SYNTHESIS OF CARBON-14 LABELLED PIRITREXIM - A POTENTIAL ANTICANCER AGENT

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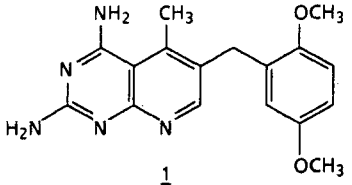
SUMMARY

Piritrexim 1 (2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]-pyrimidine) was synthesized in the [^{14}C]-labelled form with specific activity 50.8 mCi/mmol suitable for drug metabolism, transport, disposition and mechanism of action studies. The synthetic sequence involved synthesis of the novel 2-bromo-5-(2,5-dimethoxybenzyl)-4-methyl-3-pyridinecarbonitrile and condensation of this compound with [^{14}C]-guanidine hydrochloride. The radiochemical purity was >98%.

Key Words: piritrexim, PTX, 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]-pyrimidine, 301U74, anticancer agent, antipsoriasis agent.

INTRODUCTION

Piritrexim (1, 2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine, PTX, 301U74) (1,2), is a lipid-soluble dihydrofolate reductase (DHFR) inhibitor that has been evaluated as an anticancer agent, as an antipsoriasis agent and as a possible agent in the treatment of *Pneumocystis carinii* pneumonia.



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PTX enters cells rapidly by passive diffusion and is as active as methotrexate (MTX) in inhibiting DHFR and mammalian cell growth (3).

To facilitate metabolism, disposition, transport and mechanism of action studies, a carbon-14 labelled version of PTX was required. These studies required that the label be situated in the heterocyclic ring system and that the [^{14}C]-1 should have a high chemical and radiochemical purity, and a minimum specific activity of 50 mCi/mmol.

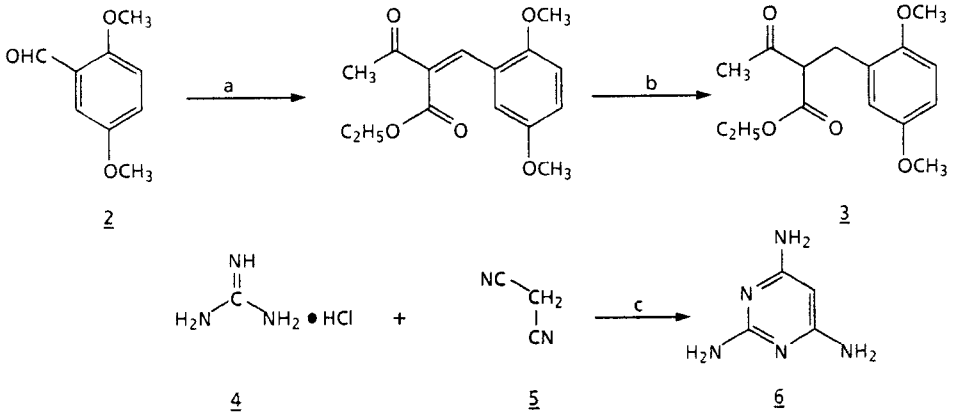
This paper describes the preparation of [^{14}C]-labelled 1 in the form of the hydrochloride salt with specific activity 50.8 mCi/mmol, and the preparation from this salt of both the free base and the isethionate salt.

RESULTS AND DISCUSSION

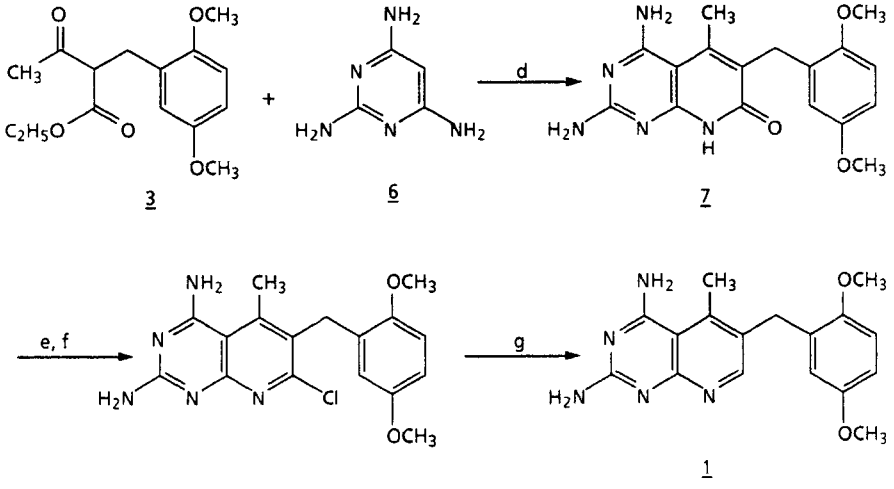
The original synthesis of 1 was accomplished using the route (1, 2) outlined in Scheme I.

In order to utilize this synthetic route to incorporate a carbon-14 label in the heterocyclic ring system, the [^{14}C]-labelled precursor would have to be guanidine hydrochloride 4, malononitrile 5, ethyl [2-^{14}C]-acetoacetate or ethyl [3-^{14}C]-acetoacetate. Use of any of these [^{14}C]-precursors involves a minimum 4-step synthetic sequence, and furthermore, at the time of this work, the overall yield of 1 from either ethyl 2-acetyl-3-(2,5-dimethoxyphenyl)propionate 3 or 2,4,6-triaminopyrimidine 6 was less than 10% (1). The above synthetic route was therefore considered to be unsuitable for the preparation of [^{14}C]-1 labelled as requested.

Scheme I



Scheme I (continued)



a) CH_3COCH_2COOEt, benzene, piperidine, AcOH, reflux ($-H_2O$)
b) EtOAc, H_2 (40 psi), 5% Pd/C
c) EtOH, NaOMe, 5°C; reflux
d) Ph_2O, 195-230°C ($-H_2O$)
e) DMF, $CHCl_3$, 0°C, $SOCl_2$
f) 7, reflux; NaOH/EtOH
g) EtOH, KOH, 5% Pd/C, H_2 (35 psi)

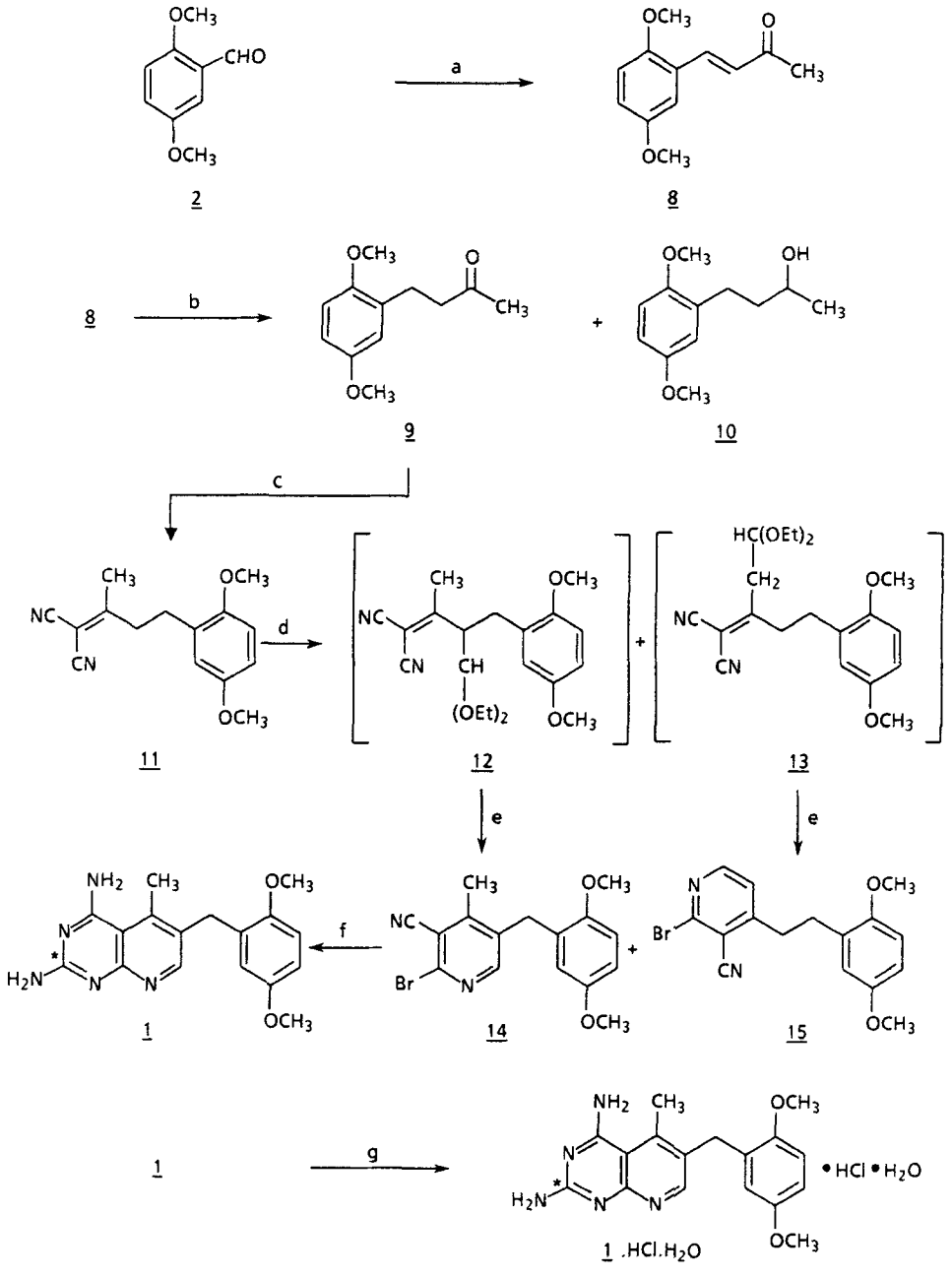
An alternative strategy for the synthesis of [^{14}C]-1 was successfully developed and is shown in Scheme II.

The key step in this synthesis is the preparation of the substituted 2-bromonicotinonitrile 14 from the acetal 12 by means of an intramolecular cyclization process first described by Bryson *et al* (4) as a general method for the synthesis of 2-halonicotinic acid derivatives.

Aldol condensation of 2,5-dimethoxybenzaldehyde 2 with excess acetone in the presence of aqueous NaOH gave an assumed quantitative yield of (*E*)-4-(2,5-dimethoxyphenyl)-3-buten-2-one 8 as a bright yellow oil (5, 6, 7).

Hydrogenation of the crude 8 in methanol over 5% Pd/C at room temperature overnight was followed by vacuum distillation to give an overall distilled yield of 59% of a mixture of 4-(2,5-dimethoxyphenyl)-2-butanone 9 (5) and the over-reduction product 4-(2,5-dimethoxyphenyl)-2-butanol 10 in a ratio of 2.8 : 1.0.

Scheme II



a) CH_3COCH_3, NaOH (aq), 25°C
b) H_2, 5% Pd/C, MeOH, 25°C
c) $NCCH_2CN$, NH_4OAc, HOAc, toluene, reflux; cyclohexane slurry
d) $(EtO)_2CHO_2CMe$, ~105°C
e) 32% HBr in HOAc, 25°C
f) *guanidine hydrochloride, NaH, *t*-BuOH, reflux
g) EtOH, H_2O, HCl, reflux

Condensation of the crude mixture of 9 and 10 with malononitrile in the presence of acetic acid and ammonium acetate in toluene at reflux with H_20 removal (Dean-Stark trap) was followed by work-up and purification to give 2-[3-(2,5-dimethoxyphenyl)-1-methylpropylidene]malononitrile 11 in 52% yield.

Optimization of conditions for alkylation of the malononitrile 11 with diethoxymethyl acetate (DEMA) proved to be difficult and required considerable development. Eventually an HPLC analysis method was developed which allowed the detection of the desired 2-[2-(diethoxymethyl)-3-(2,5-dimethoxyphenyl)-1-methylpropylidene]malononitrile 12 in the presence of the isomeric compound 2-[1-(2,2-diethoxyethyl)-3-(2,5-dimethoxyphenyl)propylidene]malononitrile 13, the starting dinitrile 11, and DEMA. After numerous attempts to effect this conversion, using HPLC analysis to monitor the progress of the reaction, the method of choice was found to be heating at 100-110°C for 3 days, and resulted in an acceptable 50% conversion to a mixture of 3 : 1 desired acetal 12 : isomeric acetal 13. Further heating results in an increase in the proportion of decomposition products rather than an increase in the yield of the desired acetal.

Earlier development work had also shown that the nitrile 14 can be separated from the isomeric nitrile 15 by chromatography and recrystallization.

Without further elaboration, the mixture of isomeric acetals was converted to the corresponding isomeric bromonicotinonitrile mixture using the general method of Bryson *et al* (4) for the synthesis of 2-halonicotinic acid derivatives by means of an intramolecular cyclization-elimination process. Treatment of a diluted solution of the mixture of acetals 12 & 13 (3 : 1) and dinitrile 11 with HBr in acetic acid followed by quench and work-up gave a crude solid consisting of 44% desired nicotinonitrile 14, 8% isomeric nicotinonitrile 15 and 47% dinitrile 11. Flash chromatography followed by recrystallization from cyclohexane resulted in the isolation of an 18% yield of white crystalline 2-bromo-5-(2,5-dimethoxybenzyl)-4-methyl-3-pyridinecarbonitrile 14 shown to be of excellent purity by TLC, HPLC and 300 MHz ^{1}H NMR.

Enough of the crude isomeric 2-bromo-4-[2-(2,5-dimethoxyphenyl)ethyl]-3-pyridinecarbonitrile 15 was isolated to allow for characterization by ^{1}H NMR.

Condensation of bromonicotinonitrile 14 with guanidine hydrochloride to produce 1 was expected to proceed in a relatively poor yield based on previous development work using a series of similar bromonicotinonitriles containing a mono- or di-halobenzyl group instead of the 2,5-dimethoxybenzyl group. An optimum yield of 38% based on bromonitrile (10.2% based on guanidine) of pyrido[2,3-*d*]-pyrimidine had been obtained when 3.5 equivalents of guanidine hydrochloride were employed. The

condensation reaction needed to be re-examined, however, with the emphasis on the yield of pyrido-[2,3-*d*]pyrimidine produced from guanidine hydrochloride as the limiting reagent. Further model studies on the available compound 2-bromo-5-(3,4-dichlorobenzyl)-4-methyl-3-pyridinecarbonitrile, using various molar ratios of guanidine : bromonicotinonitrile, eventually produced an optimum yield based on guanidine hydrochloride of 12.7% pyrido[2,3-*d*]pyrimidine when 3.7 equivalents of guanidine hydrochloride were employed per equivalent of bromonicotinonitrile.

Following the successful synthesis of 14, condensation of 14 with 3.7 equivalents guanidine hydrochloride and 3.7 equivalents NaH in *t*-butanol for 3h at reflux resulted in the eventual isolation of off-white crystalline 1.HCl.H_2O in 7.3% yield based on guanidine.HCl. The product was shown to be of excellent purity by TLC (single-spot) and HPLC. ^{1}H NMR and elemental analysis were consistent with the structure. The disappointing yield was possibly due to solubility effects, but further decreases in solvent volumes were found to be impractical.

Although the yield from guanidine hydrochloride was only 7.3%, this single-step process was greatly preferred over the original low yield multi-step process, and was used for the synthesis of [^{14}C]-piritrexim.

The radiolabelled synthesis of [^{14}C]-1.HCl.H_2O was carried out using essentially the same reaction conditions as described above, and the details are included in the experimental section.

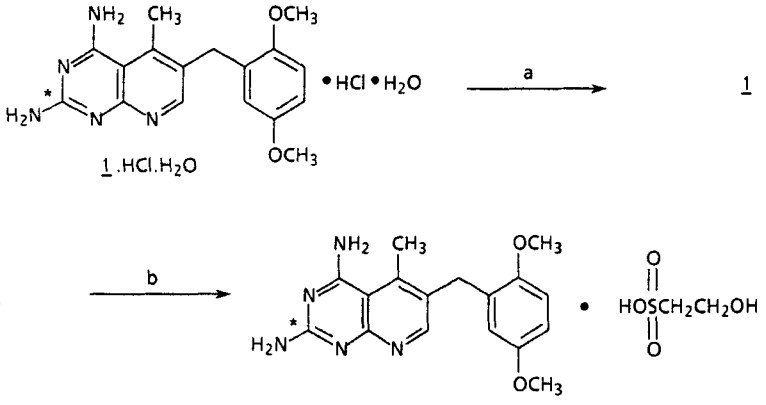
Crystallization of the [^{14}C]-1 from EtOH/H_2O/HCl gave a 23.5% yield (6.3% based on [^{14}C]-guanidine hydrochloride) of [2-^{14}C]-2,4-diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]-pyrimidine hydrochloride monohydrate (1.HCl.H_2O) with a specific activity of 50.8 mCi/mmol. The radiolabelled material was identical to an authentic sample of 1 by TLC in two systems. The radiochemical purity was > 98% by plate-scanning, and by autoradiography, scraping and scintillation counting.

Some later studies required the isolation of the [^{14}C]-piritrexim first as the free base 1, and then as the more soluble 2-hydroxyethanesulphonate salt 1.Isethionate. Conversion of 1.HCl.H_2O to free base 1 and to 1.Isethionate were routine transformations (Scheme III) and details are included in the experimental section.

The 4-year time-lapse between the synthesis of 1.HCl.H_2O and the conversion to 1.Isethionate may account for the observed decrease in radiochemical purity.

[^{14}C]-Piritrexim has been used successfully in numerous studies, including disposition and metabolism in rats (8), mice (9), and dogs (10,11), and disposition in advanced cancer patients (12).

Scheme III



1.ISETHIONATE

a) MeOH, 60°C, Et_3N, 0°C
b) MeOH, 0.43*M* isethionic acid, H_2O, Calgon NAP, 40°C

EXPERIMENTAL

[^{14}C]-Guanidine hydrochloride was obtained from California Bionuclear Corporation, Sun Valley, California. 2,5-Dimethoxybenzaldehyde was purchased from the Upjohn Company, Fine Chemical Division. Guanidine hydrochloride was purchased from R.W. Greeff & Co., Inc. Malononitrile was purchased from Eastman Kodak Company. Diethoxymethyl acetate was purchased from Aldrich Chemical Company. Sodium hydride, 61% dispersion in oil, was purchased from Ventron Corporation. Calgon NAP pulv. was purchased from Calgon Corp. All other solvents and reagents were of reagent purity and were obtained from readily available commercial sources.

High pressure liquid chromatography (HPLC) was performed using an LDC/Milton Roy mini-Pump, a Waters Lambda-Max Model 481 LC Spectrophotometer, a Hewlett-Packard 3392A Integrator, and two main sets of conditions: System A - Alltech Spherisorb Phenyl 5μ 4.6 mm x 25 cm column, mobile phase MeOH/H_2O (1/1, v/v), and flow rate 1.0 mL/min. with UV detection at 280 nm; System B - Merck LiChrosorb RP-18 5μ 4.6 mm x 25 cm column, mobile phase MeCN/H_2O (1/1, v/v), and flow rate 0.8 mL/min with UV detection at 280 nm. Thin layer chromatography (TLC) was performed on 5 x 20 cm glass plates pre-coated with 0.25 mm silica gel 60 (E. Merck). Proton NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 using a Varian FT-80A spectrometer (80 MHz) or a Varian XL-300 spectrometer (300 MHz). Melting points

were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Radiochemical purity was determined by radiochromatogram scanning of a TLC plate using a Vangard VS940 Scanner, or a Bioscan System 200 Imaging Scanner. Specific activity was determined on an accurately weighed sample by liquid scintillation counting.

(*E*)-4-(2,5-Dimethoxyphenyl)-3-buten-2-one 8

A stirred solution of 2,5-dimethoxybenzaldehyde (336 g; 2.02 mol) in acetone (672 mL; 9.18 mol) and water (185 mL) was treated dropwise with 10% (w/v) aqueous NaOH solution (55 mL) at <30°C, and the mixture was stirred at ambient temperature. The progress of the reaction was monitored by HPLC (System A: t_R 2 = 5.8 min; t_R 8 = 6.6 min; t_R intermediate alcohol = 5.0 min): after 1 h, the mixture consisted of zero 2, 73.9 % 8 and 19.0% intermediate; after 3 h, the mixture contained 86.9% 8 and 3.4% intermediate. The mixture was stored at 0°C overnight, then was warmed to 20°C and acidified to pH 3-4 with 2*N* aqueous HCl (~60 mL). The mixture was extracted with toluene (2 x 400 mL), and the toluene solution was washed with water (400 mL), saturated aqueous $NaHCO_3$ solution (400 mL), and water (400 mL). The solution was dried and evaporated to give 424 g (101.7%) of the butenone 8 (5, 6, 7) as a bright yellow oil (containing residual toluene); ^{1}H NMR (80 MHz, $CDCl_3$): δ 2.33 (s, 3 H, CH_3), 3.73 and 3.79 (2 s, 6 H, 2 OCH_3), 6.67 (d, 1 H, Ar-CH = C), 6.82-7.15 (m, 3 H, Ar-H) and 7.83 (d, 1 H, C = CH-CO).

4-(2,5-Dimethoxyphenyl)-2-butanone 9

To a mixture of the crude butenone 8 above (417.0 g theory) in MeOH (400 mL) that had been stirred at 25°C under nitrogen for 1 h, was added a suspension of 5% Pd/C (9.0 g) in cold MeOH (200 mL). After evacuation and flushing 3 times with H_2, the solution was vigorously stirred for 30 min at 0°C under H_2, then allowed to warm to ambient temperature, and stirred for 16.5 h at 25°C under H_2. After this time, H_2 absorbed was 20% greater than theory.

After removal of H_2, the reaction mixture was filtered through Celite 545 twice, and the solution was evaporated to dryness. Vacuum distillation of the residual oil (125°C /0.2 mm Hg) gave 248.2 g (58.9% weight yield) of a mixture of the butanone 9 (5) and the butanol 10 as a yellow oil; HPLC (System A): ~74% 9 (t_R = 5.7 min) and ~ 26% 10 (t_R = 5.0 min); ^{1}H NMR (80 MHz, $CDCl_3$): δ 1.14 and 1.22 (d, CH_3 of 10), and 2.13 (s, CH_3 of 9); thus the yield of 9 was 43.7%.

2-[3-(2,5-Dimethoxyphenyl)-1-methylpropylidene]malononitrile 11

To a stirred solution of the butanone-butanol mixture (2.8 : 1.0, 9/10) above (220.9 g) in toluene

(550 mL) was added malononitrile (40.7 g), NH_4OAc (5.24 g) and HOAc (19 mL). The mixture was refluxed for 5 h while H_20 (18 mL) was removed in a Dean-Stark trap. At this stage, HPLC (System A) showed 4.8% 9 (t_R = 5.7 min) and 59.3 % 11 (t_R = 6.6 min). After cooling, the toluene solution was extracted with saturated aqueous $NaHCO_3$ solution (500 mL), H_2O (500 mL), dried and concentrated to a brown oil. Et_2O (200 mL) was added and the solution stored at -15°C overnight. Evaporation of the Et_2O yielded a bright yellow solid which was allowed to stand under Et_2O-hexane (1:1, 200 mL) at 0°C overnight. After filtration, the solid was washed with Et_2O-hexane (1:1, 200 mL) and dried *in vacuo* to give 102.3 g (64.8%) of yellow solid containing 93.7% 11 by HPLC analysis. Following a slurry in cyclohexane (920 mL) and drying *in vacuo*, the propylidenemalononitrile 11 (82.5 g.; 52.2%) was obtained as a yellow solid; m.p. 73-75°C; HPLC (System A): 99.0% (t_R = 6.47 min); ^1H NMR (80 MHz, $CDCl_3$): δ 2.28 (s, 3 H, CH_3), 2.86 (s, 4 H, CH_2CH_2), 3.76 and 3.80 (2 s, 6 H, 2 OCH_3), 6.75 (m, 3 H, Ar-H). Anal. Calcd. for $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.29; N, 10.93%. Found: C, 70.11; H, 6.31; N, 10.87%.

2-Bromo-5-(2,5-dimethoxybenzyl)-4-methyl-3-pyridinecarbonitrile 14

A stirred mixture of the malononitrile 11 (25.6 g) and diethoxymethyl acetate (DEMA; 64.8 g) was heated to 105°C under nitrogen. The reaction temperature was maintained at 100-110°C by periodically boiling out low-boiling degradation products. The progress of the reaction was monitored by HPLC (System A: t_R 11 = 6.7 min; t_R 12 (desired acetal) = 8.5 min; t_R 13 (isomeric acetal) = 9.7 min): after 49 h, the mixture consisted of 58.1% 11, 28.8% 12 and 9.2% 13; after 76h, the mixture contained 49.4% 11, 32.9% 12 and 12.0% 13. After 76h, the reaction was cooled to room temperature, CH_2Cl_2 (100 mL) was added, and the resulting solution was added dropwise to stirred 32% HBr in HOAc (107 mL). After stirring for 1 h, HPLC (System A) showed that the mixture contained 39.2% 11 (t_R = 6.6 min), 42.3% desired nicotinonitrile 14 (t_R = 9.6 min) and 9.2% isomeric nicotinonitrile 15 (t_R = 8.5 min). The reaction solution was diluted with H_2O (100 mL), and after the layers were separated, the organic layer was washed with 10% aqueous Na_2CO_3 solution (150 mL) and H_2O (100 mL). The solution was dried and evaporated to give a dark brown oil which was dissolved in CH_2Cl_2 and filtered through silica gel (1 kg.) to give, after evaporation, a bright yellow solid (22.2 g) shown by HPLC (System A) to consist of 46.7% 11, 8.2% 15 and 43.9% 14.

Flash chromatography of this material in two portions on silica gel in CH_2Cl_2 eventually resulted in the isolation of three materials: a) 12.06 g of 11 (47.1% recovery) that was shown to be 98.0% pure by HPLC (System B); b) 1.10 g (3.2%) of crude yellow solid 15 shown by HPLC (System B) to consist of 82.0% 15 (t_R = 12.66 min) and 16.4% 14 (t_R = 14.65 min); ^1H NMR (300 MHz, $CDCl_3$): δ 2.95 and 3.14 (2 t, 4 H,

Ar-CH_2-CH_2-Pyr), 3.73 and 3.74 (2 s, 6 H, 2 OCH_3), 6.63 (d, 1 H, 6'-Ar-H), 6.74 (m, 2 H, 3'- and 4'-Ar-H), 7.11 (d, 1 H, 5-Pyr-H), 8.35 (d, 1 H, 6-Pyr-H); c) 8.68 g (25.0% yield) of crude 14 shown by HPLC (System B) to consist of 3.8% 11, 5.5% 15 and 90.5% 14. After recrystallization of the crude 14 from cyclohexane, the bromonicotinonitrile 14 (6.12g; 17.7%) was obtained as white crystals; m.p. 108-110°C; TLC: CH_2Cl_2, single-spot material R_f = 0.16: butanol/H_2O/HOAc (20/7/3, v/v), single-spot material R_f = 0.74; ^1H NMR (300 MH_z, $CDCl_3$): δ 2.53 (s, 3 H, CH_3), 3.73 and 3.75 (2 s, 6 H, 2 OCH_3), 3.90 (s, 2 H, Ar-CH_2), 6.51 (d, 1 H, 6'-Ar-H), 6.80 (m, 2 H, 3'- and 4'-Ar-H), 8.20 (s, 1 H, 6-Pyr-H); HPLC (System B): 97.8% (t_R = 14.67 min). Anal. Calcd. for $C_{16}H_{15}BrN_2O_2$: C, 55.35; H, 4.35; Br, 23.01; N, 8.07%. Found: C, 55.44; H, 4.40; Br, 22.95; N, 8.04%.

2,4-Diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine Hydrochloride 1.HCl.H_2O

The procedure for preparation of 1 from 14 was essentially identical to that used below in the preparation of [^{14}C]-1. From 930.9 mg of 14 and 955.3 mg of guanidine hydrochloride was obtained 277.8 mg (27.3% based on 14, 7.3% based on guanidine HCl) of off-white solid 1; ^1H NMR (80 MHz, DMSO-d_6): δ 2.66 (s, 3 H, CH_3), 3.64 and 3.74 (2 s, 6 H, 2 OCH_3), 3.99 (s, 2 H, CH_2), 6.54 (d, 1 H, 6'-Ar-H), 6.80 (d of d, 1 H, 4'-Ar-H), 6.94 (d, 1 H, 3'-Ar-H), 8.45 (s, 1 H, Pyr-H), 7.73 (bs, 2 H, 2-NH_2), 8.0 and 9.1 (bd, 2 H, 4-NH_2), 12.4 (bs, 1 H, 1-NH^+); TLC, butanol/H_2O/HOAc (20/7/3, v/v), R_f = 0.35; HPLC using Alltech Spherisorb Phenyl 5μ 4.6 mm x 25 cm column, mobile phase H_2O/MeCN/MeOH/H_2SO_4 (800/225/9/1, v/v), flow rate 1.0 mL/min, UV at 280 nm: 98.2% (t_R = 11.1 min). Anal. Calcd. for $C_{17}H_{19}N_5O_2$.HCl.H_2O: C, 53.76; H, 5.84; N, 18.44; H_2O, 4.74%. Found: C, 53.92; H, 5.88; N, 18.33; H_2O, 4.53% (Karl Fischer).

[2-^{14}C]-2,4-Diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine Hydrochloride 1.HCl.H_2O

A mixture of [^{14}C]-guanidine hydrochloride (1.337 g with specific activity ~ 50 mCi/mmol; 700 mCi; 3.73 equiv), the bromonicotinonitrile 14 (1.303 g; 1.00 equiv) and *t*-butanol (7.0 mL) was stirred vigorously for 12 min under argon in an oil-bath at room temperature. Sodium hydride (548 mg of 61% dispersion; 334 mg; 3.7 equiv) was added and the mixture was stirred for 35 min, heated to 65°C (bath temperature) during 20 min, stirred at 63-66°C for 30 min, heated to reflux during 45 min, stirred vigorously at reflux for 3 h, and allowed to cool overnight. The solid was filtered, and washed in sequence with *t*-butanol (5 mL), H_2O (7 mL) and acetone (5 mL). The resulting solid was slurried with CH_2Cl_2 (25 mL) for 60 min, then with H_2O (35 mL) for 45 min, washed with acetone (7 mL) and dried *in vacuo* to give crude [^{14}C]-1 free base as an off-white solid (482.3 mg; 39.5% yield from 14; 10.6% from [^{14}C]-guanidine.HCl). TLC using butanol/H_2O/HOAc (20/7/3, v/v) showed virtually single-spot material

with R_f = 0.43 corresponding to authentic 1. Radioactive chromatogram scanning confirmed the excellent purity with no impurities detectable within the experimental limits.

A stirred slurry of [^{14}C]-1 (482.3 mg) in EtOH (5.1 mL) and H_2O (1.6 mL) was heated to reflux under argon in an oil-bath during 45 minutes. A solution of 12*N* aqueous HCl (0.13 mL) in EtOH (1.8 mL) and H_2O (0.65 mL) was added dropwise during 30 sec. The clear yellow solution which formed was heated under reflux for 30 min then allowed to cool for 20 min. 12*N* aqueous HCl (2 drops) was added and the mixture stirred at room temperature for 60 min during which time it crystallized spontaneously. Acetone (2.6 mL) was added to increase the fluidity of the mixture which was then stirred for 2 h at 0°C.

The crystals were filtered, washed in sequence with H_2O (1.4 mL), EtOH (1.3 mL) and acetone (3.0 mL), and dried *in vacuo* at 65°C overnight. The yield of off-white crystalline solid [^{14}C]-1.HCl.H_2O was 335.6 mg (44.9 mCi; 23.5% yield from 14; 6.3% from [^{14}C]-guanidine hydrochloride) with specific activity 50.8 mCi/mmol.

TLC using butanol/H_2O/HOAc (20/7/3, v/v) showed single-spot material with R_f = 0.45 corresponding to authentic 1. No impurities were detected by radioactive scanning of the TLC plate - thus the radiochemical purity was > 98%.

TLC using $CHCl_3$/MeOH/H_2O (10/5/1, v/v) showed one major spot with R_f = 0.46 corresponding to authentic 1. Radiochemical purity by autoradiography, scraping and scintillation counting was 98.3%.

[2-^{14}C]-2,4-Diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine 1

[^{14}C]-1.HCl.H_2O synthesized above (17.5 mg; 2.33 mCi) was stirred in a mixture of MeOH (0.5 mL) and H_2O (0.5 mL) in a water-bath at 60°C under argon. A solution was made up from triethylamine (60.5 mg) in MeOH (5.0 mL), and 0.75 mL (~ 9.1 mg Et_3N; 2 equiv) was added dropwise to the reaction mixture, followed by H_2O (0.5 mL). After cooling to room temperature and standing at 0°C overnight, the mixture was filtered and the tan solid was washed with H_2O (5 mL) and dried *in vacuo* at 56°C overnight. The yield of tan solid [^{14}C]-1 free base was 12.7 mg (1.98 mCi; 84.7% yield from 1.HCl.H_2O) with specific activity assumed to be 50.8 mCi/mmol.

[2-^{14}C]-2,4-Diamino-6-(2,5-dimethoxybenzyl)-5-methylpyrido[2,3-*d*]pyrimidine 2-hydroxyethanesulphonate 1.Isethionate

[^{14}C]-1.HCl.H_2O synthesized above (40.0 mg; 5.35 mCi) was stirred in MeOH (1.0 mL) in a water-bath at 60-65°C under argon. A solution of triethylamine (12.4 mg; 1.15 equiv) in MeOH (0.6 mL) was added dropwise, and the stirred mixture was allowed to cool to room temperature. After standing at 0°C overnight, the mixture was filtered and the tan solid was air dried. The solid was stirred in MeOH (1.0 mL)

under argon, and was treated dropwise with 0.43*M* aqueous isethionic acid (0.27 mL; ≡ 14.6 mg isethionic acid; 1.1 equiv). MeOH (1.0 mL) and activated carbon (Calgon NAP pulv.; 2.5 mg) were added and the mixture was heated at 40°C for 30 min, then filtered immediately through Celite 545. Evaporation of the yellow-green solution gave a yellow crystalline solid. After slurrying with acetone (6.0 mL), the crystals were filtered, washed with acetone (2 mL), and dried *in vacuo* at 42°C overnight.

The yield of yellow crystalline solid [^{14}C]-1.Isethionate was 24.7 mg (2.78 mCi; 52.0% yield from 1.HCl.H_2O).

TLC using $CHCl_3$/MeOH/NH_4OH (85/15/0.2, v/v) showed one major spot with R_f = 0.27 corresponding to authentic 1.Isethionate, and impurities at R_f = 0.11 (trace) and R_f = 0.

Radioactive chromatogram scanning showed the radiochemical purity to be 95.0% with the balance of the radioactivity associated with the origin material.

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