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Synthesis of stable isotopes of auxinic herbicides 4-amino-3,5,6-trichloropicolinic acid and 4-amino-3,6-dichloropicolinic acid

Peter L. Johnson,* Norman R. Pearson, Bert Schuster, and Jeremy Cobb

Pentachloropyridine serves as a key intermediate in the synthesis of 4-amino-3,5,6-trichloropicolinic acid (picloram) and 4-amino-3,6-dichloropicolinic acid (aminopyralid). An M+3 stable isotope of pentachloropyridine (1, pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$) was prepared from K^{13}C^{15}N. Isotopically labeled pentachloropyridine was then carried through a seven-step synthesis to give an M+3 stable isotope of 4-amino-3,5,6-trichloropicolinic acid (2, picloram-1-^{15}N-2,6-$^{13}C_2$) in an overall yield of 42%. The chlorine atom in the 5-position of 2 was selectively removed via electrochemical reduction. Carrying out the electrochemical reduction in water provided an M+3 stable isotope of 4-amino-3,6-dichloropicolinic acid (3, aminopyralid-1-^{15}N-2,6-$^{13}C_2$), whereas conducting the reduction in deuterium oxide produced an M+4 stable isotope (4, aminopyralid-1-^{15}N-2,6-$^{13}C_2$-5-^{2}H).

Keywords: pentachloropyridine-1-^{15}N-2, 6-$^{13}C_2$; 4-amino-3, 5, 6-trichloropicolinic acid-1-^{15}N-2, 6-$^{13}C_2$; picloram-1-^{15}N-2, 6-$^{13}C_2$; 4-amino-3, 6-dichloropicolinic acid-1-^{15}N-2, 6-$^{13}C_2$; aminopyralid-1-^{15}N-2, 6-$^{13}C_2$; 4-amino-3, 6-dichloropicolinic acid-1-^{15}N-2, 6-$^{13}C_2$-5-^{2}H; aminopyralid-1-^{15}N-2, 6-$^{13}C_2$-5-^{2}H; stable isotope

Introduction

4-Amino-3,5,6-trichloropicolinic acid (picloram[1]) and 4-amino-3,6-dichloropicolinic acid (aminopyralid[2]) are plant growth regulator herbicides that display their toxicity by mimicking naturally occurring plant hormones called auxins. This leads to abnormal growth and ultimately plant death. These herbicides are used primarily in the range and pasture market for the control of broadleaf weeds. The auxin class of herbicides has enjoyed much success through the years due to their efficacy, selectivity and lack of resistance. While picloram has been on the market for many years (introduced in 1963), the registration application for aminopyralid was recently accepted by the US EPA.

Internal standards for both picloram and aminopyralid were required for registration studies. One type of internal standard that can be used for this purpose is an analog of the test material that contains at least one or more stable isotopes (^{13}C, ^{15}N, ^{2}H or ^{18}O). To aid in separating the internal standard from the test material in a mass spectral analysis, it was desirable to have the mass of the stable isotope standard at least three mass units higher than the test substance for these studies. Pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (**1**) was prepared on a multi-gram scale and utilized as a key intermediate for the synthesis of **2**. Electrochemical reduction of **2**, in either water or deuterium oxide, was used to prepare **3** and **4**, respectively. The preparation of isotopically labeled pentachloropyridine, picloram and aminopyralid will be discussed.

Results and discussion

Synthesis of pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$

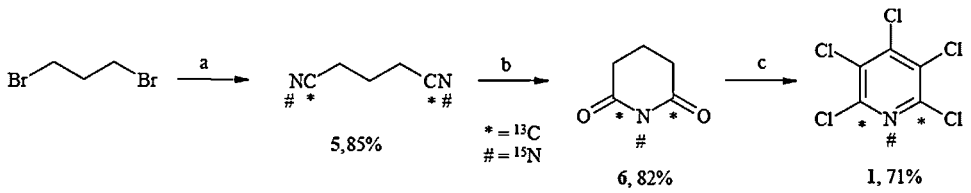
Several examples are reported in the literature for the conversion of 1,3-dibromopropane to chlorinated, carbon-14-labeled pyridine derivatives using the general route depicted in Scheme 1.[3–5] An excess of commercially available potassium cyanide-^{13}C-^{15}N was heated to reflux with 1,3-dibromopropane in a mixture of acetonitrile and water to give a high yield of isotopically labeled glutaronitrile (**5**).[6] A mixture of **5** in acetic acid and trifluoroacetic acid was heated to 230°C in a Hastelloy-C Parr pressure reactor for 2 days affording glutarimide-1-^{15}N-2,6-$^{13}C_2$ (**6**) in 82% yield after crystallization from ethanol. Treatment of **6** with excess phosphorus pentachloride and chlorine in the presence of a catalytic amount of iron (III) chloride and iodine for 28 h at 250°C and 950 psi gave a decent yield of pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (**1**) following crystallization from methanol.[7]

Synthesis of picloram-1-^{15}N-2,6-$^{13}C_2$

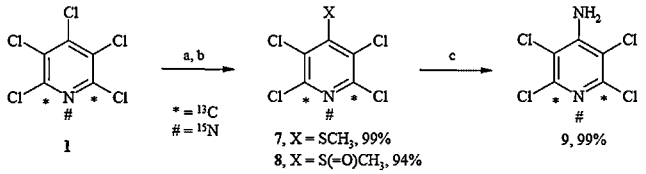
While the conversion of C-14-labeled pentachloropyridine to picloram-2,6-$^{14}C_2$ has been reported[8] no such information is available in the literature for the ^{15}N-2,6-$^{13}C_2$-labeled compound **2**. Treatment of pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (**1**) with sodium thiomethoxide at room temperature produced a near quantitative yield of the methyl sulfide derivative **7** (Scheme 2). Oxidation of **7** to the sulfoxide **8** was accomplished by treatment with a slight excess of bleach and hydrochloric acid. Reaction of **8** with ammonia in dioxane gave a near

Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46268, USA

**Correspondence to: Peter L. Johnson, Dow AgroSciences, 9330 Zionsville Road, Indianapolis, IN 46258, USA.*
E-mail: pljohnson@dow.com



Scheme 1. (a) $K^{13}C^{15}N$, CH_3CN, H_2O, reflux; (b) acetic acid, TFA, 230°C (300 psi), 48 h; and (c) PCl_5, Cl_2, $FeCl_3$ cat., I_2 cat., 250°C (950 psi), 28 h.



Scheme 2. (a) $NaSCH_3$, THF, H_2O, room temperature; (b) NaOCl, HCl, CH_2Cl_2, H_2O, 0°C; and (c) NH_3, dioxane, 50°C.

quantitative yield of 4-amino-2,3,5,6-tetrachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (**9**).

Displacement of the chlorine at the 2-position of **9** with sodium thiomethoxide in DMF/H_2O and subsequent oxidation of the methylsulfide **10** with excess bleach and hydrochloric acid gave sulfone **11** in high yield (Scheme 3). Treatment of **11** with excess sodium cyanide and hydrolysis of the resultant nitrile **12** afforded picloram-1-^{15}N-2,6-$^{13}C_2$ (**2**) in 42% overall yield from pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (**1**).

Synthesis of aminopyralid-1-^{15}N-2,6-$^{13}C_2$

Labeled aminopyralid was prepared by subjecting compound **2** to electrochemical reduction in an aqueous solution containing sodium hydroxide (Scheme 4).[9,10] In order to minimize loss from over-reduction, the reaction was stopped after HPLC analysis showed ~85% conversion to 4-amino-3,6-dichloropicolinic acid-1-^{15}N-2,6-$^{13}C_2$ (**3**). For purification purposes the crude carboxylic acid was converted to the methyl ester **13** by refluxing in methanol with a catalytic amount of sulfuric acid. The ester was readily purified by flash chromatography on silica gel and then hydrolyzed with aqueous lithium hydroxide in THF to give aminopyralid-1-^{15}N-2,6-$^{13}C_2$ (**3**) in >98% purity and an overall isolated yield of 50% from labeled picloram. Alternatively, conducting the electrochemical reduction with deuterated water in the presence of sodium deuteroxide provided 4-amino-3,6-dichloropicolinic acid-1-^{15}N-2,6-$^{13}C_2$-5-^{2}H (**5**), an M+4 isotope of aminopyralid. In this case the crude material was purified by preparative reverse-phase HPLC to give a decent yield of **4**.

Experimental

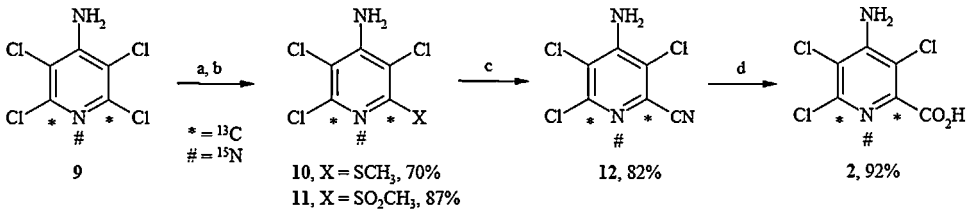
Potassium cyanide-^{15}N-^{13}C was purchased from Isotec, Inc. Nuclear magnetic resonance spectra were recorded on a Varian Gemini spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are given as δ values with reference to tetramethylsilane as the internal standard. Mass spectra were obtained using a Hewlett-Packard HP-5985 GC/MS or a Micromass ZMD single quadrupole LC/MS. Infrared spectra were recorded using a Digilabs FTS-40 spectrometer. The apparatus used for the electrochemical reduction was an EG&G Applied Research model 173 Potentiostat/Galvanostat.

Glutaronitrile-1,5-$^{15}N_2$-1,5-$^{13}C_2$ (5)

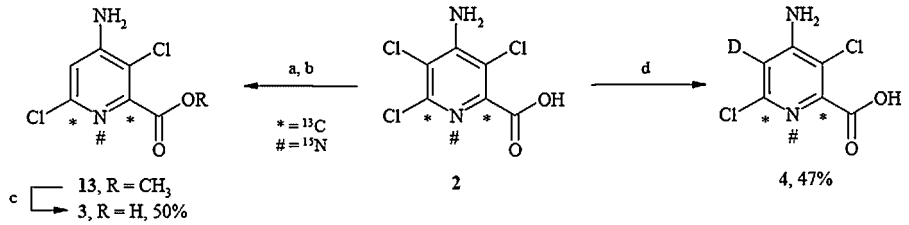
A mixture of 1,3-dibromopropane (28.0 mL, 0.276 mol) and potassium cyanide-^{15}N-^{13}C (49.5 g, 0.737 mol, 2.7 equiv.) in H_2O (525 mL) and acetonitrile (525 mL) was heated to reflux. After refluxing for 4 h, an additional 3.0 mL of 1,3-dibromopropane (0.030 mol) was added and refluxing continued for an additional 3 h. After refluxing for a total of 7 h, the reaction mixture was allowed to cool to room temperature while stirring overnight. At this time GC analysis indicated the reaction to be complete. The reaction mixture was concentrated on a rotary evaporator. The resultant yellow solution was extracted with CH_2Cl_2 (5 × 100 mL) and EtOAc (1 × 150 mL). The organic extracts were combined, dried (Na_2SO_4), filtered and concentrated *in vacuo* (50–55°C) to give 25.5 g (85% yield) of the desired product **5** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.61–2.52 (m, 4H), 2.12–1.98 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 120.3 (d, C_1, J_{CN} = 16.4 Hz), 21.9 (t, C_2, J_{CC} = 2.5 Hz), 16.5 (dt, C_2, J_{CC} = 55 Hz, J_{CN} = 3 Hz); MS (EI) 99 (M+H), 97 (M−H), 70, 68, 66, 64, 56 (base), 54; IR (NaCl) 2957, 2167, 1643, 1455, 1428, 780 cm^{-1}.

Glutarimide-1-^{15}N-2,6-$^{13}C_2$ (6)

A 1-L Hastelloy-C Parr pressure reactor fitted with a mechanical stirrer, a 2066 psi Hastelloy-C frangible and electronic pressure sensor was charged with glutaronitrile-1,5-$^{15}N_2$-1,5-$^{13}C_2$ **5** (25.0 g, 0.255 mol), acetic acid (350 mL) and trifluoroacetic acid (30 mL). The reactor was sealed and pressure tested at 115 psi of N_2. The reactor was vented to 30 psi, then closed and heated via heating jacket. The following limits were set: $pressure_{max}$ = 750 psi, $temperature_{max}$ = 240°C, time = 52 h. After cooling to room temperature the dark black reaction mixture was concentrated on the rotary evaporator to give a black, semi-solid slush. The slush was dissolved in methanol (300 mL) and then concentrated *in vacuo*. The residue was dried *in vacuo* (65°C) for 3 h. The residue was then dissolved in refluxing ethanol (200 mL) and allowed to crystallize over a period of 2 h at room temperature. After cooling to −78°C, the crude product was removed by filtration, washing with cold ethanol (2 times). The material was dried *in vacuo* to give 25.5 g (86% crude yield). The crude material was dissolved in hot methanol (1 L) and 20 g of decolorizing carbon was added. After 30 min the decolorizing carbon was removed by filtration through a plug of Celite to give a light yellow solution. The light yellow solution was concentrated *in vacuo* to give a crystalline product. The crystalline material was dissolved in hot ethanol (120 mL), allowed to cool and crystallize. The slurry was cooled to −78°C and the solid removed by vacuum filtration, washing with cold ethanol (2 × 20 mL). The solid was dried *in vacuo* (50°C) to give 24.15 g (82% yield) of the desired product **6**: ^{1}H NMR ($CDCl_3$) δ 8.84 (dt, 1H, J = 1.8 Hz, 88 Hz), 2.60–2.54 (m, 4H), 2.03–1.94 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 174.7 (d, J_{CN} = 9.2 Hz), 31.5 (dd, J_{CC} = 49.2 Hz, J_{CN} = 5.7 Hz), 17.8 (s); MS (EI) 116 (M+,



Scheme 3. (a) $NaSCH_3$, THF, H_2O, 5°C to room temperature; (b) NaOCl, HCl, CH_2Cl_2, H_2O; (c) NaCN, DMSO; and (d) 75% H_2SO_4, 140°C.



Scheme 4. (a) e^-, −1.3 V, NaOH, NaCl, H_2O; (b) CH_3OH, H_2SO_4 cat.; (c) LiOH, THF, H_2O; and (d) e^-, −1.3 V, NaOD, NaCl, D_2O.

base), 86, 73, 71, 56; IR (KBr) 3179, 2970, 2908, 2811, 1669, 1641, 1422, 1338, 1239, 1174, 1136, 1051, 834 cm^{-1}.

Pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (1)

This reaction was run in a pressure lab using a 1-L Parr reactor and a 2000 psi frangible, both made of Hastelloy-C, which should be compatible with most reagents used in this reaction. The Parr reactor was charged with glutarimide-1-^{15}N-2,6-$^{13}C_2$ **6** (23.0 g, 0.198 mol), phosphorus pentachloride (200 g, 0.96 mol), iodine (2.0 g, 0.0079 mol) and iron (III) chloride (1.75 g, 0.0108 mol). The vessel was sealed and pressure tested at 110 psi with N_2 and then vented. The Parr reactor was cooled in a dry ice/acetone bath for 20 min and then charged with chlorine gas (82 g, 1.17 mol). The Parr reactor was placed in a safety cell and the stirrer, water cooling and heat source were turned on. After heating at ~250°C for 42 h (950 psi), the reactor was allowed to cool to room temperature and opened. The contents were transferred to a 1-L flask and quenched with H_2O (temperature rose to 40°C). The resultant aqueous mixture was extracted with CH_2Cl_2 (3 × 400 mL). The extracts were combined and filtered through a pad of Celite. The filtrate was washed with 5% sodium meta-bisulfite and then dried over Na_2SO_4 and filtered. The filtrate was then treated with a 1:1 mixture of decolorizing carbon and Celite. The slurry was filtered through a plug of Celite and the filtrate concentrated to ~100 mL of CH_2Cl_2. The resultant slurry was treated with methanol (900 mL) and heated to reflux. The resultant solution was concentrated to ~500 mL, allowed to cool to room temperature and then placed in a freezer overnight. The resultant slurry was cooled to −78°C. The solid was removed by vacuum filtration, washing with cold methanol (−78°C, ~75 mL). The solid was dried to constant weight *in vacuo* to give 35.71 g (71% yield) of the desired product **1**: ^{13}C NMR (DMSO-d_6) δ 146.4 (d, J_{CN} = 7.0 Hz); MS (EI) 258, 256, 254 (base), 252 (M+), 221, 219, 217, 184, 182, 156, 154, 147, 121, 119, 112, 95, 84, 77; IR (NaCl) 1284, 1314, 1305, 1078, 809 cm^{-1}.

2,3,5,6-Tetrachloro-4-methylsulfanylpyridine-1-^{15}N-2,6-$^{13}C_2$ (7)

A solution of pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ **1** (5.0 g, 19.66 mmol) in 80 mL of THF was cooled by an ice bath (0–2°C) under N_2 and treated dropwise with a solution of sodium thiomethoxide (1.38 g, 19.66 mmol) in water (10 mL, degassed with N_2 prior to addition) over a period of 60 min. After stirring at ice bath temperature for 2.5 h, GC analysis still showed ~20% starting material present. The reaction mixture was treated with a solution of sodium thiomethoxide (0.21 g, 0.15 equiv.) in 2 mL of H_2O. After an additional 90 min, GC analysis still showed 8% starting material present. The reaction mixture was treated with an additional 0.07 g of sodium thiomethoxide (0.05 equiv.) in 2 mL of H_2O. After 60 min, GC analysis showed that all of the starting material had been consumed. The reaction mixture was poured into 50 mL of H_2O and extracted with Et_2O (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 5.16 g (99% yield) of the desired product **7**: ^{1}H NMR ($CDCl_3$) δ 2.61 (s); MS (EI) 268, 266 (base), 264, 233, 231, 216, 193, 144, 119, 104.

2,3,5,6-Tetrachloro-4-methylsulfinylpyridine-1-^{15}N-2,6-$^{13}C_2$ (8)

A solution of 2,3,5,6-tetrachloro-4-methylsulfanylpyridine-1-^{15}N-2,6-$^{13}C_2$ **7** (5.16 g, 19.41 mmol) in 70 mL of CH_2Cl_2 was cooled by an ice bath (0–5°C) with stirring as H_2O (15 mL) and concentrated HCl (3.5 mL) were added via addition funnel. The resultant mixture was treated dropwise with sodium hypochlorite (39.5 mL, 23.3 mmol, 1.2 equiv., 4% solution) over a period of 60 min. After 2.5 h, GC-MS analysis indicated that all of the starting material had been consumed. The reaction mixture was poured into saturated sodium bisulfite and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic extracts were washed with H_2O (2 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 5.13 g (94% yield) of the desired product **8**: ^{1}H NMR ($CDCl_3$) δ 3.08 (s); MS (EI) 284, 282, 280, 268, 266 (base), 264, 233, 231, 214, 193, 174, 156,144, 119, 104.

2,3,5,6-Tetrachloropyridin-4-ylamine-1-^{15}N-2,6-$^{13}C_2$ (9)

A solution of 2,3,5,6-tetrachloro-4-methylsulfinylpyridine-1-^{15}N-2,6-$^{13}C_2$ **8** (5.13 g, 18.2 mmol) in 25 mL of 1,4-dioxane was treated with 50 mL of a 0.5 M solution of ammonia in 1,4-dioxane. The resultant solution was placed under an atmosphere of ammonia and heated to 45–50°C. After stirring

overnight, GC-MS analysis indicated that all of the starting material had been consumed. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (50 mL) and washed with saturated NaCl (50 mL). The organic phase was extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with H_2O (2 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 4.25 g (99% yield) of the desired product **9**: ^{1}H NMR (DMSO-d_6) δ 7.37 (bs); MS (EI) 237, 235 (base), 233, 200, 198, 171, 135, 100, 87.

2,3,5-Trichloro-6-methylsulfanylpyridin-4-ylamine-1-^{15}N-2,6-$^{13}C_2$ (10)

A solution of 2,3,5,6-tetrachloropyridin-4-ylamine-1-^{15}N-2,6-$^{13}C_2$ **9** (4.25 g, 18.1 mmol) in 75 mL of DMF was cooled to 0°C under N_2 and treated dropwise with a solution of sodium thiomethoxide (1.26 g, 18.1 mmol) in 12 mL of H_2O (degassed with N_2 prior to addition) over a period of 45 min. The reaction mixture was gradually allowed to warm to room temperature. After stirring overnight the reaction mixture was treated with an additional 0.2 equiv. of sodium thiomethoxide (0.25 g in 2 mL of H_2O) in an effort to drive the reaction to completion. The reaction mixture was poured into 100 mL of H_2O and extracted with Et_2O. The combined Et_2O extracts were washed with H_2O (3 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude material was chromatographed over silica gel, eluting with 90% hexanes/10% EtOAc. Isolation of the major product gave 3.14 g (70% yield) of the desired product **10**: ^{13}C NMR (DMSO-d_6) δ 153.9, 153.1, 148.6, 145.2 (d, J = 6 Hz), 13.2; MS (EI) 247, 245, 212, 210 (base), 200, 198, 166, 164, 138, 87.

2,3,5-Trichloro-6-methanesulfonylpyridin-4-ylamine-1-^{15}N-2,6-$^{13}C_2$ (11)

A solution of 2,3,5-trichloro-6-methylsulfanylpyridin-4-ylamine-1-^{15}N-2,6-$^{13}C_2$ **10** (3.14 g, 12.75 mmol) in 65 mL of CH_2Cl_2 was cooled by an ice bath (0°C) under N_2 and treated dropwise with 2 M HCl (37.5 mL) followed by sodium hypochlorite (44 mL, 25.5 mmol, ~4% aqueous solution) over a period of 30 min. After 5 h, GC-MS analysis showed the reaction mixture to contain ~75% desired product and ~25% sulfoxide intermediate. The reaction mixture was treated with an additional 0.3 equiv. of the sodium hypochlorite solution. The reaction mixture was gradually allowed to warm to room temperature. After stirring overnight, the reaction mixture was poured into saturated sodium bisulfite (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with H_2O (2 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude material was purified via silica gel chromatography, eluting with 60% hexanes/40% EtOAc. Isolation of the major product gave 3.09 g (87% yield) of the desired product **11**: ^{1}H NMR (DMSO-d_6) δ 7.04 (bs, 2H), 3.34 (s, 3H); MS (EI) 281, 279, 277, 217, 215 (base), 213, 200, 198, 187, 185, 171, 142, 128, 115, 100, 87, 63.

4-Amino-3,5,6-trichloropyridine-2-carbonitrile-1-^{15}N-2,6-$^{13}C_2$ (12)

A solution of 2,3,5-trichloro-6-methanesulfonylpyridin-4-ylamine-1-^{15}N-2,6-$^{13}C_2$ **11** (3.09 g, 11.1 mmol) in 30 mL of DMSO was warmed to 45–55°C under N_2 and treated in portions with sodium cyanide (3 × 0.54 g, 33.3 mmol, 3 equiv.) at 90-min intervals. After 4.5 h, the reaction mixture was allowed to cool to room temperature. The reaction mixture was poured into saturated NaCl (100 mL) and extracted with EtOAc (7 × 100 mL). The combined organic extracts were washed with saturated NaCl (5 × 100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. The crude material was dissolved in EtOAc (150 mL) and passed through a plug of silica gel in order to remove colored impurities. The filtrate was concentrated *in vacuo* to give 2.05 g (82% yield) of the desired product **12**: ^{1}H NMR (DMSO-d_6) δ 7.64 (bs); MS (EI) 228, 226, 224 (base), 191, 189, 162, 135, 91, 78, 64.

4-Amino-3,5,6-trichloropyridine-2-carboxylic acid-1-^{15}N-2,6-$^{13}C_2$ (picloram-1-^{15}N-2,6-$^{13}C_2$, 2)

4-Amino-3,5,6-trichloropyridine-2-carbonitrile-1-^{15}N-2,6-$^{13}C_2$ **12** (2.05 g, 9.10 mmol) was dissolved in 75 mL of 75% sulfuric acid. The resultant solution was heated to 140°C. After stirring at 140°C overnight, TLC analysis showed the reaction to be complete. The reaction mixture was allowed to cool to room temperature and poured into 150 mL of H_2O. The resulting mixture was extracted with EtOAc (4 × 100 mL). The combined organic extracts were washed with H_2O (1 × 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 2.04 g (92% yield) of the desired product **2**: ^{1}H NMR (DMSO-d_6) δ 13.92 (bs, 1H), 7.26 (bs, 2H); MS (API, ES-Pos) 248, 246, 244, 230, 228, 226 (base), 200, 149; IR (KBr) 3476, 3375, 1706, 1601, 1527, 1431, 1366, 1279, 1225, 1100, 974, 890 cm^{-1}.

Methyl 4-amino-3,6-dichloropyridine-2-carboxylate-^{15}N-2,6-$^{13}C_2$ (13)

The cathode (Ag mesh) was activated immediately prior to use by running at +0.60 V and then reversing the polarity and running at −1.30 V in a 2% NaOH–1% NaCl (w/w) solution. Picloram-1-^{15}N-2,6-$^{13}C_2$ **2** (0.50 g, 2.0 mmol) was dissolved in 16.5 mL of a 2% NaOH/1% NaCl (w/w) solution in a 20 mL vial equipped with a magnetic stir bar. This solution was electrolyzed at −1.30 applied volts using a silver mesh cathode/graphite anode and a silver chloride reference electrode. After 6 h, HPLC analysis showed 12% **2** remaining and 77% desired product **13**. The reaction was stopped at this point and the brown mixture was filtered through a plug of Celite in a disposable pipet. The filtrate was cooled using an ice bath and acidified with concentrated HCl. The resultant solution was extracted with EtOAc (4 × 50 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 0.38 g of a light brown solid. The crude material (0.38 g) was dissolved in methanol (5 mL), treated with four drops of concentrated sulfuric acid and heated to reflux. After refluxing for 17 h, HPLC analysis showed only a trace amount of the starting material remaining. The reaction mixture was allowed to cool and then concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (10 mL) and 5% $NaHCO_3$ (10 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 0.36 g of an orange solid. The crude material was chromatographed on silica gel, eluting with 70% hexanes/30% EtOAc. Isolation of the major product gave 0.250 g (56% yield over two steps) of the desired product **13** as a beige solid: ^{1}H NMR ($CDCl_3$) δ 6.74 (d, 1H, J = 2.7 Hz), 4.9 (bs, 2H), 3.97 (s, 3H); MS (EI) 225, 223, 194, 192, 167, 166 (base), 137, 129, 108, 101, 94, 68.

4-Amino-3,6-dichloropyridine-2-carboxylic acid-1-^{15}N-2,6-$^{13}C_2$ (aminopyralid-1-^{15}N-2,6-$^{13}C_2$, 3)

A solution of lithium hydroxide hydrate (55 mg, 1.32 mmol) in 1 mL of H_2O was added to a solution of methyl

4-amino-3,6-dichloropyridine-2-carboxylate-^{15}N-2,6-$^{13}C_2$ **13** (0.248 g, 1.10 mmol) in 2 mL of THF at room temperature. After 2 h at room temperature, HPLC analysis indicated that all of the ester starting material had been consumed. The reaction mixture was poured into H_2O (10 mL) and washed with Et_2O (1 × 10 mL). The aqueous phase was acidified (pH 1–2) with 2 M HCl and extracted with EtOAc (5 × 10 mL). The combined EtOAc extracts were washed with saturated NaCl (1 × 10 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 0.210 g (91% yield) of the desired product **3** as a light peach colored solid: ^{1}H NMR (DMSO-d_6) δ 13.74 (bs, 1H), 6.95 (bs, 2H), 6.73 (d, 1H, J = 2.4 Hz); MS (API, ES-Pos) 212, 210 (M+H), 194, 192, 166, 164; IR (KBr) 3480, 3348, 1723, 1617, 1559, 1523, 1441, 1364, 1299, 1202, 1131, 864 cm^{-1}.

4-Amino-3,6-dichloropyridine-2-carboxylic acid-1-^{15}N-2,6-$^{13}C_2$-5-^{2}H (aminopyralid-1-^{15}N-2,6-$^{13}C_2$-5-^{2}H, 4)

The cathode (Ag mesh) was activated immediately prior to use by running at +0.60 V and then reversing the polarity and running at −1.30 V in 10 mL of D_2O consisting of 2% NaOD/1% NaCl (w/w). Picloram-1-^{15}N-2,6-$^{13}C_2$ **2** (250 mg, 1.02 mmol) was dissolved in a solution consisting of 10 mL of D_2O, 2 wt% NaOD and 1 wt% NaCl. The electrode was placed in this solution and −1.30 V was slowly applied in the cathode mode while maintaining 0–80 mA. After 210 min, HPLC analysis showed 82% **4** and 18% **2**. The reaction was stopped at this point. The reaction mixture was acidified with concentrated HCl and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with saturated NaCl (1 × 15 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 0.179 g of a tan solid.

Preparative HPLC conditions: Varian Prepstar model SD-1 solvent delivery module; Varian model 320 UV–Vis detector set at 254 nm; YMC ODS-AQ Guard-Pack 50 × 50 mm, S-10P μm guard column, YMC ODS-AQ 50 × 250 mm, 120 Å, 10P μm preparative column; flow rate = 100 mL/min; isocratic mobile phase consisting of 85% H_2O with 0.05% TFA and 15% CH_3CN.

The crude material (179 mg) was dissolved in a solution consisting of 2 mL of CH_3CN/1 mL of CH_3OH and loaded onto the preparative HPLC column (one injection). The fraction containing the desired product (200–250 mL) was concentrated *in vacuo*. The residue was taken up in CH_3CN and concentrated (multiple times to remove H_2O). The resultant solid was dried under high vacuum to give 100 mg (47% yield) of the desired product **4** as a light tan solid: ^{1}H NMR (DMSO-d_6) δ 13.76 (bs, 1H), 6.96 (bs, 2H); MS (ES-Neg) 211, 209 (M−H), 167, 165.

Conclusion

A three-step sequence was used to transform commercially available, and relatively inexpensive, potassium cyanide-^{15}N,^{13}C into pentachloropyridine-1-^{15}N-2,6-$^{13}C_2$ (**1**) on a multi-gram scale. A seven-step synthesis was then used to convert **1** into 4-amino-3,5,6-trichloropicolinic acid-1-^{15}N-2,6-$^{13}C_2$ (**2**). While the sequence was somewhat lengthy, each step was performed in high chemical yield and purity. Electrochemical reduction of **2**, either in water or deuterium oxide, provided 4-amino-3,6-dichloropicolinic acid-1-^{15}N-2,6-$^{13}C_2$ (**3**) or 4-amino-3,6-dichloropicolinic acid-1-^{15}N-2,6-$^{13}C_2$-5-^{2}H (**4**), respectively. The stable isotopes of picloram and aminopyralid were obtained in sufficient quantities and isotopic purity to be used in method development studies related to the registration of these molecules.

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References

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