SYNTHESIS OF TRIPLE ($^{13}C_2$,^{15}N), SINGLE (^{14}C), AND DOUBLE ($^{14}C_2$) LABELED TRIMETREXATE

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SUMMARY

A method was developed for the synthesis of triple ($^{13}C_2$,^{14}N) labeled trimetrexate. A method for single carbon-14 labeled synthesis is also described. Modifications of the triple labeled synthesis with carbon-14 produced a doubled carbon-14 labeled trimetrexate.

INTRODUCTION

Trimetrexate, 5-methyl-6-[[(3,4,5-trimethoxyphenyl)amino]methyl]-2,4-quinazolinediamine (CI-898), was first synthesized by Elslager *et al.*[1,2] It was found to be a potent folate antagonist and was under development for treatment of malaria. It was shown to also have antineoplastic activity in breast cancer, head and neck cancer, and non-small-cell lung cancer.[3] More recently it was found to be active in treatment of *Pneumocystis carinii* pneumonia in AIDS patients when administered in combination with leucovorin.[3]
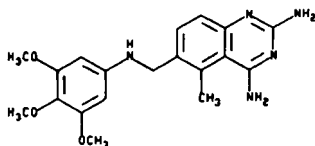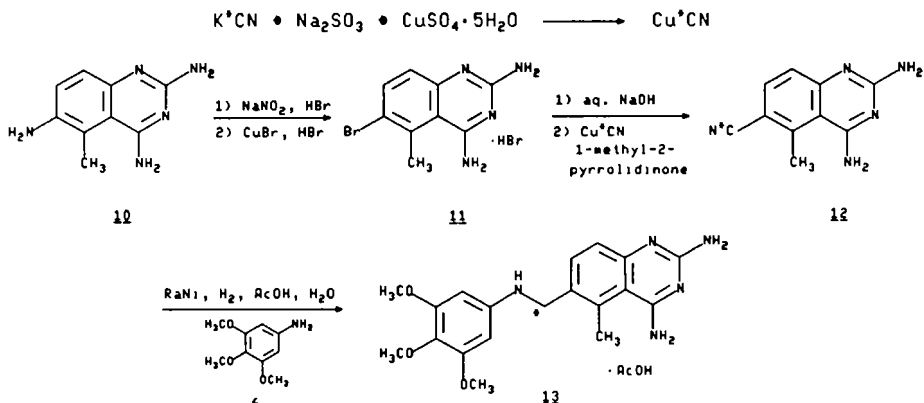

Trimetrexate (CI-898)
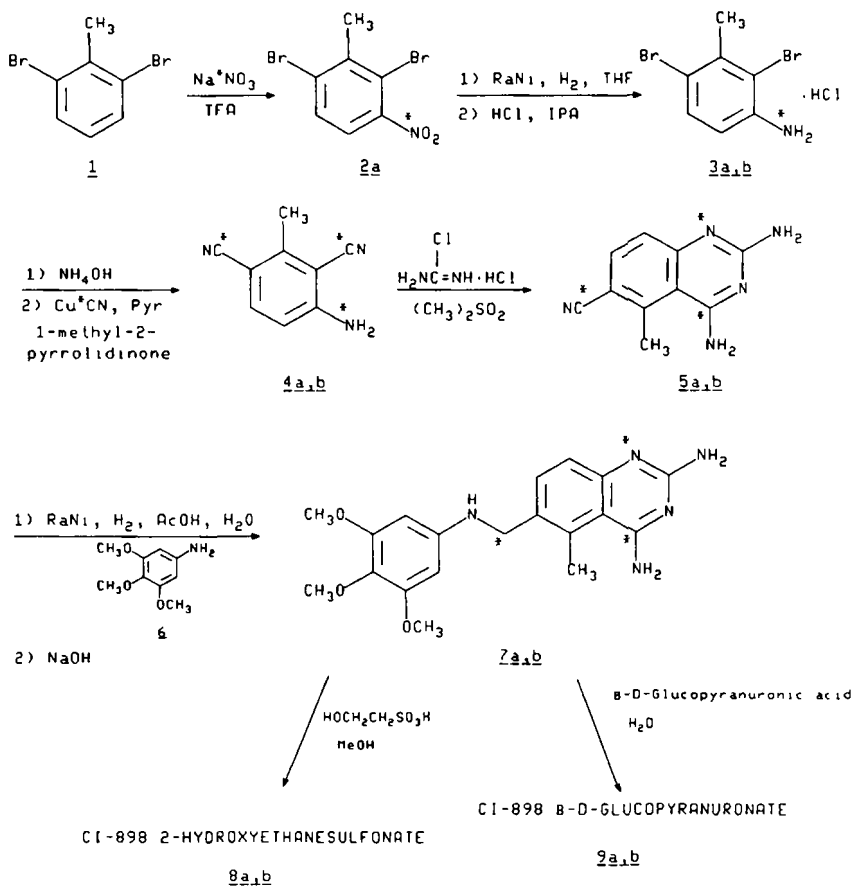
Scheme 1 a (* - denotes position of carbon-13 and nitrogen-15)
          b (* - denotes position of carbon-14, nitrogen unlabeled)

Isotope labeled material was synthesized to facilitate the study of the pharmacokinetics and metabolism of the drug. Animal studies have shown that the drug accumulated at high levels in certain organs. For human pharmacokinetic and metabolism studies this could cause unacceptable radiation doses to these organs if carbon-14 labeled drug were administered at the specific activity necessary for good detection limits. Thus it was desirable to synthesize a stable isotope labeled compound for these studies. Since the stable isotope labeled studies used mass spectrometry for detection, the preference was to label the CI-898 such that the mass was at least three units greater than that of the parent drug. The original synthesis did not lend itself readily to introduction of three labels. A new synthesis was developed which allowed the

introduction of three stable isotope labels, two carbon–13's, and one

nitrogen–15. (*Scheme 1*).

A modification of the original synthesis of trimetrexate was used for the

first carbon–14 labeled synthesis (*Scheme 2*). Incorporation of carbon–14 by

means of potassium [^{14}C]cyanide, gave a low overall yield of the drug. The

sequence for the stable isotope synthesis was also well suited for the

incorporation of carbon–14; it allowed the introduction of two labeled carbons

and thus provided higher specific activity (*Scheme 1*).

Scheme 2 (* - denotes position of carbon-14)

RESULTS AND DISCUSSION

TRIPLE ($^{13}C_2$, ^{15}N) LABELED SYNTHESIS

The new synthesis of trimetrexate started with the nitration of

2,6–dichloro– or 2,6–dibromotoluene using nitric acid/sulfuric acid conditions.

To incorporate a nitrogen–15, the nitration of 1 was done using the procedure

described by Spitzer and Stewart[4] using sodium [^{15}N]nitrate in trifluoroacetic

acid to give 1,3–dibromo–2–methyl–4–[^{15}N]nitrobenzene (2a) in a 93% yield. This

material was reduced by catalytic hydrogenation using Raney nickel in

tetrahydrofuran. A 73.4% yield of 2,4–dibromo–3–methyl–[^{15}N]benzenamine

monohydrochloride (3a) was obtained after conversion to the hydrochloride salt.

The displacement of the bromine with cyanide was done with the free base of 3a

and cuprous [^{13}C]cyanide in 1–methyl–2–pyrrolidinone based on work of Newman and

Boden[5]. 1–Methyl–2–pyrrolidinone was used because of its advantages of high

boiling point and ability to dissolve CuCN. The addition of pyridine was found
to enhance the reaction yield. The reaction of cuprous [^{13}C]cyanide and the free
base of 3a at 180-185 °C gave a 68% yield of 4-[^{15}N]amino-2-methyl-1,3-benzene-
[^{13}C$_2$]dicarbonitrile (4a). The chemical purity was 94.4% based on UV
densitometry at 253 nm. The impurity was identified as the monobromo—monocyano
derivative by ^{1}H-NMR. The reaction was treated with sodium cyanide to break up
the copper halide—nitrile complexes and remove the copper salts.[6] Carbamimidoyl
chloride hydrochloride and 4a in methyl sulfone were heated at 145 °C to give a
84.5% yield of 2,4-diamino-5-methyl-6-[1-^{15}N,4-^{13}C]quinazoline [^{13}C]carbonitrile
(5a). 5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino]-[^{13}C]methyl]-[1-^{15}N,4-^{13}C]-2,4-
quinazolinediamine, [^{13}C$_2$,^{15}N]CI-898 (7a) was formed by reductive amination of 5a
with 3,4,5-trimethoxybenzeneamine (6). The corresponding acetate salt was
isolated directly from the reaction mixture. This was converted to the free
base (7a) and recrystallized in a 52% yield. The 2-hydroxyethanesulfonate salt
(8a) was formed in an 83% yield and >99% purity. An impurity, formed as a side-
product of the reduction and also as a decomposition product, was 2,4-diamino-5-
methyl-6-[1-^{15}N,4-^{13}C]quinazoline [^{13}C]carboxaldehyde. This impurity reached an
unacceptable level during formation of the β-D-glucopyranuronate salt (9a) and
was removed by treating the solution of 9a with saturated aqueous sodium
bisulfite. Next the free base 7a was isolated, and then the salt 9a was formed
and isolated in >99% purity. The isotopic purity was determined on 5a due to
the interference of an M$^+$-1 peak in the mass spectrum of the final product. The
analysis showed 0.6 atom-% excess of one label, 1.3 atom-% excess of two labels,
and 98.1 atom-% excess of three labels.

SINGLE LABELED CARBON-14 SYNTHESIS

An intermediate, 10, in the original synthesis of trimetrexate[1] was
converted to 6-bromo-3-methyl-2,4-quinazolinediamine hydrobromide (11) by a
Sandmeyer reaction using cuprous bromide in a 70% yield. A 100 mCi lot of
potassium [^{14}C]cyanide was dissolved in an aqueous solution of sodium sulfite and
sodium hydroxide and treated with cupric sulfate pentahydrate to produce cuprous
[^{14}C]cyanide in 86% yield.[7] The conversion of 11 to 6-[^{14}C]cyano-3-methyl-2,4-

quinazolinediamine (12) used an excess of cuprous [^{14}C]cyanide resulting in a 23% radiochemical yield. The nitrile 12 seemed to decompose under the reaction conditions. The reductive coupling reaction of 12 with 3,4,5-trimethoxyaniline (6) produced 13 which after purification had a radiochemical purity of >97% and radiochemical yield of 36%. The overall yield for the entire synthesis of 13 from potassium [^{14}C]cyanide was 7%.

DOUBLED CARBON-14 LABELED SYNTHESIS

A 1.5 Ci lot of potassium [^{14}C]cyanide was converted to cuprous [^{14}C]cyanide similar to that described above. Treatment of 3b with ammonium hydroxide produced the corresponding free amine which was then reacted with the cuprous [^{14}C]cyanide in 1-methyl-2-pyrrolidinone and pyridine to give 4b in an 82% yield and a radiochemical purity of 86%. The subsequent reaction with carbamimidoyl chloride hydrochloride in methyl sulfone gave the double labeled quinazoline 5b in a 78% yield. The radiochemical purity had not changed from that of 4b.

The coupling reaction of 5b with 6 to yield trimetrexate acetate salt was run in acetic acid-water under hydrogen gas and catalyzed by Raney nickel. The product was isolated as the free base 7b by treatment with sodium hydroxide in a 55% yield.

Extensive purification was required to improve the radiochemical purity of 77%. Crystallization improved the radiochemical purity but not sufficiently. The results of flash chromatography using EtOAc:MeOH (3:2) on silica gel were satisfactory. The free base 7b had limited solubility in solvents suitable for chromatographic purification. The acetate salt was used for purification because of its greater solubility than 7b. Even so, many chromatographic runs were necessary to purify sufficient material. Reconversion of the purified product to the free base with sodium hydroxide resulted in 70.8 mCi of purified [^{14}C]CI-898, with a specific activity of 282 μCi/mg (104 mCi/mmol) and a radiochemical purity of 98.9%. Portions of 7b were used to prepare the 2-hydroxyethanesulfonic acid and β-D-glucopyranuronate salts, 8b and 9b, respectively.

EXPERIMENTAL

GENERAL

Sodium [^{15}N]nitrate was purchased from Cambridge Isotope Laboratories at 99%
isotopic abundance. Potassium [^{13}C]cyanide was purchased from Isotec Inc. at
99.1% isotopic abundance. Potassium [^{14}C]cyanide at a specific activity of
50 mCi/mmol was purchased from California Bionuclear and at a specific activity
of 56.6 mCi/mmol from American Radiolabeled Chemicals. Other starting materials
were purchased from the following suppliers and used without further
purification: 2,6-dibromotoluene, Lancaster Synthesis, Ltd; 3,4,5-trimethoxy-
aniline, Aldrich Chemical Co. or Transworld Chemical; Raney nickel, Davison
Chemical; β-D-glucopyranuronic acid, Spectrum Chemicals; methyl sulfone, Aldrich
Chemical Co.; 3-methyl-2,4,6-quinazolinetriamine, Preparations Laboratory,
Warner-Lambert/Parke-Davis. Triple distilled water was used in the purification
of the product and for the salt formations.

Cuprous [^{13}C]cyanide was made from potassium [^{13}C]cyanide using minor
modifications of the method described by Vogel[7] in an 87% yield. Carbamimidoyl
chloride hydrochloride was made by treatment of cyanamide with HCl gas in
diethyl ether.[8]

Melting points were determined on a Fisher-Johns capillary melting point
apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were determined on a
Varian XL-200 (200 MHz) spectrometer. Chemical shifts were reported in δ units
downfield from tetramethylsilane. Mass spectra were recorded on a Finnigan
Series 4000 GC-MS or a VG Trio-2 mass spectrometer interfaced with a Hewlett-
Packard 5890 gas chromatograph. Liquid scintillation counting was performed
with a Packard Tricarb 4530 liquid scintillation counter using Beckman
Readysolve or Ready Safe liquid scintillation cocktail. Thin layer
chromatography plates were analyzed for radiochemical purity (RCP) using a
Berthold LB 2832 automatic TLC-analyzer. Silica gel plates (0.25 mm) were
purchased from E. Merck. Reverse phase (C$_{18}$) LKC-18F plates (0.20 mm) were
purchased from Whatman. High pressure liquid chromatography (HPLC) was
performed using a Spectra Physics SP8700 solvent delivery system, Kratos

Spectroflow 773 variable wavelength UV detector, and Radiomatic Beta Flow 1 radioactivity flow detector. Gas chromatographic analysis was performed using a Hewlett-Packard 5790 instrument with a 0.025 mm x 30 m J & W DB-5 column, injector @ 225 °C, and FID @ 325 °C. Ultraviolet densitometry was done with the Shimadzu Thin-layer Chromato Scanner model CS-930 @ 253 nm.

TRIPLE ($^{13}C_2$,^{15}N) LABELED SYNTHESIS

1,3-Dibromo-2-methyl-4-[^{15}N]nitrobenzene (2a). Sodium [^{15}N]nitrate (25.02 g, 291 mmol) was mixed with trifluoroacetic acid (500 mL). To this mixture was added **1** (71.5 g, 286 mmol) over 40 min. The internal temperature was kept below 30 °C by controlling the rate of addition of the arene. After the addition was complete, the mixture consisted of two liquid phases and a solid phase. The mixture was stirred vigorously for 24 h. The resulting yellow solution was cooled in an ice bath. Water (750 mL) was added slowly and the product crystallized. After stirring for 15 min, the product was isolated by filtration, rinsed with cold water (2 x 300 mL), and dried *in vacuo* at room temperature to provide **2** (78.74 g, 93% yield) as a light yellow solid. mp 45-46 °C (lit.[9] for unlabeled material 51 °C). Anal. Calc. for $C_7H_5Br_2$$^{15}NO_2$: C, 28.41; H, 1.70; N, 4.73. Found: C, 28.47; H, 1.67; N, 4.61.

^{1}H-NMR (CDCl$_3$) δ 7.67(d, 1H̲); 7.40(dd, 1H̲); 2.70(s, 3H̲).

MS (EI) *m/e* (%) 298(28), 296(57), 294(29), 267(25), 265(52), 263(27), 237(19), 170(38), 168(39), 158(20), 89(100), 88(22), 77(20), 63(62), 62(33).

2,4-Dibromo-3-methyl-[^{15}N]benzenamine monohydrochloride (3a). A mixture of **2** (78.2 g, 264 mmol) and Raney nickel (10 g) in tetrahydrofuran (1.6 L) was placed in a Parr apparatus under hydrogen (31 psi) at room temperature. After shaking for 19 h, additional Raney nickel (10 g) was added. After 22 h, GC (isothermal @ 180 °C) showed the disappearance of **2a**. The reaction was filtered through Celite and the solvent evaporated *in vacuo* to an oil which solidified upon standing. The solid was dissolved in hot isopropanol (650 mL) and filtered through Celite to remove some dark insoluble material. The filtrate was cooled in an ice bath and treated with HCl gas. The resulting salt was collected and washed with isopropanol. The off-white solid was dried *in vacuo* at room

temperature to provide <u>3a</u> (58.6 g, 73.4% yield). TLC of free base: R_f=0.42,

silica gel, hexane:EtOAc 4:1. Anal. Calc. for $C_7H_7Br_2{}^{15}N \cdot HCl$: C, 27.80;

H, 2.67; N, 4.63. Found: C, 27.99, H, 2.65; N, 4.84.

^{1}H-NMR (DMSO-d_6) δ 7.35(d, 1$\underline{H}$); 6.83(s, 3$\underline{H}$); 6.79(d, 1$\underline{H}$); 2.5(s, 3H).

MS(EI) m/e(%) 268(49), 266(100), 264(52), 187(29), 185(32), 106(37), 105(43),

78(16), 77(27), 51(22).

4-[^{15}N]Amino-2-methyl-1,3-benzene-[^{13}C$_2$]dicarbonitrile (4a). The free base of <u>3a</u>

was isolated by treatment of the HCl salt (58.4 g, 193 mmol) in MeOH (600 mL)

with concentrated ammonium hydroxide. The resulting solution was evaporated *in*

vacuo to give a quantitative recovery of the free base. A mixture of the free

base, cuprous [^{13}C]cyanide (38.8 g, 429 mmol), pyridine (25 mL) and 1-methyl-2-

pyrrolidinone (105 mL) was heated at 180–185 °C for 75 min. The reaction

mixture was poured into ice–NH$_4$OH and stirred for one hour. The resulting solid

was isolated by filtration. The solid was stirred for 16 h in a solution of

NaCN (32 g) and water (300 mL) to facilitate the removal of the copper salts.

The solid was filtered, washed with NH$_4$OH and dried *in vacuo* at 70 °C to give the

crude product. The material was passed through a column of silica gel by

continuous elution with refluxing CH$_2$Cl$_2$. The volume of the solvent was reduced

to 150 mL and the crystallized material was collected. Vacuum drying at 50 °C

overnight gave <u>4a</u> (20.97 g, 68% yield). TLC: R_f=0.17, chemical purity 94.4%

silica gel, hexane:EtOAc (2:1).   Anal. Calc. for $C_7{}^{13}C_2H_7N_2{}^{15}N$: C, 67.50;

H, 4.41; N, 26.24. Found: C, 65.78, H, 4.25; N, 25.65. ^{1}H-NMR(DMSO-d_6)

δ 7.55(m, 1$\underline{H}$); 6.95(d, 2$\underline{H}$); 6.69(d, 1$\underline{H}$); 2.48(s, 3$\underline{H}$). ^{13}C-NMR(DMSO-d_6) δ 118,

116. MS(EI) m/e(%) 161(41), 160(100), 159(32), 132(5), 131(7).

2,4-Diamino-5-methyl-6-[1-^{15}N, 4-^{13}C]quinazoline-[^{13}C]carbonitrile (5a). Methyl

sulfone (80 g) was heated to 140 °C. A mixture of <u>4a</u> (20.8 g, 130 mmol) and

carbamimidoyl chloride hydrochloride (24 g, 210 mmol) was added to the melted

solvent. The mixture was heated at 145 °C forming a complete solution after 20

min. After 45 min total reaction time the reaction was poured into ice–water

(1.5 L) and stirred for 4 h. The mixture was filtered through Celite to remove

a small amount of flocculent material. The filtrate was treated with 2 N NaOH

to pH–11. The resulting suspension was stirred for 45 min, filtered, and air

dried overnight. The product was purified by trituration with MeOH (200 mL), then water (350 mL), and again with MeOH (200 mL). The isolated material was dried *in vacuo* at 70 °C to give 5a as a hydrate (25.47 g, 84.5% yield). TLC: R_f=0.42, chemical purity 95 %, silica gel, CH_2Cl_2:MeOH 9:1. Anal. Calc. for $C_8{}^{13}C_2H_9N_4{}^{15}N\cdot0.9\ H_2O$: C, 55.00; H, 4.95; N, 32.07. Found: C, 53.92, H, 4.75; N, 31.94. ^1H-NMR(DMSO-d_6) δ 7.63(d, 1H); 7.09(d, 3H); 6.50(s, 2H); 2.88(s, 3H). ^{13}C-NMR(DMSO-d_6) δ 163, 119.

MS(EI) m/e(%) 203(10), 202(100), 201(28), 184(10), 159(12), 131(10).

5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{13}C]methyl]-[1-^{15}N,4-^{13}C]-2,4-quinazolinediamine, [$^{13}C_2$,^{15}N]CI-898 (7a). A mixture of 5a (25.6 g, 127 mmol), 6 (35.15 g, 192 mmol), and Raney nickel (30 g) in water (150 mL) and acetic acid (850 mL) was shaken on a Parr apparatus under hydrogen (50 psi). After 176 min HPLC analysis showed no 5a. The mixture was filtered through Celite and the filtrate was evaporated *in vacuo*. The resulting residue was slurried in toluene and evaporated. This procedure was repeated twice to aid in removing water and AcOH. The dry solid was heated in toluene (500 mL), filtered, rinsed with diethyl ether (2 x 500 mL) and dried *in vacuo* to give the crude acetate salt. The product was again heated on a steam bath in toluene (500 mL) for 60 min, filtered, rinsed with diethyl ether and air dried. The material was suspended in water (500 mL) and 2 *M* NaOH (100 mL) was added. The slurry was stirred under N_2 for 17 h. The solid was isolated by filtration, rinsed with 1 *M* NaOH and water, and air dried. The solid (50.4 g) was recrystallized (charcoal) from 60% ethanol/water (3.5 L) to give 7a (24.24 g, 52% yield) in three crops. HPLC: t_R=16.7 min, chemical purity >98.5%. Alltech C_{18}, 10μ, 4.6 mm ID x 25 cm, 70:30 CH_3CN:(14 parts AcOH, 1.54 g sodium octanesulfonate in 1 L water), flow 1.0 mL/min, uv @ 254 nm. Anal. Calc. for $C_{17}{}^{13}C_2H_{23}N_4{}^{15}NO_3\cdot1.2\ H_2O$: C, 57.92; H, 6.49; N, 17.77. Found: C, 58.26, H, 6.38; N, 17.99. ^1H-NMR(DMSO-d_6) δ 7.42(m, 1H); 7.03(d, 1H); 6.77(s,2H); 5.93(s,4H); 5.67(s, 1H); 4.17(dd, 2H); 3.67(s, 6H); 3.51(s, 3H); 2.66(s, 3H). IR(KBr) cm^{-1} 3419, 1609, 1566, 1542, 1508, 1452, 1414, 1238, 1128, 1000. MS(EI) m/e(%) 372(26), 191(10), 190(100), 183(14), 173(13), 168(10), 148(11).

5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{13}C]methyl]-[1-^{15}N,4-^{13}C]-2,4-
quinazolinediamine 2-hydroxyethanesulfonate, [^{13}C$_2$,^{15}N]CI-898 2-hydroxy-
ethanesulfonate (8a). 2-Hydroxyethanesulfonic acid (30 mL, 51 mmol, 1.72 M in
MeOH) was added to a suspension of 7a in 100 mL of MeOH. After stirring for
90 min, the solution was cooled and the resulting solid isolated by filtration.
After drying in a high vacuum at room temperature for 2.5 days, the salt 8a
(11.88 g, 83% yield) was isolated. mp 192–193 °C; TLC: R_f=0.58, silica gel,
isopropanol:water:AcOH (3:1:1); R_f=0.56, C$_{18}$, MeOH:0.5 M NaCl (3:2).
HPLC: t_R=17.3 min, chemical purity 99.75%. Anal. Calc. for
C$_{17}$^{13}C$_2$H$_{23}$N$_4$^{15}NO$_3$·C$_2$H$_6$SO$_4$: C, 50.60; H, 5.86; N, 14.05; S, 6.43. Found: C, 50.58,
H, 5.85; N, 14.13; S, 6.69. ^{1}H-NMR(DMSO-d_6) δ 12.13(s, 1H); 11.95(s, 1H);
9.01(s, 1H); 7.70(m, 3H); 7.24(d, 1H); 5.90(s, 3H); 4.50(s, 1H); 4.28(dd, 2H);
3.65(m, 7H); 3.50(s, 3H); 2.66(m, 4H). ^{13}C-NMR(DMSO-d_6) δ 164, 45.
IR(KBr) cm^{-1} 3383, 3174, 2938, 1654, 1612, 1510, 1458, 1233, 1206, 1185, 1160,
1149, 1128, 1032. MS(EI) *m/e*(%) 372(32), 190(100), 183(11), 173(11), 168(10).
5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{13}C]methyl]-[1-^{15}N,4-^{13}C]-2,4-
quinazolinediamine β-D-glucopyranuronate, [^{13}C$_2$,^{15}N]CI-898 β-D-
glucopyranuronate(9a). β-D-Glucopyranuronic acid (4.32 g, 22.3 mmol) was
dissolved in water (100 mL) and purged with Ar. To the solution was added a
suspension of 7a (7.95 g, 21.4 mmol) in water (250 mL). A solution formed in
15 min. Saturated aqueous sodium bisulfite (10 mL) was added to the solution
and stirred for 90 min. The pH was adjusted to 11 with 3 M NaOH (20 mL) and
stirred for 30 min. The resulting precipitate of 7a was filtered, washed with
water (3 x 300 mL) and dried *in vacuo* at room temperature for 48 h. A portion
of 7a (5.35 g, 14.4 mmol) was transferred as a water (50 mL) suspension to an Ar
purged solution of β-D-glucopyranuronic acid (2.82 g 14.5 mmol) in water(30 mL).
After stirring for 30 min, the mixture was filtered through Celite.
The filtrate was lyophilized over 25 h to give 9a (8.26 g, 98% recovery).
HPLC analysis showed the purity was >98%. Repeating the conversion to 7a then
back to 9a two more times resulted in material of >99% purity (3.94 g).
TLC: R_f=0.18, silica gel, EtOAc:EtOH:Et$_3$N (50:50:1); R_f=0.52,
isopropanol:water:AcOH (3:1:1). HPLC: t_R=17.3 min, chemical purity >99.4%.

Anal. Calc. for $C_{17}{}^{13}C_2H_{23}N_4{}^{15}NO_3 \cdot C_6H_{10}O_7 \cdot 1.2$ H$_2$0: C, 51.05; H, 6.07; N, 11.91.

Found: C, 49.57, H, 5.96; N, 11.54. ^{1}H-NMR(DMSO-d_6) δ 7.65(s, 1$\underline{H}$); 7.61(m, 1$\underline{H}$);

7.32(s, 2$\underline{H}$); 7.14(d, 1$\underline{H}$); 5.92(s, 2$\underline{H}$); 5.77(s, 1$\underline{H}$); 4.93(d, 1$\underline{H}$); 4.44(s, 1$\underline{H}$);

4.12(dd, 1$\underline{H}$); 3.98(s, 1$\underline{H}$); 3.67(s, 3$\underline{H}$); 3.52(s, 3$\underline{H}$); 3.43(t, 1$\underline{H}$); 3.26(, 1$\underline{H}$);

3.15(t, 1$\underline{H}$); 2.93(t, 1$\underline{H}$); 2.69(s, 3$\underline{H}$). ^{13}C-NMR(DMSO-d_6) δ 163, 46.

IR(KBr) 3361, 3340, 3229, 2933, 1661, 1509, 1456, 1427, 1413, 1234, 1127.

MS(EI) *m/e*(%) 372(20), 190(100), 183(13), 173(11), 168(12), 148(7).

SINGLE CARBON-14 LABELED SYNTHESIS

<u>6-Bromo-3-methyl-2,4-quinazolinediamine hydrobromide (11)</u>. To a solution of
3-methyl-2,4,6-quinazolinetriamine (1.245 g, 5.00 mmol) in 49% hydrobromic acid:
water (2.5 ml:7.5 ml) at 10-15 °C, a chilled aqueous solution (5 mL) of sodium
nitrite (380 mg, 5.50 mmol) was added dropwise. The solution was stirred for
10 min at 10-15 °C until it became homogeneous. To prepare a cuprous bromide
solution, cupric sulfate pentahydrate (1.37 g, 5.50 mmol) and sodium bromide
(2.06 g, 20 mmol) were dissolved in hot water (5 mL). To this solution was
added a solution of sodium metabisulfite (270 mg, 1.40 mmol) and sodium
hydroxide (195 mg) in water (5 mL). The bluish-green solution was maintained at
about 75 °C while the cold diazonium solution was added dropwise. After the
solution was thoroughly mixed, 49% hydrobromic acid was added and the mixture
was stirred at 75 °C for one hour. The resulting yellow solid was filtered,
washed with water, and the crude product was recrystallized from 15% acetic acid
to afford <u>11</u> (1.17 g, 70% yield): mp 350 °C(dec.).

Anal. calcd. for $C_9H_{10}N_4Br_2$: C, 32.36; H, 3.02; N, 16.77; Br, 47.85.

Found: C, 32.33; H, 2.97; N, 16.73; Br, 47.61.

MS *m/e* 254(M$^+$ Br^{81}), 252(M$^+$ Br^{79}), 173(M$^+$-Br).

<u>Cuprous [^{14}C]cyanide</u>. A solution of potassium [^{14}C]cyanide (100 mCi, 130 mg),
potassium cyanide (504 mg, 7.75 mmol) and sodium sulfite (1.16 g, 9.2 mmol) in
0.5 *M* sodium hydroxide (69 mL) was treated with 6 *M* sulfuric acid to the
phenolphthalein end point. This solution was slowly added to a cooled (0 °C)
solution of cupric sulfate pentahydrate (3.375 g, 13.5 mmol) in water (22 mL)
containing 12 *M* sulfuric acid (22 drops). The mixture was stirred at room

temperature for 3 h. After cooling to 0 °C, the colorless cuprous [^{14}C]cyanide
was filtered, washed with water (80 mL) and dried to afford 725 mg (86 mCi,
86% yield).

2,4-Diamino-3-methyl-6-[^{14}C]quinazolinecarbonitrile (12). The free base of 11
was prepared from the hydrobromide salt by suspending the salt in 1 M NaOH for
1 h, filtering and washing with water. The product (777 mg, 3.07 mmol) was
added to a solution of cuprous [^{14}C]cyanide (725 mg, 8.10 mmol, 86 mCi) in
1-methyl-2-pyrrolidinone at 120 °C. The heterogeneous solution was stirred at
170 °C for 22 h. The reaction mixture was poured into an aqueous potassium
cyanide solution (1 g/50 mL). The mixture was cooled to 0 °C, filtered and
washed with water. The resulting dark brown solid was partially dissolved in
hot dimethylformamide and purified by dry column chromatography on silica gel
with ethyl acetate:methanol (75:25) as the eluent. The yield of partially
purified 12 was 446 mg, 20 mCi (73% chemical yield, 23% radiochemical yield).
TLC: R$_f$-0.38; RCP-85% silica gel EtOAc:MeOH (75:25).

5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{14}C]methyl]-[4-^{14}C]-2,4-
quinazolinediamine monoacetate monohydrate, (13). A heterogeneous suspension of
the partially purified nitrile 12 (446 mg, 20 mCi, 2.24 mmol) and 6 (409 mg,
2.24 mmol) in glacial acetic acid (16 mL) was added to a vial containing Raney
nickel (700 mg). The reaction mixture was subjected to hydrogen at 58 psi for
16 h at room temperature. The resulting clear solution was filtered to remove
the catalyst and then freeze-dried. The solid was dissolved in 95% ethanol and
purified by dry column chromatography on silica gel (200 g). The column was
first eluted with ethyl acetate:methanol (75:25) to remove the unreacted 6 and
impurities, and then eluted with ethyl acetate:methanol:triethylamine (75:25:1)
to give 13 (340 mg, 7.1 mCi, 36% yield) as a yellow powder.

DOUBLE CARBON-14 LABELED SYNTHESIS

2,4-Dibromo-3-methylbenzenamine monohydrochloride. A 9 g lot of 2,4-dibromo-3-
methylbenzenamine monohydrochloride was prepared as described for 2a and 3a
above. A portion of this product was used in subsequent steps of the carbon-14
synthesis.

Cuprous [^{14}C]cyanide. Potassium [^{14}C]cyanide (1.5 Ci, 26.7 mmol) was similarly converted to cuprous [^{14}C]cyanide (1909 mg, 80% yield) as described previously.

4-Amino-2-methyl-1,3-benzene-[^{14}C$_2$]dicarbonitrile (4b). The free base of 2,4-dibromo-3-methylbenzenamine monohydrochloride (3.2 g, 10.7 mmol) was generated as described above. The methanol was removed and the crude product was partitioned between water and diethyl ether. The diethyl ether extractant was backwashed with saturated sodium chloride, dried (Na$_2$SO$_4$), filtered, and stripped to give the free base. Cuprous [^{14}C]cyanide (1198 mCi, 1909 mg, 21.3 mmol) was added to a solution of the free base in 1-methyl-2-pyrrolidinone (50 mL) and pyridine (1.3 mL). The mixture was heated at 108 °C for 135 min and then poured into an ice cold solution of NaCN (2.1 g, 43 mmol) in water (250 mL) and stirred for 18 h. The resulting solid was isolated by filtration and slurried repeatedly in weak ammonium hydroxide followed by stirring for 48 h in water (250 mL) containing NaCN (8 g). The isolated solid was air dried under suction for 8 h at room temperature to give the product 4b (1.4 g, 82% yield), which was used as is in the next step. TLC: R$_f$=0.18, RCP=86% silica gel, hexane:EtOAc (2:1).

2,4-Diamino-5-methyl-6-[4-^{14}C]quinazoline-[^{14}C]carbonitrile (5b). The intermediate 4b (982 mCi, 1400 mg, 8.7 mmol) was treated as described above for 5a resulting in 5b (1.36 g, 78% yield). TLC: R$_f$=0.39, RCP=88%, silica gel, EtOAc:MeOH (3:2); R$_f$=0.43, RCP=85%, silica gel, MeOH:EtOAc (3:1).

5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{14}C]methyl]-[4-^{14}C]-2,4-quinazolinediamine, [^{14}C$_2$]CI-898 (7b). A mixture of 5b (769 mCi, 1364 mg, 6.85 mmol), 6 (1260 mg, 6.85 mmol), Raney nickel (5 g) and acetic acid:water (7:1, 40 mL) was shaken on a Parr apparatus under hydrogen (50 psi) for 18 h at room temperature. The mixture was filtered to remove the catalyst; the filtrate was treated with NaOH to pH 11 producing a flocculent precipitate which was isolated by filtration, washed with water and dried at room temperature under high vacuum for 18 h to give crude 7b (293 mCi, 1237 mg, 237 μCi/mg, 87.45 mCi/mmol, 49% yield, RCP=77%). Portions of crude 7b were converted to the acetate salt, using AcOH in the mobile phase and flash chromatographed (silica gel, EtOAc:MeOH (3:2). The pure fractions of the various runs were

combined and stripped to a yellow–white solid. The solid was dissolved in water
(100 mL), filtered and treated dropwise with 4 *N* NaOH to pH 11 producing a white
flocculent precipitate. The solids were filter isolated, washed with water to
pH 7 and dried at room temperature under high vacuum for 3 days to give purified
7b (70.776 mCi, 251 mg, 282 μCi/mg, 104 mCi/mmol). TLC: R_f=0.30, RCP=99.5%,
silica gel, EtOAc:MeOH:Et$_3$N (75:25:1). HPLC: t_R=6.0 min, RCP=98.9%, chemical
purity >98.2%. Alltech C$_{18}$, 10μ, 4.6 mm ID x 25 cm, 40:60 CH$_3$CN:(10 parts AcOH,
1.08 g PIC B–8 in 1 L water), flow 1.0 mL/min, uv @ 254 nm.

^{1}H–NMR(DMSO-d_6) δ 7.44(d), 7.03(d), 6.72(s), 5.94(s), 5.86(s), 5.67(t), 4.16(d),
3.68(s), 3.52(s), 3.34(s), 2.67(s).

5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{14}C]methyl]-[4-^{14}C]-2,4-
quinazolinediamine 2-hydroxyethanesulfonate. [^{14}C$_2$]CI–898 2-hydroxy-
ethanesulfonate (8b). A portion of 7b (13 mCi, 46.1 mg, 0.125 mmol) was
slurried in anhydrous methanol (5 mL). 2-Hydroxyethanesulfonic acid (0.37 mL,
0.131 mmol, 0.35 *M* in MeOH) was added to the slurry and momentarily produced
clarification. After stirring the slurry at room temperature, the product was
isolated by filtration and dried at room temperature for 48 h under high vacuum
to give 8b (6.3 mCi, 29.54 mg, 212 μCi/mg, 105 mCi/mmol, 48% yield).
TLC: R_f=0.20, RCP=100%, silica gel, EtOAc:MeOH:Et$_3$N (75:25:1); R_f=0.60,
RCP=100%, C$_{18}$, MeOH:0.5 *M* NaCl (3:2). HPLC: t_R=6.1 min, RCP=99.54%, chemical
purity 100%. Alltech C$_{18}$, 10μ, 4.6 mm ID x 25 cm, 40:60 CH$_3$CN:(10 parts AcOH,
1.08 g PIC B–8 in 1 L water), flow 1.0 mL/min, uv @ 254 nm.

^{1}H–NMR(DMSO-d_6) δ 12.04(s), 7.72(d), 7.59(s), 7.27(d), 5.90(s), 4.46(s), 4.27(d),
3.67(s), 3.52(s), 3.34(s), 2.71(s), 2.61(t).

5-Methyl-6-[[(3,4,5-trimethoxyphenyl)amino][^{14}C]methyl]-[4-^{14}C]-2,4-
quinazolinediamine β-D-glucopyranuronate. [^{14}C$_2$]CI–898 β-D-glucopyranuronate
(9b). A portion of 7b (22 mCi, 78.01 mg, 0.211 mmol) was added to an aqueous
(5 mL) solution of β-D-glucopyranuronic acid (43.09 mg, 0.222 mmol) resulting in
a clear yellow solution which was lyophilized over 18 h to give 9b (20.95 mCi,
122 mg, 179 μCi/mg, 100.8 mCi/mmol). TLC: R_f=0.20, RCP=100%, silica gel,
EtOAc:MeOH:Et$_3$N (75:25:1); R_f=0.28, RCP=99.6. HPLC: t_R=6.0 min, RCP=98.5%.
Alltech C$_{18}$, 10μ, 4.6 mm ID x 25 cm, 40:60 CH$_3$CN:(10 parts AcOH, 1.08 g PIC B–8

in 1 L water), flow 1.0 mL/min, uv @ 254 nm. ^{1}H–NMR(DMSO–d_6) δ 7.58(d),

7.55(s), 7.15(d), 7.05(s), 5.93(s), 5.77(t), 4.94(s), 4.33(d), 4.20(s), 3.93(d),

3.81(d), 3.67(s), 3.52(s), 3.1–3.6(m), 2.96(t), 2.69(s).

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