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2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine Analogues of Trimethoprim as Inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* Dihydrofolate Reductase

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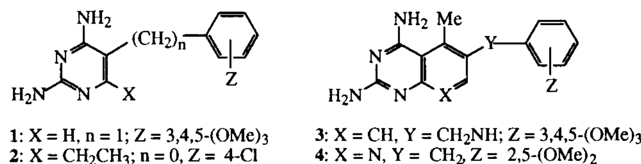
Three previously unreported (*R,S*)-2,4-diamino-5-[(3,4,5-trimethoxyphenyl)alkyl]-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidines **15a**−**c** were synthesized as analogues of trimethoprim (TMP) and were tested as inhibitors of *Pneumocystis carinii*, *Toxoplasma gondii*, and rat liver dihydrofolate reductase (DHFR). The length of the alkyl bridge between the cyclopenta[*d*]pyrimidine and trimethoxyphenyl moiety ranged from one in **15a** to three carbons in **15c**. The products were tested as competitive inhibitors of the reduction of dihydrofolate by *Pneumocystis carinii*, *Toxoplasma gondii*, and rat liver DHFR. Compounds **15a**−**c** had IC_{50} values of >32, 1.8 and 1.3 μM, respectively, against *P. carinii* DHFR, as compared to 12 μM for TMP. Against the *T. gondii* enzyme, **15a**−**c** had IC_{50} values of 21, 0.14 and 0.14 μM, respectively, as compared to 2.7 μM for TMP. Inhibitors **15b** and **15c** with two- and three-carbon bridges were significantly more potent than **15a** against all three enzymes. Unlike TMP, **15b** and **15c** were better inhibitors of the rat liver enzyme than of the microbial enzymes. The potency of **15b** and **15c** against rat liver DHFR was less than has been reported for the corresponding 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidines with a classical *p*-aminobenzoyl-L-glutamate side chain as inhibitors of bovine, murine, and human DHFR.

The opportunistic microbes *Pneumocystis carinii* and *Toxoplasma gondii* cause life-threatening illnesses in individuals with a compromised immune system, such as AIDS patients, immunosuppressed organ transplant recipients, and patients receiving cancer chemotherapy.[1−3] Despite the very promising advances made recently in the treatment of AIDS with various three-drug cocktails combining nucleosides and protease inhibitors, some important problems still remain. The new regimens are very expensive and not universally effective. In addition, there is the prospect of eventual viral resistance even with multidrug regimens. Another problem with the new treatments is their inaccessibility in underdeveloped countries where the spread of AIDS continues to accelerate at an alarming rate. Thus, until worldwide control of HIV-1 by antiviral vaccination or antiviral chemotherapy becomes possible,[4] the development of new drugs for the management of *P. carinii*, *T. gondii*, and other opportunistic infections in AIDS patients remains an important goal.



The standard clinical agents for treatment and prophylaxis of *P. carinii* pneumonia (PCP) and toxoplasmosis are the lipophilic dihydrofolate reductase (DHFR) inhibitors trimethoprim (TMP, **1**) and pyrimethamine (PM, **2**).[5−8] TMP is most often used against PCP, whereas PM is more often prescribed for toxoplasmosis. While these drugs have a high degree of binding selectivity for *P. carinii* and *T. gondii* DHFR versus mammalian DHFR, they are not very potent and are not effective when used as single agents. Thus, TMP and PM are generally used in combination with sulfa drugs, which in some patients are so poorly tolerated that treatment has to be discontinued. The lipophilic antifolates trimetrexate (TMQ, **3**) and piritrexim (PTX, **4**) have recently been used in clinical trials against *P. carinii* and *T. gondii* infections in AIDS patients.[9−11] These compounds were originally developed as anticancer agents and bind very tightly to mammalian DHFR. They are much better inhibitors of *P. carinii* and *T. gondii* DHFR than TMP or PM and thus can be used without sulfa drugs. However, they are more potent against the mammalian enzyme than against the *P. carinii* or *T. gondii* enzyme and thus have an unfavorable binding selectivity which requires use of leucovorin to avoid myelosuppression. The rationale for this host-selective protection of host tissues by leucovorin is that *P. carinii* and *T. gondii* cells lack an active transport carrier for reduced folates and thus cannot utilize leucovorin as a cofactor for one-carbon metabolism. Because they would presumably avoid the need for leucovorin, lipophilic DHFR inhibitors combining the potency of TMQ and PTX with the selectivity of TMP and PM would be very desirable.

Several classical antifolates containing 6/5 fused heterocyclic rings have recently been shown to be potent DHFR inhibitors with good anticancer activity. These

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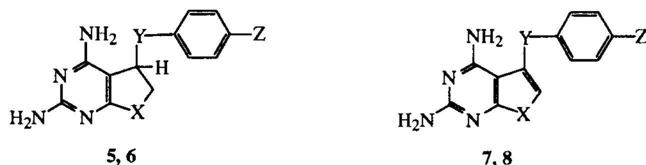
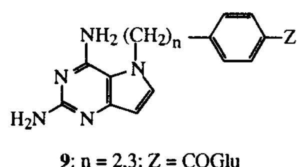
include the cyclopenta[*d*]pyrimidines **5**,[12-14] the pyrrolo[2,3-*d*]pyrimidines **6** and **7**,[15-20] the furo[2,3-*d*]pyrimidines **8**,[21] and the pyrrolo[3,2-*d*]pyrimidine **9**.[14] Unfortunately these agents, whose glutamate side chain makes them efficient substrates for the reduced folate carrier, are unlikely to be useful against *P. carinii* or *T. gondii* because these organisms cannot actively transport classical antifolates any better than they can transport the natural reduced folates.

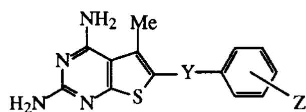


5: X = CH_2; Y = $(CH_2)_n$ (n = 2,3), CH_2CHR (R = Me, Et), CH_2NR (R = H, Me, Et); Z = COGlu
6: X = NH; Y = $(CH_2)_n$ (n = 2,3), CHMe, CH_2NR (R = H, Me); Z = COGlu
7: X = NH; Y = $(CH_2)_n$ (n = 2-4), CH_2CH_2CHMe, CH_2NR (R = H, Me); Z = COGlu
8: X = O; Y = CH_2NR (R = H, Me); Z = COGlu

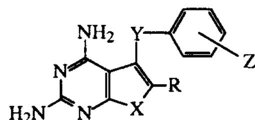


9: n = 2,3; Z = COGlu

Lipophilic nonclassical 6/5 fused antifolates, which are believed to enter cells by diffusion, have also been reported. The carbon-bridged thieno[2,3-*d*]pyrimidines **10** and **11** were the first of this type to be described.[22] Analogues containing a nitrogen atom in the bridge, the furo[2,3-*d*]pyrimidines **12**,[23] pyrrolo[2,3-*d*]pyrimidines **13**,[24] and thieno[2,3-*d*]pyrimidines **14**,[25] have also been reported. Herein we report the synthesis and DHFR



10: X = S; Y = none, CH_2, CH_2CH_2; Z = 3,4,5-$(OMe)_3$, 2,5-$(OMe)_2$



11: X = S; R = H, Y = CH_2, CH_2CH_2; Z = 2,5-$(OMe)_2$
12: X = O; R = H; Y = CH_2NH; Z = 3,4,5-$(OMe)_3$, 2,5-$(OMe)_2$, 3,4-Cl_2, 3,4,5-Cl_3
13: X = NH; R = H; Y = CH_2NH; Z = H, 4-OMe, 3,4-$(OMe)_2$, 2,5-$(OMe)_2$, 2,5-$(OEt)_2$, 3,4,5-$(OMe)_3$, 3,4-Cl_2, 3,4,5-Cl_3
14: X = S; R = H, Br; Y = CH_2NH, CH_2NMe; Z = 3,4,5-$(OMe)_3$, 2,5-$(OMe)_2$, 3,5-Cl_2-4-(1-pyrrolo)

inhibitory activity of three 6/5 fused cyclopenta[*d*]pyrimidines (**15a**–**c**) that may be viewed as TMP analogues by virtue of the fact that they all contain a 3,4,5-trimethoxyphenyl ring and lack a nitrogen atom in the bridge. To the best of our knowledge these are the first reported 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine DHFR inhibitors with a lipophilic side-chain of the TMP type, and **15a** is the first example of this class with a one-carbon bridge.

Chemistry

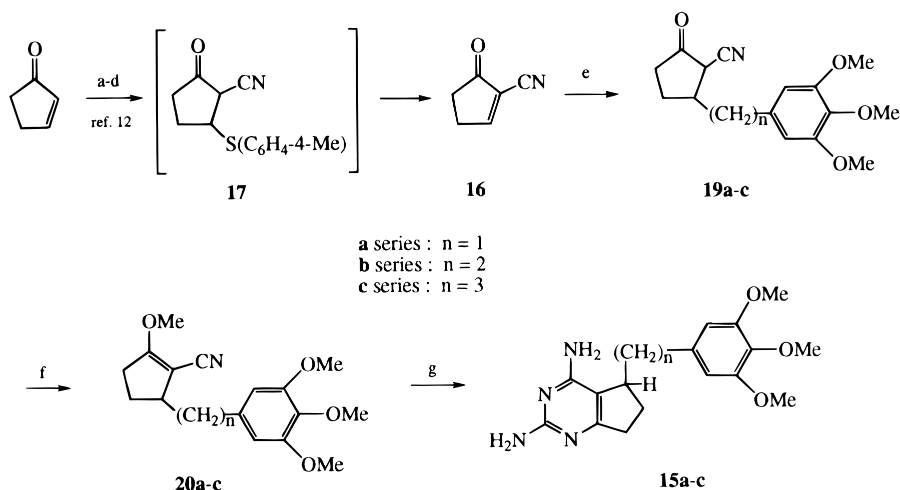
The preparation of racemic **15a**–**c**, which was adapted from the route used by Kotake and co-workers[12] to obtain the classical analogues **5**, is given in Schemes 1 and 2. The key starting material 2-cyano-2-cyclopentenone (**16**) was prepared from 2-cyclopentenone via the 3-(arylthio)-2-cyanocyclopentanone intermediate **17** as described[12] and was subjected to a carbon–carbon radical coupling reaction with aryl iodides **18a**–**c** to form the 3-aralkyl-2-cyanoketones **19a**–**c** as mixtures of diastereomers in yields ranging from 30 to 40%. Reaction of **19a**–**c** with (trimethysilyl)diazomethane in the presence of i-Pr_2NH, followed by heating with guanidine carbonate, yielded the enantiomer mixtures **20a**–**c** and **15a**–**c**, respectively. We also attempted to use this route to prepare the analogue with a trimethoxyphenyl group joined directly to the heterocycle; however, reaction of **16** with 5-iodo-1,2,3-trimethoxybenzene under the same conditions as with the other iodides gave only a complex mixture from which none of the desired product could be recovered.

Aralkyl iodides **18a**–**c** were prepared by mesylation of the corresponding alcohols **21a**–**c** followed by treatment with NaI in acetone. Athough this gave high yields of **18b** and **18c**, the benzyl analogue **18a** tended to decompose during workup and was therefore isolated in low yield by this method. The instability of **18a** is presumably due to the formation of tarry byproducts via a quinonemethide intermediate. 1-Iodo-3,4,5-trimethoxybenzene (**18d**) was prepared in 36% yield by diazotization of 3,4,5-trimethoxyaniline in a modified Sandmeyer reaction using nitrosonium tetrafluoroborate. Alcohol **21b** was obtained from 3,4,5-trimethoxybenzaldehyde by reaction with methylenetriphenylphosphorane followed by hydroboration of the Wittig product **22** with 9-BBN and oxidative workup with alkaline H_2O_2. Alcohol **21a** is commercially available, and **21c** was prepared earlier in our laboratory in connection with other work[26] by $LiAlH_4$ reduction of 3,4,5-trimethoxycinnamic acid.[27]

Dihydrofolate Reductase Inhibition

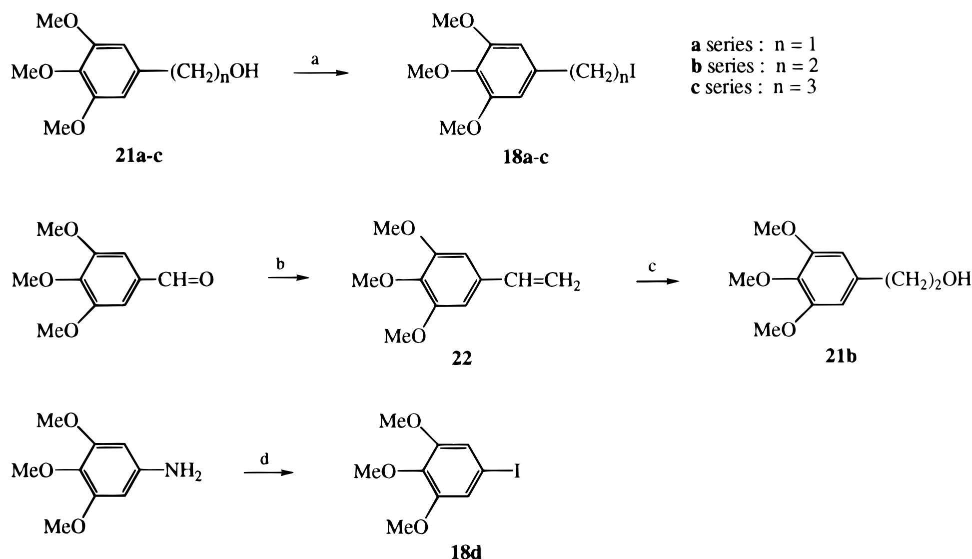
Compounds **15a**–**c**, as the nonresolved 5*R*,5*S*-enantiomers, were tested as inhibitors of DHFR from *P. carinii*, *T. gondii*, and rat liver as described in earlier publications from this program.[22,25,28-33] As shown in Table 1, the arylethyl and arylpropyl analogues **15b** and **15c** were 7-fold and 9-fold more potent than TMP against *P. carinii* DHFR, but were less potent than TMQ or PTX. Activity decreased by more than 18-fold when the bridge was shortened to a single CH_2 as in **15a**. Similarly, **15b** and **15c** were more potent than TMP against *T. gondii* DHFR, and again activity decreased, in this case by 150-fold, when the bridge was shortened to one carbon as in **15a**. The reason for the decrease in selectivity is that, unlike TMP, these compounds are good inhibitors of the rat liver enzyme as well as the parasite enzymes. The selectivity of **15b** and **15c** was ca. 10-fold greater than that of TMQ and PTX, but this

Scheme 1[a]



[a] Reagents: (a) 4-MeC_6H_4SH; (b) Me_3Al; (c) TsCN; (d) SiO_2; (e) 3,4,5-$(MeO)_3C_6H_2(CH_2)_nI$ (**18a**–**c**), $(n\text{-}Bu)_3SnH$/AIBN; (f) $TMSCHN_2$/i-Pr_2NH; (g) guanidine carbonate.

Scheme 2[a]



[a] Reagents: (a) (i) MsCl, (ii) NaI; (b) $Ph_3P{=}CH_2$; (c) (i) 9-BBN, (ii) H_2O_2/NaOH; (d) (i) $NOBF_4$, (ii) NaI.

was achieved at the expense of a ca. 20-fold loss in potency. Overall, the standard agent with which these compounds appeared to share the greatest similarity in terms of potency and selectivity was PM.

Compounds **15a**–**c** were much less potent than the corresponding 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidines with a classical *p*-aminobenzoyl-L-glutamate (PABG) side-chain. This decreased activity may reflect, at least in part, the lack of the side-chain α-COOH group, which, in addition to the length of the bridge, is known to play a role in binding to DHFR in antifolates with a PABG side-chain. The low activity of **15a**–**c** relative to analogues of the classical type may also reflect inappropriate positioning of the trimethoxyphenyl ring in the active site. The 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine **5** ($n = 3$) is reported to have IC_{50} values of 2.5 nM against bovine DHFR and 7.1 nM against DHFR from P388 mouse leukemia cells, as compared with 1.3 and 6.6 nM for MTX.[12] The corresponding IC_{50} values for **5** ($n = 2$) were reported to be 22 and 72 nM.[12] Thus, with respect to these enzymes, there was a ca. 10-fold decrease in potency between **5** ($n = 3$) and **5** ($n = 2$). In another study[14] the IC_{50} of **5** ($n = 3$) against human DHFR has been found to be 0.6 nM as compared with 0.9 nM for MTX. A modest difference in potency between **15b** and **15c** was similarly obtained in the present work using rat liver DHFR. However, in contrast to the similar potencies of **5** ($n = 3$) and MTX against bovine and murine DHFR, the potency of **15c** obtained against rat liver DFHR was ca. 100-fold lower than that previously reported for MTX against this enzyme.[28] Taken together with the previous work of our group and others,[34] these results underscore the subtlety and complexity of interspecies differences where structure–activity correlations among DHFR inhibitors are concerned.

Experimental Section

IR spectra were obtained on a Perkin-Elmer Model 781 double-beam recording spectrophotometer. ^1H NMR spectra were recorded at 60 MHz on a Varian Model EM360 spectrometer using Me_4Si as the reference or at 500 MHz on a Varian VX500 instrument. TLC analyses were done on Whatman MK6F silica gel plates, using 254-nm illumination to visualize the spots. Column chromatography was on Baker 7024 flash

Table 1. Inhibition of *P. carinii*, *T. gondii*, and Rat Liver Dihydrofolate Reductase

	IC_{50} (μM)[b]			Selectivity Ratio[c]	
compd[a]	rat liver	*P. carinii*	*T. gondii*	*P. carinii*	*T. gondii*
15a	69	>32	21	ND	3.29
15b	0.51	1.8	0.14	0.28	3.64
15c	0.22	1.3	0.14	0.17	1.57
TMP (**1**)	130	12	2.7	11	48
PM (**2**)	1.5	2.4	0.39	0.62	5.9
TMQ (**3**)	0.003	0.042	0.01	0.07	0.30
PTX (**4**)	0.015	0.031	0.017	0.048	0.088

[a] Compounds **15a** had an IC_{50} value of >32 μM against the *P. carinii* enzyme. Higher concentrations could not be tested because of insufficient aqueous solubility. Data shown for TMP, TMQ, and PTX for comparison purposes are from ref 29. The IC_{50} values for PM are slightly lower than those in ref 29 because they are an updated historical average (see footnote *b*). [b] Enzyme activity was determined spectrophotometrically at 340 nm according to a standardized and highly reliable method which has been in continuous use in this program for a number of years.[22,25,26,30−33] Each concentration of drug was tested in triplicate. As an illustration of the reproducibility of the assay, the IC_{50} values (mean ± standard error) obtained over a 5-year period by S.F.Q. with PM against rat liver, *P. carinii*, and *T. gondii* DHFR has been 2.39 ± 0.42, 0.50 ± 0.23, and 1.52 ± 0.32 μM, respectively. [c] IC_{50} (rat liver)/IC_{50} (*P. carinii* or *T. gondii*).

silica gel (40 μm particle size). Chemicals and solvents for moisture-sensitve reactions were purchased from Aldrich. Melting points were determined in Pyrex capillary tubes using a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are not corrected. Elemental analyses were performed by QTI Laboratories, Whitehouse, NJ, or Robertson Laboratories, Madison, NJ, and were within ±0.4% of theoretical values unless otherwise indicated.

(*R*,*S*)-1-Methoxy-2-cyano-3-(3,4,5-trimethoxybenzyl)cyclopentene (20a). A solution of methanesulfonyl chloride (7.69 g, 0.0672 mol) in dry CH_2Cl_2 (100 mL) was added dropwise over 20 min to a stirred solution of 3,4,5-trimethoxybenzyl alcohol (10.0 g, 0.0505 mol) and Et_3N (7.73 g, 0.0783 mol) in dry CH_2Cl_2 (300 mL) cooled in an ice bath. When addition was complete the bath was removed and stirring was continued for 1 h. The reaction mixture was washed with H_2O (500 mL), and the organic layer was dried (Na_2SO_4) and evaporated. The yellow residue was taken up in Me_2CO (70 mL), solid NaI (2.39 g, 0.0161 mol) was added, and the mixture was stirred under reflux overnight and filtered. The filter cake was rinsed several times with EtOAc, the combined filtrate and wash solution were evaporated, and the residue containing iodide **18a** was chromatographed on silica gel (1:1 EtOAc−heptane). Decomposition of the product occurred in some of the fractions during rotary evaporation with mild heating, but one fraction afforded the pure iodide as a light-yellow solid (1.26 g, 8%); IR (KBr) 2995, 2930, 1830, 1580, 1500, 1460, 1330, 1240, 1145, 1180, 990, 825, 650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.9 (s, 1H, OH), 4.4 (s, 2H, CH_2), 6.6 (s, 2H, aromatic protons). This material was used directly in the next step.

To a solution of **18a** (194 mg, 0.623 mmol) in refluxing deoxygenated anhydrous benzene (50 mL) were added dropwise at the same time a solution of **16** (100 mg, 0.934 mmol)[12] in benzene (10 mL) and a solution of n-Bu_3SnH (266 mg, 0.90 mmol) and AIBN (11 mg, 0.064 mmol) in benzene (10 mL). When the simultaneous additions were complete (1 h), the reaction mixture was heated at reflux for an additional 1 h and then cooled to room temperature and diluted with Et_2O (30 mL). The mixture was washed with saturated aqueous KF (20 mL) and then with H_2O (20 mL) and finally with brine (20 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue was chromatographed on silica gel (1:2 EtOAc−heptane, then 1:1 EtOAc−heptane) to obtain **19a** as an oil (47 mg, 26%); IR (thin film) ν 2920, 2860, 2220, 1745, 1580, 1500, 1450, 1410, 1325, 1230, 1120, 995, 900, 720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5−3.0 (m, 8H aliphatic protons), 3.8 (s, 9H, OMe), 6.4 (s, 2H, aromatic protons). The product was used directly in the next step.

A solution of **19a** (130 mg, 0.453 mmol), i-Pr_2NH (42 mg, 0.33 mmol), and (trimethylsilyl)diazomethane (1.12 mL of 2 M solution in hexanes, 2.24 mmol) in 6 mL of a 1:1 mixture of dry MeOH and MeCN was stirred at room temperature for 2.5 h. Volatiles were removed, and the residue was purified by silica gel chromatography (1:1 EtOAc−heptane) to obtain **20a** as a yellow oil (114 mg, 83%); IR (thin film) ν 2920, 2820, 2180, 1620, 1580, 1500, 1450, 1410, 1345, 1320, 1235, 1120, 1000, 970, 770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.0−3.2 (m, 7H, aliphatic protons), 3.9 (s, 9H, aromatic OMe), 4.1 (s, 3H, vinyl OMe), 6.4 (s, 2H, aromatic protons). Anal. ($C_{17}H_{21}NO_4$·$0.25H_2O$) C, H, N.

2-(3,4,5-Trimethoxyphenyl)ethyl Iodide (18b). n-BuLi (20.9 mL of 2.5 M solution in hexanes, 0.52 mol) was added dropwise over 25 min to a stirred suspension of methyltriphenylphosphonium bromide (19.1 g, 0.0535 mol) in dry THF (100 mL) in a dry ice/acetone cooling bath. When addition was complete, the dry ice/acetone bath was replaced with an ice bath, stirring was continued for 1 h, and 3,4,5-trimethoxybenzaldehyde (10.0 g, 0.0510 mol) was added dropwise over 10 min. After 0.5 h the reaction mixture was washed with aqueous NH_4Cl, the organic layer was removed, and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. Silica gel column chromatography (1:1 EtOAc−heptane) afforded 3,4,5-trimethoxystyrene (**22**) as a white solid (9.70 g, 98%); mp 152−153 °C; IR (KBr) ν 3040, 1590, 1480, 1440, 1310, 1190, 1120, 995, 745, 720, 695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.9 (s, 9H, OCH_3), 5.2 (d, J_{cis} = 11 Hz, 1H, CH=CH_2), 5.7 (d, J_{trans} = 18 Hz, 1 H, CH=CH_2), 6.5−6.9 (m, 3H, aromatic protons, *CH*=CH_2).

A 0.5 M solution of 9-BBN in THF (61 mL, 0.031 mol) was added dropwise over 15 min to a stirred solution of **22** (5.0 g, 0.026 mol) in THF (40 mL). Stirring was continued for 2 h, and H_2O (9 mL) followed by 6 N NaOH (5.1 mL) were added. This was followed by dropwise addition of 30% H_2O_2 (10.4 mL) at a rate that maintained the internal temperature at <50 °C. After the addition, stirring was continued for 1 h at room temperature, and the reaction mixture was poured into water and extracted with Et_2O. The organic layer was dried (Na_2SO_4) and evaporated. Silica gel column chromatography (1:1 EtOAc−heptane) yielded **21b** as a yellow oil (2.62 g, 48%); IR (thin film) ν 3450, 2930, 2840, 1590, 1500, 1460, 1415, 1330, 1310, 1230, 1180, 1120, 1040, 1000, 850, 780, 660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.8 (t, J = 6 Hz, 2H, PhCH_2), 3.0 (m, 12H, OMe, PhCH_2CH_2, and OH), 6.5 (s, 2H, aromatic protons).

A solution of methanesulfonyl chloride (1.63 g, 0.0143 mol) in dry CH_2Cl_2 (20 mL) was added dropwise over 20 min to a stirred solution of **21b** (2.00 g, 0.0107 mol) and Et_3N (1.68 g, 0.0166 mol) in dry CH_2Cl_2 (75 mL) cooled in an ice bath. When addition was complete, the cooling bath was removed and stirring was continued for 1 h. The reaction mixture was washed with H_2O (100 mL), and the organic layer was dried (Na_2SO_4) and evaporated to a yellow residue which was dissolved in Me_2CO (70 mL). Solid NaI (2.39 g, 0.0161 mol) was added, and the resulting mixture was heated at reflux overnight. The reaction mixture was suction filtered, and the salt cake was rinsed several times with EtOAc. Evaporation under reduced pressure followed by column chromatography of the residue on silica gel (1:1 EtOAc/heptane) gave iodide **18b** as a yellow oil (1.50 g, 47%); IR (thin film) ν 2950, 2925, 2820, 1585, 1500, 1420, 1330, 1230, 1130, 1000, 960, 820, 730 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.3 (m, 4H, CH_2CH_2), 3.9 (s, 9H, OMe), 6.4 (s, 2H, aromatic protons). Anal. ($C_{11}H_{15}IO_3$) C, H.

(*R*,*S*)-1-Methoxy-2-cyano-3-[2-(3,4,5-trimethoxyphenyl)ethyl]cyclopentene (20b). To a solution of iodide **18b** (431 mg, 1.34 mmol) in refluxing deoxygenated anhydrous benzene (50 mL) were added dropwise at the same time a solution of **16** (300 mg, 2.80 mmol) in benzene (10 mL) and a solution of n-Bu_3SnH (622 mg, 2.11 mmol) and AIBN (24 mg, 0.14 mmol) in benzene (10 mL). When the simultaneous additions were complete (70 min), the reaction was heated at reflux for an additional 25 min. After cooling to room temperature, the benzene was removed by rotary evaporation

and replaced with Et_2O (300 mL). The solution was washed with saturated aqueous KF (50 mL) and then water (2 × 50 mL). The organic layer was dried ($MgSO_4$) and evaporated. Silica column chromatography (1:2 EtOAc–heptane, then 1:1 EtOAc–heptane) gave **19b** as an oil (145 mg, 36%); IR (thin film) ν 2990, 2840, 2240, 1750, 1590, 1505, 1455, 1330, 1235, 1125, 1000, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–3.0 (m, 10H, aliphatic protons), 3.8 (s, 9H, OMe), 6.4 (s, 2H, aromatic protons). This material was used directly in the next step.

A solution of **19b** (106 mg, 0.349 mmol), i-Pr_2NH (35 mg, 0.27 mmol), and (trimethylsilyl)diazomethane (0.88 mL of 2 M solution in hexanes, 1.8 mmol) in 6 mL of a 1:1 mixture of dry MeOH and MeCN was stirred at room temperature for 6 h under N_2. Volatiles were removed, and the product was purified by silica gel column chromatography (1:3 EtOAc–hexane) to obtain **20b** as a yellow oil (85 mg, 77%); IR (thin film) ν 2915, 2815, 2200, 1620, 1585, 1500, 1450, 1415, 1350, 1235, 1125, 1005 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–3.1 (m, 9H, aliphatic protons), 3.8 (s, 9H, aromatic OMe), 4.1 (s, 3H, vinyl OMe), 6.4 (s, 2H, aromatic protons). Anal. ($C_{18}H_{23}NO_4$) C, H, N.

1-Iodo-3-(3,4,5-trimethoxyphenyl)propane (18c). A slightly warm solution of 3,4,5-trimethoxycinnamic acid (5.0 gm, 21 mmol) in THF (12 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (1.2 g, 32 mmol) in THF (30 mL) at 0 °C. After another 2 h, the reaction mixture was allowed to come to room temperature and cooled again to 0 °C while excess reagent was quenched slowly with 1:1 H_2O–THF. The reaction mixture was diluted with THF (30 mL), Celite (2.5 g) was added, and the slurry was filtered and washed with Et_2O. Evaporation of the filtrate yielded alcohol **21c** as an oil which was used directly in the next step; yield 4.4 g (92%); ^1H NMR ($CDCl_3$) δ 1.5–2.0 (m, 2H, CH_2*CH_2*CH_2), 2.5–3.0 (m, 2H, $ArCH_2$), 3.4–3.7 (m, 2H, CH_2O), 3.8 (s, 9H, OMe), 6.4 (s, 2H, aromatic protons).

A solution of **21c** (2.50 g, 0.0118 mol) and Et_3N (1.86 g, 0.0184 mol) in dry CH_2Cl_2 (75 mL) was added slowly (20 min) with cooling in an ice bath to a solution of methanesulfonyl chloride (1.80 g, 0.0158 mol) in 20 mL of dry CH_2Cl_2. After addition was complete, the cooling bath was removed and stirring was continued for 1 h. The reaction mixture was washed with H_2O (100 mL), and the organic layer was dried (Na_2SO_4) and evaporated to a yellow residue which was redissolved in Me_2CO (70 mL). Solid NaI (2.66 g, 0.0179 mol) was added, and the resulting mixture was heated at reflux overnight. The reaction mixture was filtered, and the filter cake was rinsed several times with EtOAc. The combined filtrate and wash solutions were evaporated, and the residue was purified on a silica gel column (1:1 EtOAc–heptane) to obtain iodide **18c** as a yellow oil (1.44 g, 36%); IR (thin film) ν 2990, 2930, 2830, 1585, 1505, 1460, 1420, 1350, 1330, 1235, 1130, 1005, 820, 780, 660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.0 (m, 2H, CH_2*CH_2*CH_2), 2.6 (t, J = 8 Hz, 2H, CH_2I), 3.2 (t, J = 7.5 Hz, 2H, $ArCH_2$), 3.8 (s, 9H, OMe), 6.4 (s, 2H, aromatic protons). Anal. ($C_{12}H_{17}IO_3$) C, H.

(*R,S*)-1-Methoxy-2-cyano-3-[3-(3,4,5-trimethoxyphenyl)propyl]cyclopentene (20c). To a solution of **18c** (450 mg, 1.40 mmol) in refluxing deoxygenated anhydrous benzene under N_2 were added at the same time a solution of **16** (300 mg, 2.80 mmol) in benzene (10 mL) and a solution of n-Bu_3SnH (622 mg, 2.11 mmol) and AIBN (24 mg, 0.14 mmol) in benzene (10 mL). When addition was complete (70 min), the reaction was heated at reflux for another 20 min and then cooled to room temperature and diluted with Et_2O (100 mL). The mixture was washed with saturated aqueous KF (50 mL) and H_2O (2 × 50 mL) and then dried ($MgSO_4$), and evaporated. Purification on a silica gel column (1:1 EtOAc–heptane) afforded cyano ketone **19c** as an oil (170 mg, 38%); IR (thin film) ν 2930, 2880, 2230, 1760, 1595, 1515, 1465, 1430, 1340, 1245, 1140, 1010, 920, 840, 740 cm^{-1}; ^1H NMR ($CDCl_2$) δ 1.2–3.1 (m, 12H, aliphatic protons), 3.8 (s, 9H, OMe), 6.4 (s, 2H, aromatic protons). The product was used directly in the next step.

A solution of **19c** (65 mg, 0.205 mmol), i-Pr_2NH (24 mg, 0.20 mmol), and (trimethylsilyl)diazomethane (0.24 mL of 2 M solution in hexanes, 0.47 mmol) in 15 mL of a 1:1 mixture of dry MeOH and MeCN was stirred overnight at room temperature under N_2. Analytical TLC showed that the reaction was incomplete. Another 0.24 mL of (trimethylsilyl)diazomethane and 26 mg of i-Pr_2NH were added, and stirring was resumed for 1.5 h, at which time the reaction was essentially complete. Glacial AcOH (0.5 mL) was added, the volatiles were removed, and Et_2O (20 mL) was added. The solution was washed with H_2O (20 mL) and brine (10 mL), and the organic layer was dried ($MgSO_4$) and evaporated. Purification on a silica gel column (1:1 EtOAc–hexane) afforded **20c** as a yellow oil (35 mg, 51%); IR (thin film) ν 2930, 2840, 2200, 1625, 1590, 1505, 1455, 1420, 1350, 1240, 1130, 1010 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2–3.1 (m, 9H, aliphatic protons), 3.8 (s, 9H, aromatic OMe), 4.1 (s, 3H, vinyl OMe), 6.4 (s, 2H, aromatic protons). Anal. ($C_{19}H_{25}NO_4 \cdot 0.33H_2O$) C, H, N.

1-Iodo-3,4,5-trimethoxybenzene (18d). Nitrosonium tetrafluoroborate (3.15 g, 13.5 mmol) was added to a stirred solution of 3,4,5-trimethoxyaniline (4.50 g, 12.3 mmol) in MeCN (80 mL) cooled to 0 °C in an ice bath. The cooling bath was removed, and the dark solution was left to stir overnight. Solid NaI (3.15 g, 13.5 mmol) was added, the mixture was stirred at room temperature for one more day, and the MeCN was removed by rotary evaporation. The residue was dissolved in Et_2O (200 mL), and the solution was washed with H_2O (100 mL), dried ($MgSO_4$), and evaporated. Silica gel column chromatography (1:1 EtOAc–heptane) gave the iodide as a light-yellow solid (0.73 g, 21%); mp 84.5–85.5 °C (lit.[35] 86–87 °C); IR (KBr) ν 2990, 2930, 2830, 1570, 1490, 1450, 1430, 1400, 1300, 1220, 1170, 980, 800, 760, 725 cm^{-1}; ^1H NMR ($CDCl_3$) d 3.9 (s, 9H, OMe), 6.9 (s, 2H, aromatic protons). Anal. ($C_9H_{11}IO_3$) C, H.

Attempts to couple **18d** and **16** under the same conditions as were used with **18a–c** yielded complex mixtures from which no identifiable product could be isolated.

(*R,S*)-2,4-Diamino-5-(3,4,5-trimethoxybenzyl)-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (15a). Method A. A solution of **20a** (59 mg, 0.20 mmol) and guanidine carbonate (208 mg, 1.16 mmol) in *tert*-butyl alcohol (100 mL) was heated at 160 °C (internal temperature) in a Teflon-lined stainless steel autoclave for 3 days. The reaction mixture was filtered, and the filtrate was concentrated to dryness. Preparative TLC on silica gel (1.5:8.5 MeOH–$CHCl_3$) yielded **15a** as a white solid (22 mg, 33%); mp 167 °C dec; IR (KBr) ν 3330, 3200, 2930, 1650, 1590, 1510, 1450, 1430, 1330, 1240, 1125, 1005 cm^{-1}; ^1H NMR (CD_3COCD_3) δ 0.9–3.4 (m, 7 H, aliphatic protons), 3.7 (s, 3H, OMe), 3.8 (s, 6H, OMe), 5.3 (s, 2H, NH_2), 5.5 (s, 2H, NH_2), 6.5 (s, 2H, aromatic protons). Anal. ($C_{17}H_{22}N_4O_3 \cdot 1.5H_2O$) C, H, N.

Method B. A solution of **20a** (10 mg, 0.033 mmol) and guanidine carbonate (18 mg, 0.10 mmol) in diethylene glycol monoethyl ether (2 mL) was heated at 160 °C for 2 days under N_2.

The reaction mixture mixture was cooled to room temperature, diluted with $CHCl_3$ (2 mL), and chromatographed on a column of silica gel (EtOAc, followed by 8.5:1.5 $CHCl_3$–MeOH) to obtain pure **15a** as a white solid (10 mg, 92%). This method was advantageous in that it did not require use of an autoclave.

(*R,S*)-2,4-Diamino-5-[2-(3,4,5-trimethoxyphenyl)ethyl]-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine (15b). A solution of **20b** (55 mg, 0.17 mmol) and guanidine carbonate (156 mg, 0.867 mmol) in *tert*-butyl alcohol (100 mL) was heated in an autoclave at 160 °C for 14 h. Another 156 mg (0.867 mmol) of guanidine carbonate was added, heating was resumed for 3 days, and the product was worked up as in the preceding experiment to obtain a white solid (17 mg, 29%); mp 208–209 °C; IR (KBr) ν 3360, 2920, 2830, 1625, 1585, 1500, 1440, 1415, 1230, 1120, 1000 cm^{-1}; ^1H NMR (CD_3COCD_3) δ 0.9–3.4 (m, 9H, aliphatic protons), 3.7 (s, 3H, OMe), 3.8 (s, 6H, OMe), 5.30 (s, 2H, NH_2), 5.5 (s, 2H, NH_2), 6.6 (s, 2H, aromatic protons). Anal. ($C_{18}H_{24}N_4O_3 \cdot H_2O$) C, H, N.

(*R,S*)-2,4-Diamino-5-[3-(3,4,5-trimethoxyphenyl)propyl]-5*H*-6,7-dihydrocyclopenta[*d*]pyrimidine (15c). A solution of 60 mg (0.18 mmol) of **20c** (60 mg, 0.18 mmol) and guanidine carbonate (168 mg, 0.934 mmol) in *tert*-butyl alcohol (100 mL)

was heated in an autoclave at 160 °C for 7 days. The reaction mixture was filtered, the filtrate was evaporated, and the product was worked up as in the synthesis of **15a** and **15b**. Preparative TLC on silica gel (1.5: 8.5 MeOH−$CHCl_3$) yielded a white solid (22 mg, 34%); mp 117−118 °C; IR (KBr) ν 3340, 3180, 2920, 2820, 1610, 1580, 1550, 1440, 1410, 1230, 1120, 1000 cm^{-1}; ^1H NMR (CD_3COCD_3) δ 1.1−3.2 (m, 11H, aliphatic protons), 3.7 (s, 3H, OMe), 3.8 (s, 6H, OMe), 5.5 (s, 2H, NH_2), 5.6 (s, 2H, NH_2), 6.5 (s, 2H, aromatic protons). Anal. ($C_{19}H_{26}N_4O_3\cdot H_2O$) C, H, N.

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