ 161.89 (s, C-7), 159.37 (s, C-4), 158.05 (d, C-2), 157.88 (s, C-3′), 154.70 (s, C-8a), 148.44 (d, C-5), 140.20 (s, C-1′), 129.22 (d, C-5′), 114.61, 109.02, 108.23 (3 d, C-2′,4′,6′), 103.99 (s, C-4a), 97.03 (d, C-8), 55.11 (q, OCH_3). Anal. ($C_{14}H_{13}N_5O$) C, H, N.

Similar reaction of **12** (35 mg, 0.18 mmol) and *p*-anisidine

(0.51 g, 4.14 mmol) at 170 °C for 30 min, followed by chromatography of the product on silica gel (6% MeOH/CH_2Cl_2), gave 7-amino-4-[(4-methoxyphenyl)amino]pyrido[4,3-*d*]pyrimidine (**7m**) (28 mg, 58%): mp (MeOH/$CHCl_3$) 254–255.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.82 (br s, 1 H, NH), 9.30 (s, 1 H, H-5), 8.32 (s, 1 H, H-2), 7.64 (d, J = 8.9 Hz, 2 H, ArH), 6.96 (d, J = 9.1 Hz, 2 H, ArH), 6.59 (s, 2 H, 7-NH_2), 6.41 (s, 1 H, H-8), 3.77 (s, 3 H, OCH_3); ^{13}C NMR δ 161.83 (s, C-7), 157.98 (d, C-2), 157.88 (s, C-4), 155.90 (s, C-4'), 154.25 (s, C-8a), 148.34 (d, C-5), 131.61 (s, C-1'), 124.47 (d, 2 C, C-2',6'), 113.63 (d, 2 C, C-3',5'), 103.74 (s, C-4a), 96.69 (d, C-8), 55.22 (q, OCH_3). Anal. ($C_{14}H_{13}N_5O$·0.75H_2O) C, H, N.

Similar reaction of **12** (348 mg, 1.91 mmol) and 2-(dimethylamino)aniline [prepared by hydrogenation of a solution of 2-(dimethylamino)nitrobenzene (2.80 g, 16.9 mmol) and 5% Pd/C (0.5 g) in MeOH (100 mL) at 60 psi and 20 °C for 1 h followed by filtration of the solution over Celite, drying over Na_2SO_4, and evaporation] at 190 °C for 1 h, followed by chromatography of the product on alumina (1% MeOH/CH_2Cl_2) and then on silica gel (3–4% MeOH/CH_2Cl_2), gave 7-amino-4-[[2-(dimethylamino)phenyl]amino]pyrido[4,3-*d*]-pyrimidine (**7q**) (202 mg, 40%): mp (CH_2Cl_2/light petroleum) 198–200.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.50 (br s, 1 H, NH), 9.25 (s, 1 H, H-5), 8.29 (s, 1 H, H-2), 7.69 (br d, J = 7.4 Hz, 1 H, ArH), 7.16 (m, 2 H, ArH), 7.04 (td, J = 7.1, 2.4 Hz, 1 H, ArH), 6.58 (s, 2 H, 7-NH_2), 6.43 (s, 1 H, H-8), 2.65 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR δ 161.88 (s, C-7), 158.32 (sd, 2 C, C-2,4), 154.58 (s, C-8a), 147.99 (d, C-5), 147.55 (s, C-2'), 131.12 (s, C-1'), 126.86, 125.68 (2 d, C-4',6'), 122.09, 118.76 (2 d, C-3',5'), 104.12 (s, C-4a), 96.92 (d, C-8), 43.36 (q, 2 C, $N(CH_3)_2$). Anal. ($C_{15}H_{16}N_6$·0.25H_2O) C, H, N.

Similar reaction of **12** (245 mg, 1.28 mmol) and *N,N*-dimethyl-1,3-phenylenediamine (1.60 g, 11.8 mmol) under N_2 at 190 °C for 1 h, followed by chromatography (twice) of the product on alumina (3% EtOH/$CHCl_3$), gave 7-amino-4-[[3-(dimethylamino)phenyl]amino]pyrido[4,3-*d*]pyrimidine (**7r**) (113 mg, 32%): mp (EtOH/$CHCl_3$/light petroleum) 266–270 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.66 (br s, 1 H, NH), 9.33 (s, 1 H, H-5), 8.36 (s, 1 H, H-2), 7.22 (br d, J = 7.8 Hz, 1 H, ArH), 7.16 (m, 2 H, ArH), 6.57 (s, 2 H, 7-NH_2), 6.51 (ddd, J = 8.0, 2.3, 1.2 Hz, 1 H, ArH), 6.42 (s, 1 H, H-8), 2.91 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR δ 161.91 (s, C-7), 158.34 (d, C-2), 158.18 (s, C-4), 154.79 (s, C-8a), 150.94 (s, C-3'), 148.51 (d, C-5), 139.68 (s, C-1'), 128.96 (d, C-5'), 111.18, 108.64, 106.93 (3 d, C-2',4',6'), 104.16 (s, C-4a), 97.15 (d, C-8), 40.36 (q, 2 C, $N(CH_3)_2$). Anal. ($C_{15}H_{16}N_6$) C, H, N.

Similar reaction of **12** (256 mg, 1.33 mmol) and *N,N*-dimethyl-1,4-phenylenediamine (1.95 g, 14.4 mmol) under N_2 at 190 °C for 20 min, and chromatography of the resulting product on alumina (3–7% EtOH/$CHCl_3$), gave 7-amino-4-[[4-(dimethylamino)phenyl]amino]pyrido[4,3-*d*]pyrimidine (**7s**) (198 mg, 53%): mp (EtOH/$CHCl_3$/light petroleum) 250–251 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.67 (br s, 1 H, NH), 9.27 (s, 1 H, H-5), 8.27 (s, 1 H, H-2), 7.51, 6.75 (2 d, J = 8.9 Hz, 2 × 2 H, ArH), 6.51 (s, 2 H, 7-NH_2), 6.39 (s, 1 H, H-8), 2.89 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR δ 161.70 (s, C-7), 158.25 (d, C-2), 157.85 (s, C-4), 154.63 (s, C-8a), 148.09 (d, C-5), 147.59 (s, C-4'), 128.07 (s, C-1'), 124.26 (d, 2 C, C-2',6'), 112.29 (d, 2 C, C-3',5'), 103.94 (s, C-4a), 96.98 (d, C-8), 40.43 (q, 2 C, $N(CH_3)_2$). Anal. ($C_{15}H_{16}N_6$) C, H, N.

Similar reaction of **12** (215 mg, 1.12 mmol) and 3-fluoroaniline (1.16 g, 10.4 mmol) at 160 °C for 30 min, and chromatography of the product on silica gel (6–7% MeOH/CH_2Cl_2), gave 7-amino-4-[(3-fluorophenyl)amino]pyrido[4,3-*d*]-pyrimidine (**7t**) (185 mg, 65%): mp (CH_3CN/water) 308–311 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.94 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.46 (s, 1 H, H-2), 7.91 (br d, J_{HF} = 11.9 Hz, 1 H, ArH), 7.63 (br d, J = 8.1 Hz, 1 H, ArH), 7.41 (dd, J = 15.7, 7.7 Hz, 1 H, ArH), 6.93 (td, J = 8.5, 2.4 Hz, 1 H, ArH), 6.68 (s, 2 H, 7-NH_2), 6.38 (s, 1 H, H-8). ^{13}C NMR δ 161.97 (s, C-7), 161.94 (d, J_{CF} = 241 Hz, C-3'), 157.81 (d, C-2), 157.69 (s, C-4), 154.59 (s, C-8a), 148.53 (d, C-5), 140.93 (d, J_{CF} = 11 Hz, C-1'), 129.95 (dd, J_{CF} = 10 Hz, C-5'), 117.59 (d, C-6'), 109.84 (dd, J_{CF} = 21 Hz, C-4'), 108.76 (dd, J_{CF} = 26 Hz, C-2'), 103.86 (s, C-4a), 96.92 (d, C-8). Anal. ($C_{13}H_{10}FN_5$) C, H, N.

Similar reaction of **12** (208 mg, 1.08 mmol) and 3-chloroaniline (1.21 g, 9.48 mmol) at 150 °C for 20 min, and chromatography of the product on alumina (5–10% MeOH/CH_2Cl_2), gave 7-amino-4-[(3-chlorophenyl)amino]pyrido[4,3-*d*]-pyrimidine (**7u**) (177 mg, 60%): mp (MeOH/$CHCl_3$/light petroleum) 303–305 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.92 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.08 (br s, 1 H, ArH), 7.79 (br d, J = 8.0 Hz, 1 H, ArH), 7.40 (t, J = 8.1 Hz, 1 H, ArH), 7.16 (dd, J = 7.9, 1.3 Hz, 1 H, ArH), 6.68 (s, 2 H, 7-NH_2), 6.46 (s, 1 H, H-8); ^{13}C NMR δ 161.99 (s, C-7), 157.89 (d, C-2), 157.70 (s, C-4), 154.67 (s, C-8a), 148.55 (d, C-5), 140.67 (s, C-1'), 132.74 (s, C-3'), 130.11 (d, C-5'), 123.12, 121.46, 120.36 (3 d, C-2',4',6'), 103.88 (s, C-4a), 96.98 (d, C-8). Anal. ($C_{13}H_{10}ClN_5$) C, H, N.

Similar reaction of **12** (72 mg, 0.37 mmol) and 3-iodoaniline (1.25 g, 5.71 mmol) at 160 °C for 30 min, and chromatography of the product on silica gel (5–7% MeOH/CH_2Cl_2), gave 7-amino-4-[(3-iodophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7v**) (83 mg, 61%): mp (MeOH/CH_2Cl_2) 293–296 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.84 (br s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.30 (br s, 1 H, ArH), 7.90 (dd, J = 7.9, 0.8 Hz, 1 H, ArH), 7.47 (d, J = 7.7 Hz, 1 H, ArH), 7.18 (t, J = 8.0 Hz, 1 H, ArH), 6.66 (s, 2 H, 7-NH_2), 6.46 (s, 1 H, H-8). ^{13}C NMR δ 161.93 (s, C-7), 157.89 (d, C-2), 157.63 (s, C-4), 154.66 (s, C-8a), 148.49 (d, C-5), 140.58 (s, C-1'), 131.93, 130.44, 130.06 (3 d, C-2',4',5'), 121.30 (d, C-6'), 103.85 (s, C-4a), 96.96 (d, C-8), 94.12 (s, C-3'). Anal. ($C_{13}H_{10}IN_5$) C, H, N.

Similar reaction of **12** (299 mg, 1.56 mmol) and 3-aminophenol (1.60 g, 14.7 mmol) at 160 °C for 15 min, and chromatography of the product on silica gel (9% MeOH/CH_2Cl_2), gave 7-amino-4-[(3-hydroxyphenyl)amino]pyrido[4,3-*d*]pyrimidine (**7w**) (108 mg, 18%): mp (CH_3CN/water) >270 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.69, 9.44 (2 s, 2 × 1 H, NH, OH), 9.33 (s, 1 H, H-5), 8.38 (s, 1 H, H-2), 7.37 (t, J = 2.1 Hz, 1 H, ArH), 7.21 (br d, J = 8.4 Hz, 1 H, ArH), 7.14 (t, J = 8.0 Hz, 1 H, ArH), 6.59 (s, 2 H, 7-NH_2), 6.53 (ddd, J = 7.9, 2.2, 0.8 Hz, 1 H, ArH), 6.43 (s, 1 H, H-8); ^{13}C NMR δ 161.83 (s, C-7), 158.04 (d, C-2), 157.82, 157.37 (2 s, C-4,3'), 154.70 (s, C-8a), 148.45 (d, C-5), 139.98 (s, C-1'), 129.03 (d, C-5'), 113.11, 110.85, 109.51 (3 d, C-2',4',6'), 103.97 (s, C-4a), 96.97 (d, C-8). Anal. ($C_{13}H_{11}N_5O$) C, H, N.

Similar reaction of **12** (217 mg, 1.13 mmol) and *m*-toluidine (1.50 g, 14.0 mmol) at 155 °C for 30 min, and chromatography of the product on silica gel (5% MeOH/CH_2Cl_2), gave 7-amino-4-[(3-methylphenyl)amino]pyrido[4,3-*d*]pyrimidine (**7x**) (190 mg, 67%): mp (CH_3CN/water) 272–273 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.81 (br s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.38 (s, 1 H, H-2), 7.60 (s, 2 H, ArH), 7.26 (dd, J = 8.5, 7.6 Hz, 1 H, ArH), 6.95 (d, J = 7.4 Hz, 1 H, ArH), 6.63 (s, 2 H, 7-NH_2), 6.44 (s, 1 H, H-8), 2.33 (s, 3 H, 3'-CH_3); ^{13}C NMR δ 161.90 (s, C-7), 157.94 (sd, 2 C, C-2,4), 154.34 (s, C-8a), 148.50 (d, C-5), 138.81, 137.65 (2 s, C-1',3'), 128.32 (d, C-5'), 124.58, 123.06, 119.80 (3 d, C-2',4',6'), 103.86 (s, C-4a), 96.73 (d, C-8), 21.20 (q, CH_3). Anal. ($C_{14}H_{13}N_5$) C, H, N.

Similar reaction of **12** (243 mg, 1.27 mmol) and 3-aminopyridine (1.58 g, 16.8 mmol) at 160 °C for 30 min, and chromatography of the product on silica gel (10–15% MeOH/CH_2Cl_2), gave 7-amino-4-[(3-pyridyl)amino]pyrido[4,3-*d*]-pyrimidine (**7y**) (159 mg, 53%): mp (MeOH/$CHCl_3$) 338–341 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.00 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.96 (s, 1 H, ArH), 8.42 (s, 1 H, H-2), 8.32 (d, J = 4.7 Hz, 1 H, ArH), 8.26 (br d, J = 8.1 Hz, 1 H, ArH), 7.42 (dd, J = 8.3, 4.7 Hz, 1 H, ArH), 6.69 (s, 2 H, 7-NH_2), 6.47 (s, 1 H, H-8); ^{13}C NMR δ 162.01 (s, C-7), 157.91 (sd, 2 C, C-2,4), 154.59 (s, C-8a), 148.58 (d, C-5), 144.38, 143.71 (2 d, C-2',6'), 135.81 (s, C-1'), 129.41 (d, C-4'), 123.31 (d, C-5'), 103.85 (s, C-4a), 96.89 (d, C-8). Anal. ($C_{12}H_{10}N_6$) C, H, N.

Similar reaction of **12** (244 mg, 1.27 mmol) and 3-aminoacetanilide (1.23 g, 8.20 mmol) under N_2 at 200 °C for 15 min, and direct crystallization of the product from MeOH/iPrOH/CH_2Cl_2, gave 7-amino-4-[(3-acetamidophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7z**) (174 mg, 47%): mp 318.5–320 °C. The remaining material was chromatographed on alumina (10–20% EtOH/$CHCl_3$) to give further product (78 mg, 21%). An analytical sample was obtained by recrystallization: mp (MeOH/H_2O) 329–330.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.98, 9.84 (2 s, 2 H, 2 NH), 9.35 (s, 1 H, H-5), 8.37 (s, 1 H, H-2), 8.08 (s, 1 H, ArH), 7.44 (d, J = 8.1 Hz, 1 H, ArH), 7.33 (d, J = 8.2 Hz,

1 H, ArH), 7.27 (t, J = 8.0 Hz, 1 H, ArH), 6.59 (s, 2 H, 7-NH_2), 6.43 (s, 1 H, H-8), 2.06 (s, 3 H, $COCH_3$); ^{13}C NMR δ 168.32 (s, NHCO), 161.85 (s, C-7), 158.02 (d, C-2), 157.92 (s, C-4), 154.73 (s, C-8a), 148.55 (d, C-5), 139.39, 139.17 (2 s, C-1',3'), 128.46 (d, C-5'), 117.52, 114.69, 113.41 (3 d, C-2',4',6'), 103.94 (s, C-4a), 96.95 (d, C-8), 24.04 (q, CH_3). Anal. ($C_{15}H_{14}N_6O$) C, H, N.

Similar reaction of **12** (138 mg, 0.72 mmol) and 4-aminoacetanilide (1.50 g, 10.0 mmol) under N_2 at 200 °C for 1 h, and chromatography of the product on alumina (8–10% MeOH/CH_2Cl_2), gave 7-amino-4-[(4-acetamidophenyl)amino]-pyrido[4,3-*d*]pyrimidine (**7aa**) (110 mg, 52%): mp (MeOH/CH_2Cl_2) 305–307 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.94, 9.79 (2 s, 2 H, 2 NH), 9.31 (s, 1 H, H-5), 8.34 (s, 1 H, H-2), 7.69, 7.57 (2 d, J = 8.9 Hz, 2 × 2 H, ArH), 6.57 (s, 2 H, 7-NH_2), 6.43 (s, 1 H, H-8), 2.05 (s, 3 H, $COCH_3$); ^{13}C NMR δ 168.10 (s, NHCO), 161.84 (s, C-7), 158.16 (d, C-2), 157.79 (s, C-4), 154.69 (s, C-8a), 148.33 (d, C-5), 135.41, 133.96 (2 s, C-1',4'), 123.06, 119.07 (2 d, 2 × 2 C, C-2',3',5',6'), 103.90 (s, C-4a), 97.02 (d, C-8), 23.96 (q, CH_3). Anal. ($C_{15}H_{14}N_6O$·0.5H_2O) C, H, N.

Similar reaction of **12** (500 mg, 2.60 mmol) and 2-nitrobenzylamine (1.52 g, 10.1 mmol) at 170 °C for 2 min, followed by chromatography of the product on alumina (3–7% MeOH/CH_2Cl_2) and then on silica gel (10% MeOH/CH_2Cl_2), gave 7-amino-4-[[(2-nitrophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (**8b**) (70 mg, 9%): mp (MeOH/CH_2Cl_2) 248–250 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.14 (s, 1 H, H-5), 8.94 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.20 (s, 1 H, H-2), 8.07 (dd, J = 8.1, 0.9 Hz, 1 H, ArH), 7.70 (td, J = 7.5, 1.0 Hz, 1 H, ArH), 7.58 (d, J = 7.6 Hz, 1 H, ArH), 7.54 (dd, J = 8.1, 7.4 Hz, 1 H, ArH), 6.53 (s, 2 H, 7-NH_2), 6.38 (s, 1 H, H-8), 5.01 (d, J = 5.7 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.88, 159.39 (2 s, C-4,7), 158.34 (d, C-2), 154.36 (s, C-8a), 148.13 (s, C-2'), 148.03 (d, C-5), 134.34 (s, C-1'), 133.82 (d, C-5'), 129.18, 128.21 (2 d, C-4',6'), 124.62 (d, C-3'), 103.75 (s, C-4a), 97.00 (d, C-8), 40.68 (t, NCH_2). Anal. ($C_{14}H_{12}N_6O_2$·0.25H_2O) C, H, N.

Similar reaction of **12** (228 mg, 1.19 mmol) and 3-nitrobenzylamine (0.81 g, 5.33 mmol) under N_2 at 150–160 °C for 1.5 h, and then chromatography of the product on silica gel (5–10% EtOH/EtOAc), gave 7-amino-4-[[(3-nitrophenyl)methyl]-amino]pyrido[4,3-*d*]pyrimidine (**8c**) (151 mg, 43%): mp (CH_3-CN/water) 237–240 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.11 (s, 1 H, H-5), 8.98 (t, J = 5.5 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 8.22 (br s, 1 H, ArH), 8.12 (dd, J = 8.0, 1.8 Hz, 1 H, ArH), 7.83 (d, J = 7.7 Hz, 1 H, ArH), 7.63 (t, J = 7.9 Hz, 1 H, ArH), 6.50 (s, 2 H, 7-NH_2), 6.38 (s, 1 H, H-8), 4.85 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.77, 159.26 (2 s, C-4,7), 158.32 (d, C-2), 154.28 (s, C-8a), 147.87 (d, C-5), 147.74 (s, C-3'), 141.86 (s, C-1'), 133.97 (d, C-6'), 129.78 (d, C-5'), 121.74 (d, 2 C, C-2',4'), 103.66 (s, C-4a), 96.93 (d, C-8), 42.60 (t, NCH_2); HREIMS calcd for $C_{14}H_{12}N_8O_2$ *m/z* (M^+) 296.1023, found 296.1023.

Similar reaction of **12** (220 mg, 1.15 mmol) and 4-nitrobenzylamine (0.81 g, 5.33 mmol) under N_2 at 150–160 °C for 1.5 h, and chromatography of the product on silica gel (10–20% EtOH/EtOAc) and then on alumina (5–10% EtOH/$CHCl_3$), gave 7-amino-4-[[(4-nitrophenyl)methyl]amino]pyrido[4,3-*d*]-pyrimidine (**8d**) (19 mg, 6%): mp (CH_3CN/water) 271 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.12 (s, 1 H, H-5), 8.99 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.23 (s, 1 H, H-2), 8.19 (d, J = 8.5 Hz, 2 H, ArH), 7.60 (d, J = 8.6 Hz, 2 H, ArH), 6.49 (s, 2 H, 7-NH_2), 6.37 (s, 1 H, H-8), 4.85 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.85, 159.36 (2 s, C-4,7), 158.40 (d, C-2), 154.38 (s, C-8a), 147.92 (d, C-5), 147.73 (s, C-4'), 146.43 (s, C-1'), 128.12 (d, 2 C, C-2',6'), 123.52 (d, 2 C, C-3',5'), 103.76 (s, C-4a), 97.01 (d, C-8), 42.89 (t, NCH_2). Anal. ($C_{14}H_{12}N_6O_2$) C, H, N.

Similar reaction of **12** (228 mg, 1.19 mmol) and 3-bromobenzylamine (0.84 g, 4.52 mmol) under N_2 at 140 °C for 1 h, and chromatography of the product on silica gel (2–10% EtOH/EtOAc), gave 7-amino-4-[[(3-bromophenyl)methyl]amino]-pyrido[4,3-*d*]pyrimidine (**8f**) (203 mg, 52%): mp (CH_2Cl_2/light petroleum) 208–210 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.09 (s, 1 H, H-5), 8.86 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 7.54 (s, 1 H, ArH), 7.44 (d, J = 7.8 Hz, 1 H, ArH), 7.36 (d, J = 7.6 Hz, 1 H, ArH), 7.29 (t, J = 7.7 Hz, 1 H, ArH), 6.48 (s, 2 H, 7-NH_2), 6.37 (s, 1 H, H-8), 4.73 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.72, 159.22 (2 s, C-4,7), 158.36 (d, C-2), 154.31 (s, C-8a), 147.80 (d, C-5), 142.28 (s, C-1'), 130.44, 129.82, 129.55, 126.23 (4 d, C-2',4',5',6'), 121.51 (s, C-3'), 103.68 (s, C-4a), 96.93 (d, C-8), 42.59 (t, NCH_2). Anal. ($C_{14}H_{12}BrN_5$) C, H, N.

Similar reaction of **12** (234 mg, 1.22 mmol) and 4-bromobenzylamine (0.84 g, 4.52 mmol) under N_2 at 140 °C for 1 h, and chromatography of the product on silica gel (10% EtOH/EtOAc), gave 7-amino-4-[[(4-bromophenyl)methyl]amino]-pyrido[4,3-*d*]pyrimidine (**8g**) (192 mg, 48%): mp (EtOH/EtOAc/light petroleum) 245–246.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.09 (s, 1 H, H-5), 8.87 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.25 (s, 1 H, H-2), 7.51 (d, J = 8.3 Hz, 2 H, ArH), 7.31 (d, J = 8.3 Hz, 2 H, ArH), 6.46 (s, 2 H, 7-NH_2), 6.37 (s, 1 H, H-8), 4.70 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.69, 159.22 (2 s, C-4,7), 158.37 (d, C-2), 154.32 (s, C-8a), 147.77 (d, C-5), 138.85 (s, C-1'), 131.05, 129.37 (2 d, 2 × 2 C, C-2',3',5',6'), 119.66 (s, C-4'), 103.70 (s, C-4a), 96.92 (d, C-8), 42.55 (t, NCH_2). Anal. ($C_{14}H_{12}BrN_5$·0.25EtOH) C, H, N.

Similar reaction of **12** (225 mg, 1.17 mmol) and 2-(trifluoromethyl)benzylamine (0.90 mL, 6.42 mmol) under N_2 at 150 °C for 1 h, and chromatography of the product on silica gel (5% EtOH/EtOAc), gave 7-amino-4-[[[2-(trifluoromethyl)-phenyl]methyl]amino]pyrido[4,3-*d*]pyrimidine (**8h**) (0.22 g, 59%): mp (CH_2Cl_2/light petroleum) 198–199 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.16 (s, 1 H, H-5), 8.88 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.23 (s, 1 H, H-2), 7.75 (d, J = 7.7 Hz, 1 H, ArH), 7.62 (t, J = 7.5 Hz, 1 H, ArH), 7.50 (d, J = 7.4 Hz, 1 H, ArH), 7.47 (t, J = 7.6 Hz, 1 H, ArH), 6.51 (s, 2 H, 7-NH_2), 6.39 (s, 1 H, H-8), 4.92 (d, J = 5.5 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.78, 159.41 (2 s, C-4,7), 158.39 (d, C-2), 154.33 (s, C-8a), 147.91 (d, C-5), 137.41 (s, C-1'), 132.61, 128.00, 127.22 (3 d, C-4',5',6'), 126.02 (q, J_{CF} = 30 Hz, C-2'), 125.75 (dq, J_{CF} = 5.8 Hz, C-3'), 124.49 (q, J_{CF} = 274 Hz, 2'-CF_3), 103.69 (s, C-4a), 96.94 (d, C-8), 39.89 (tq, J_{CF} = 3.4 Hz, NCH_2). Anal. ($C_{15}H_{12}F_3N_5$·0.5H_2O) C, H, N.

Similar reaction of **12** (225 mg, 1.17 mmol) and 3-(trifluoromethyl)benzylamine (0.63 mL, 4.40 mmol) under N_2 at 140 °C for 1 h, followed by chromatography of the product on silica gel (3–5% EtOH/EtOAc), gave 7-amino-4-[[[3-(trifluoromethyl)phenyl]methyl]amino]pyrido[4,3-*d*]pyrimidine (**8i**) (0.24 g, 63%): mp (CH_2Cl_2/light petroleum) 189–190.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.10 (s, 1 H, H-5), 8.92 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 7.71 (s, 1 H, ArH), 7.66 (d, J = 7.4 Hz, 1 H, ArH), 7.62 (d, J = 7.8 Hz, 1 H, ArH), 7.57 (t, J = 7.6 Hz, 1 H, ArH), 6.49 (s, 2 H, 7-NH_2), 6.38 (s, 1 H, H-8), 4.82 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.75, 159.30 (2 s, C-4,7), 158.37 (d, C-2), 154.32 (s, C-8a), 147.82 (d, C-5), 140.92 (s, C-1'), 131.35, 129.34 (2 d, C-5',6'), 128.94 (q, J_{CF} = 32 Hz, C-3'), 124.20 (q, J_{CF} = 272 Hz, 3'-CF_3), 123.71, 123.50 (2 dq, J_{CF} = 3.8 Hz, C-2',4'), 103.70 (s, C-4a), 96.96 (d, C-8), 42.80 (t, NCH_2). Anal. ($C_{15}H_{12}F_3N_5$·0.5H_2O) C, H, N.

Similar reaction of **12** (225 mg, 1.17 mmol) and 4-(trifluoromethyl)benzylamine (0.63 mL, 4.42 mmol) under N_2 at 140 °C for 1 h, and chromatography of the product on alumina (5–10% EtOH/$CHCl_3$) and then on silica gel (2–10% EtOH/EtOAc), gave 7-amino-4-[[[4-(trifluoromethyl)phenyl]methyl]-amino]pyrido[4,3-*d*]pyrimidine (**8j**) (0.21 g, 56%): mp (CH_2Cl_2/light petroleum) 208–211 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.12 (s, 1 H, H-5), 8.94 (t, J = 5.8 Hz, 1 H, N*H*CH_2), 8.24 (s, 1 H, H-2), 7.69 (d, J = 8.1 Hz, 2 H, ArH), 7.56 (d, J = 8.1 Hz, 2 H, ArH), 6.48 (s, 2 H, 7-NH_2), 6.38 (s, 1 H, H-8), 4.82 (d, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.73, 159.30 (2 s, C-4,7), 158.35 (d, C-2), 154.32 (s, C-8a), 147.80 (d, C-5), 144.34 (s, C-1'), 127.74 (d, 2 C, C-2',6'), 127.36 (q, J_{CF} = 32 Hz, C-4'), 125.10 (dq, J_{CF} = 3.8 Hz, 2 C, C-3',5'), 124.27 (q, J_{CF} = 272 Hz, 4'-CF_3), 103.70 (s, C-4a), 96.94 (d, C-8), 42.82 (t, NCH_2). Anal. ($C_{15}H_{12}F_3N_5$·0.5H_2O) C, H, N.

7-Amino-4-[[2-(trifluoromethyl)phenyl]amino]pyrido-[4,3-*d*]pyrimidine (7h): Example of Amine Hydrochloride Method. A mixture of **12** (300 mg, 1.56 mmol), 2-aminobenzotrifluoride hydrochloride (1.00 g, 5.06 mmol), and 2-aminobenzotrifluoride (2.00 g, 12.4 mmol) was stirred at 160 °C for 10 min. The resulting mixture was neutralized with excess $NaHCO_3$, dissolved in MeOH/$CHCl_3$, dried onto silica gel, and chromatographed over silica gel (eluting with a gradient of 6–7% MeOH in CH_2Cl_2) to give 7-amino-4-[[2-(trifluoromethyl)phenyl]amino]pyrido[4,3-*d*]pyrimidine (**7h**) (194 mg, 41%),

mp (MeOH/$CHCl_3$/light petroleum) 126–130 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 10.60 (br s, 1 H, NH), 9.17 (br s, 1 H, H-5), 8.13 (br s, 1 H, H-2), 7.76, 7.69 (2 m, 2 × 1 H, ArH), 7.45 (m, 2 H, ArH), 6.66 (s, 2 H, 7-NH_2), 6.36 (s, 1 H, H-8); ^{13}C NMR δ 162.07 (s, C-7), 158.75 (br d, C-2), 156.27 (br s, C-4), 152.20 (br s, C-8a), 148.76 (d, C-5), 133.09 (d, C-5′), 130.30 (br d, C-3′), 126.46 (d, C-4′), 125.85 (br d, C-6′), 123.89 (q, J_{CF} = 273 Hz, 2′-CF_3), 103.93 (br s, C-4a), 94.81 (br s, C-8). Anal. ($C_{14}H_{10}F_3N_5 \cdot 0.5H_2O$) C, H, N.

Similar reaction of **12** (390 mg, 2.03 mmol), 4-aminobenzotrifluoride hydrochloride (0.40 g, 2.02 mmol), and 4-aminobenzotrifluoride (1.61 g, 10.0 mmol) at 180 °C for 2 min, followed by neutralization of the cooled mixture with excess $NaHCO_3$, gave a product which was dissolved in MeOH/$CHCl_3$, dried onto alumina, and chromatographed over alumina (4–7% MeOH/CH_2Cl_2) to give 7-amino-4-[[4-(trifluoromethyl)phenyl]amino]pyrido[4,3-*d*]pyrimidine (**7j**) (390 mg, 63%): mp (MeOH/CH_2Cl_2) 276.5–277.5 °C. Analytically pure material was obtained by further chromatography over silica gel (5% MeOH/CH_2Cl_2): mp (MeOH/$CHCl_3$) 280–282 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.09 (s, 1 H, NH), 9.40 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 8.13 (d, J = 8.2 Hz, 2 H, ArH), 7.74 (d, J = 8.7 Hz, 2 H, ArH), 6.72 (s, 2 H, 7-NH_2), 6.40 (s, 1 H, H-8); ^{13}C NMR δ 162.03 (s, C-7), 157.77 (d, C-2), 157.71 (s, C-4), 154.71 (s, C-8a), 148.71 (d, C-5), 142.94 (s, C-1′), 125.66 (dq, J_{CF} = 3.3 Hz, 2 C, C-3′,5′), 124.49 (q, J_{CF} = 272 Hz, 4′-CF_3), 123.15 (q, J_{CF} = 32 Hz, C-4′), 121.63 (d, 2 C, C-2′,6′), 103.93 (s, C-4a), 96.93 (d, C-8); HREIMS calcd for $C_{14}H_{10}F_3N_5$ m/z (M^+) 305.0888, found 305.0870.

Similarly, a mixture of **12** (220 mg, 1.15 mmol) and 2-nitroaniline (2.00 g, 14.5 mmol) was heated to 100 °C, and then excess dry HCl gas was added to the hot stirred solution and the mixture stirred at 160 °C for 20 min. The resulting product was neutralized with excess $NaHCO_3$, dissolved in MeOH/$CHCl_3$, dried onto silica gel, and chromatographed over silica gel (2–4% MeOH/CH_2Cl_2) to give 7-amino-4-[(2-nitrophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7b**) (108 mg, 33%): mp (MeOH/CH_2Cl_2) 279–280.5 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.40 (br s, 1 H, NH), 9.24 (br s, 1 H, H-5), 8.20 (br s, 1 H, H-2), 8.12 (br s, 1 H, ArH), 8.01 (br s, 2 H, ArH), 7.75 (br s, 1 H, ArH), 6.70 (s, 2 H, 7-NH_2), 6.43 (br s, 1 H, H-8); ^{13}C NMR δ 162.14 (s, C-7), 157.62 (sd, 2 C, C-2,4), 154.65 (s, C-8a), 148.76 (d, C-5), 143.76 (s, C-2′), 133.92 (d, C-5′), 131.99 (s, C-1′), 127.32, 125.67, 124.91 (3 d, C-3′,4′,6′), 103.85 (s, C-4a), 96.85 (d, C-8). Anal. ($C_{13}H_{10}N_6O_2$) C, H, N.

7-Amino-4-[[(3-aminophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (8o) by Hydrogenation. A solution of 7-amino-4-[[(3-nitrophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (**8c**) (120 mg, 0.41 mmol) and 5% Pd/C (0.2 g) in EtOH/THF (2:1, 200 mL) was hydrogenated (60 psi/20 °C/3 h). The solution was filtered over Celite, washing thoroughly, and then dried onto alumina and chromatographed on alumina, eluting with a gradient of 7–15% EtOH in $CHCl_3$, to give 7-amino-4-[[(3-aminophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (**8o**) (33 mg, 31%): mp (MeOH/$CHCl_3$/light petroleum) 184–188 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.12 (s, 1 H, H-5), 8.80 (t, J = 5.7 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 6.95 (t, J = 7.6 Hz, 1 H, ArH), 6.45 (m, 5 H, ArH, 7-NH_2), 6.36 (s, 1 H, H-8), 4.98 (br s, 2 H, 3′-NH_2), 4.61 (d, J = 5.7 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.75, 159.40 (2 s, C-4,7), 158.57 (d, C-2), 154.35 (s, C-8a), 148.70 (s, C-3′), 147.96 (d, C-5), 139.93 (s, C-1′), 128.81 (d, C-5′), 114.66 (d, C-6′), 112.45 (d, 2 C, C-2′,4′), 103.88 (s, C-4a), 96.92 (d, C-8), 43.32 (t, NCH_2). Anal. ($C_{14}H_{14}N_6$) C, H, N.

Similar hydrogenation of **7c** as a suspension in MeOH/THF (4:1, 250 mL) over 5% Pd/C (2 g) at 60 psi and 20 °C for 1 d, followed by filtration over Celite, washing thoroughly (hot MeOH), drying onto alumina, and chromatography on alumina (4–8% EtOH/$CHCl_3$), gave 7-amino-4-[(3-aminophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7o**) (16%): mp (MeOH) 257–260 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.57 (br s, 1 H, NH), 9.30 (s, 1 H, H-5), 8.33 (s, 1 H, H-2), 7.04 (t, J = 2.0 Hz, 1 H, ArH), 6.99 (t, J = 8.0 Hz, 1 H, ArH), 6.88 (br d, J = 8.0 Hz, 1 H, ArH), 6.55 (s, 2 H, 7-NH_2), 6.40 (s, 1 H, H-8), 6.34 (dd, J = 7.9, 1.3 Hz, 1 H, ArH), 5.10 (br s, 2 H, 3′-NH_2); ^{13}C NMR δ 161.77 (s, C-7), 158.17 (d, C-2), 157.97 (s, C-4), 154.73 (s, C-8a), 148.84 (s, C-3′), 148.42 (d, C-5), 139.41 (s, C-1′), 128.70 (d, C-5′), 110.66, 110.09, 108.43 (3 d, C-2′,4′,6′), 104.03 (s, C-4a), 97.00 (d, C-8). Anal. ($C_{13}H_{12}N_6 \cdot CH_3OH$) C, H, N.

7-Amino-4-[(4-aminophenyl)amino]pyrido[4,3-*d*]pyrimidine (7p) by Hydrolysis. A solution of 7-amino-4-[(4-acetamidophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7aa**) (0.30 g, 1.02 mmol) in aqueous NaOH (2 M, 10 mL) and MeOH (10 mL) was stirred at 100 °C for 7 h. The resulting product was chromatographed over alumina, eluting with a gradient of 3–4% EtOH in $CHCl_3$, to give 7-amino-4-[(4-aminophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7p**) (86 mg, 33%): mp (CH_3CN/water) >235 °C dec; ^1H NMR [$(CD_3)_2SO$] δ 9.58 (s, 1 H, NH), 9.24 (s, 1 H, H-5), 8.25 (s, 1 H, H-2), 7.31, 6.58 (2 d, J = 8.6 Hz, 2 × 2 H, ArH), 6.48 (s, 2 H, 7-NH_2), 6.39 (s, 1 H, H-8), 5.00 (br s, 2 H, 4′-NH_2); ^{13}C NMR δ 161.68 (s, C-7), 158.37 (d, C-2), 157.98 (s, C-4), 154.68 (s, C-8a), 148.06 (d, C-5), 145.72 (s, C-4′), 127.29 (s, C-1′), 124.74 (d, 2 C, C-2′,6′), 113.60 (d, 2 C, C-3′,5′), 103.97 (s, C-4a), 97.03 (d, C-8). Anal. ($C_{13}H_{12}N_6$) C, H, N.

7-Amino-4-[(phenylmethyl)amino]pyrido[4,3-*d*]pyrimidine (8a): Example of Formic Acid Method. A mixture of the acetate salt of 3-cyano-4,6-diaminopyridine (**9**) (8.78 g, 45 mmol), formic acid (10.66 g, 0.204 mol), and benzylamine (45 mL, 0.41 mol) was heated at 200 °C under N_2 for 2 h and cooled. The resulting solid was stirred with water (500 mL) for 20 min at 0 °C, filtered, and washed with water. Recrystallization from iPrOH gave 7-amino-4-[(phenylmethyl)amino]pyrido[4,3-*d*]pyrimidine (**8a**) (8.29 g, 73%): mp 248–249 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.11 (s, 1 H, H-5), 8.85 (t, J = 5.6 Hz, 1 H, N*H*CH_2), 8.26 (s, 1 H, H-2), 7.33 (m, 4 H, ArH), 7.24 (t, J = 6.8 Hz, 1 H, ArH), 6.45 (s, 2 H, 7-NH_2), 6.37 (s, 1 H, H-8), 4.74 (d, J = 5.7 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 161.66 (s, C-7), 159.29 (s, C-4), 158.44 (d, C-2), 154.35 (s, C-8a), 147.78 (d, C-5), 139.33 (s, C-1′), 128.21, 127.15 (2 d, 2 × 2 C, C-2′,3′,5′,6′), 126.69 (d, C-4′), 103.76 (s, C-4a), 96.94 (d, C-8), 43.12 (t, NCH_2). Anal. ($C_{14}H_{13}N_5$) C, H, N.

7-Amino-4-[(4-nitrophenyl)amino]pyrido[4,3-*d*]pyrimidine (7d): Example of NaH Method. A mixture of **12** (301 mg, 1.57 mmol), 4-nitroaniline (1.52 g, 11.0 mmol), and NaH (80%, 0.33 g, 11.0 mmol) in DMF (3 mL) was stirred at 120 °C for 20 min. The resulting product was neutralized with dilute HCl and excess $NaHCO_3$ and then diluted with water and cooled to give a solid, which was collected by vacuum filtration. This material was chromatographed on neutral alumina, eluting with a gradient of 8–12% MeOH in CH_2Cl_2, to give 7-amino-4-[(4-nitrophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7d**) (150 mg, 34%): mp (CH_3CN/water) 332–335 °C; ^1H NMR [$(CD_3)_2SO$] δ 10.29 (br s, 1 H, NH), 9.42 (s, 1 H, H-5), 8.54 (s, 1 H, H-2), 8.28 (d, J = 9.2 Hz, 2 H, ArH), 8.20 (d, J = 9.2 Hz, 2 H, ArH), 6.78 (s, 2 H, 7-NH_2), 6.49 (s, 1 H, H-8); ^{13}C NMR δ 162.11 (s, C-7), 157.52 (d, C-2), 157.46 (s, C-4), 154.68 (s, C-8a), 148.93 (d, C-5), 145.88 (s, C-4′), 141.83 (s, C-1′), 124.57 (d, 2 C, C-2′,6′), 120.88 (d, 2 C, C-3′,5′), 104.00 (s, C-4a), 96.77 (d, C-8). Anal. ($C_{13}H_{10}N_6O_2$) C, H, N.

7-Amino-4-[(2-aminophenyl)amino]pyrido[4,3-*d*]pyrimidine (7n) and Its Rearrangement Product 15. A mixture of **12** (192 mg, 1.00 mmol) and 1,2-phenylenediamine (2.10 g, 19.4 mmol) was stirred at 115 °C for 10 min. The resulting product, which contained unreacted starting material by TLC, was chromatographed over silica gel (eluting with 10–11% MeOH in CH_2Cl_2) to give a 1:1 mixture of two products (76 mg, 32%). Preparative reversed-phase (C-18) HPLC (50% CH_3CN/water) gave, as the more polar component, 7-amino-4-[(2-aminophenyl)amino]pyrido[4,3-*d*]pyrimidine (**7n**) (32 mg, 13%): mp (MeOH/$CHCl_3$) 319–320 °C; ^1H NMR [$(CD_3)_2SO$] δ 9.45 (br s, 1 H, NH), 9.25 (s, 1 H, H-5), 8.19 (s, 1 H, H-2), 7.07 (d, J = 7.7 Hz, 1 H, ArH), 7.00 (t, J = 7.6 Hz, 1 H, ArH), 6.77 (d, J = 8.0 Hz, 1 H, ArH), 6.58 (t, J = 7.5 Hz, 1 H, ArH), 6.49 (s, 2 H, 7-NH_2), 6.39 (s, 1 H, H-8), 4.95 (br s, 2 H, 2′-NH_2); ^{13}C NMR δ 161.69 (s, C-7), 158.99 (s, C-4), 158.32 (d, C-2), 154.55 (s, C-8a), 148.76 (d, C-5), 144.57 (s, C-2′), 128.20, 126.99 (2 d, C-4′,6′), 123.14 (s, C-1′), 115.94, 115.63 (2 d, C-3′,5′), 104.07 (s, C-4a), 96.72 (d, C-8); HREIMS calcd for $C_{13}H_{12}N_6$ m/z [H^+] 252.1123, found 252.1107.

The less polar component from preparative reversed-phase HPLC was 2-(4′,6′-diamino-3-pyridyl)benzimidazole (**15**): mp (MeOH/$CHCl_3$) 329–334 °C; ^1H NMR [$(CD_3)_2SO$] δ 12.46 (br

s, 1 H, NH), 8.41 (s, 1 H, H-2'), 7.56 (d, J = 7.4 Hz, 1 H, ArH), 7.49 (br s, 2 H, 6'-NH_2), 7.42 (d, J = 7.5 Hz, 1 H, ArH), 7.16, 7.12 (2 t, J = 7.5 Hz, 2 × 1 H, ArH), 5.82 (s, 2 H, 4'-NH_2), 5.75 (s, 1 H, H-5'); ^{13}C NMR δ 160.39 (s, C-4'), 153.82, 151.95 (2 s, C-2,6'), 148.11 (d, C-2'), 142.91 (s, C-7a), 133.36 (s, C-3a), 121.78, 121.07, 117.50, 110.33 (4 d, C-4,5,6,7), 100.68 (s, C-1'), 89.29 (d, C-5'); HREIMS calcd for $C_{12}H_{11}N_5$ m/z (H^+) 225.1014, found 225.1011. Anal. ($C_{12}H_{11}N_5$·0.25H_2O) C, H, N.

When the condensation reaction was carried out at 150 °C for 10 min, the only product obtained after chromatography over silica gel, eluting with a gradient of 10–13% MeOH/CH_2Cl_2, was the benzimidazole **15** (51% yield).

Reaction of 12 with 3-Aminobenzonitrile. A mixture of **12** (199 mg, 1.04 mmol) and 3-aminobenzonitrile (2.04 g, 17.3 mmol) was stirred at 160 °C for 30 min. The resulting product was chromatographed over silica gel, eluting with a gradient of 9–10% MeOH/CH_2Cl_2, to give 7-amino-4-[[(3-aminophenyl)methyl]amino]pyrido[4,3-*d*]pyrimidine (**8o**) (90 mg, 33%), identical with an authentic sample by TLC and ^{1}H NMR spectrometry.

Enzyme Assay. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles by immunoaffinity chromatography as previously described,[23] and the assays were carried out as reported previously.[13] The substrate used was based on a portion of phospholipase C-γ1, 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 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 ca. 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%.

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