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Research Article

Highly efficient preparation of carbon-14 labeled, auxin herbicide 4-amino-3,5,6-trichloropicolinic acid

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Summary

A highly efficient, seven-step route has been developed for the preparation of 2,6-[$^{14}C_2$]-4-amino-3,5,6-trichloropicolinic acid ([$^{14}C_2$]-**1**, 2,6-[$^{14}C_2$]-picloram) from 2,6-[$^{14}C_2$]-pentachloropyridine. The method involves the stepwise, highly selective and high yield introduction of amino and carboxylic acid groups to the 4- and 2-positions, respectively, of pentachloropyridine affording [$^{14}C_2$]-**1** in an overall radiochemical yield of >70% with the use of only one formal purification step. Copyright © 2006 John Wiley & Sons, Ltd.

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Introduction

4-Amino-3,5,6-trichloropicolinic acid (**1**, picloram) is the active ingredient in several registered herbicide products used to control broadleaf and woody plant species and is a member of the auxin class of herbicides that act by disrupting plant growth metabolic processes. Tordon™, Surmount™ and Grazon™ are registered herbicides of Dow AgroSciences, LLC that contain picloram as an active ingredient. In order to maintain the registration of **1** in the markets in which it is used, it is necessary to periodically conduct

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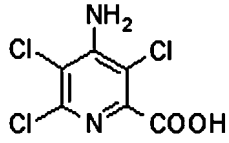
environmental fate and metabolism studies with it. To be able to conduct these registration studies in an efficient manner, a significant amount of the radiolabeled standard of **1** was required. Herein we report an efficient and highly selective preparation of carbon-14 labeled **1**.



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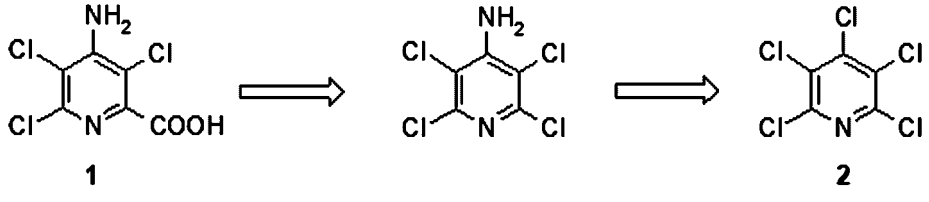
Results and discussion

When preparing radiotracers for use in regulatory studies, it is important to place the isotopic label(s) in positions that have good stability to both chemical and metabolic degradation so that the label cannot be expelled from the molecule of interest before relevant chemical species can be detected and quantitated in the test system being studied. In this case, the carbon atoms of the pyridine ring of **1** were especially suitable for this purpose. Pentachloropyridine (**2**) was utilized as the starting material for this preparative sequence owing to the ready availability of its carbon-14 isotope[1] and its suitability for the selective introduction of the 4-amino and 2-carboxylic acid groups of **1** in a controlled, stepwise manner as illustrated in Scheme 1.



Scheme 1.

The synthetic route developed was a highly optimized modification of an unpublished method developed by W. W. Muelder and J. C. Van Heertum of the Dow Chemical Company in the 1970s. As illustrated in Scheme 2 below, 2,6-[$^{14}C_2$]-pentachloropyridine ([$^{14}C_2$]-**2**) was treated with sodium thiomethoxide in THF-water at 0–5°C to yield, after workup, a very high, crude yield of 4-methylthiotetrachloropyridine **3a** (>95% selective formation). Careful attention to temperature, addition rate and stoichiometry were necessary to achieve the high yield in this and subsequent reactions. Crude **3a** was then oxidized to sulfoxide **3b** in high yield with sodium hypochlorite treated with

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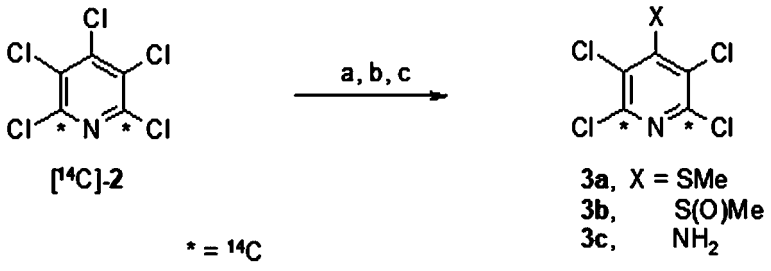

Scheme 2. (a) NaSMe, THF-H_2O; (b) NaOCl, CH_2Cl_2-H_2O/HCl; (c) NH_3, dioxane

hydrochloric acid. It was not possible to prepare the corresponding sulfone under these reaction conditions. Compound **3b** was used without purification in the next step where it was treated with ammonia in dioxane to afford 2,6-[$^{14}C_2$]-4-amino-2,3,5,6-tetrachloropyridine (**3c**). After three chemical steps, a nearly quantitative conversion of [$^{14}C_2$]-**2** to **3c** (on a weight basis) had been realized and HPLC analysis indicated >88% radiochemical purity for **3c**. Had one treated [$^{14}C_2$]-**2** with ammonia directly, a mixture of 2- and 4-amino isomers would have resulted with the 4-isomer predominating (70:30, 4-amino : 2-amino), but after isolation and separation, a far lower yield (<50%) of the desired **3c** would have resulted than in the present case (unpublished work of W. W. Muelder and J. A. Gilpin of the Dow Chemical Company).

In a manner similar to that described above for [$^{14}C_2$]-**2**, **3c** was efficiently converted to 2-methylthio-4-aminopyridine **4a** with sodium thiomethoxide in DMF (see Scheme 3 below). This time, upon treatment with >2 equivalents of sodium hypochlorite-hydrochloric acid, the methylthio group of **4a** oxidized very readily to sulfone **4b** and did not stop at the intermediate sulfoxide stage as was observed during the conversion of **3a** to **3b**. The sulfur atom of the methylthio group attached at the 2-position of the pyridine ring of **4a** appears more oxidizable with *in situ* generated hypochlorous acid than does the methylthio group of **3a** (sulfur atom at 4-position). These positional, reactivity differences are not unusual in pyridine chemistry.[2] At this point the crude sulfone **4b**, isolated after five chemical steps, was purified by preparative HPLC to afford **4b** in 77% overall yield from **2** and in >99% radiochemical purity. Purified **4b** was then treated with sodium cyanide in DMSO and the resulting 2-cyanopyridine **4c** was isolated in crude form and subjected to final hydrolysis with 75% aqueous sulfuric acid to furnish [$^{14}C_2$]-**1**. Upon workup and isolation, 34.6 mCi of the desired product [$^{14}C_2$]-**1** (23.8 mCi/mmol specific activity) was obtained in an overall radiochemical yield of nearly 71% for the seven-step route. Reverse phase HPLC analysis indicated the radiochemical purity of [$^{14}C_2$]-**1** to be >99%.

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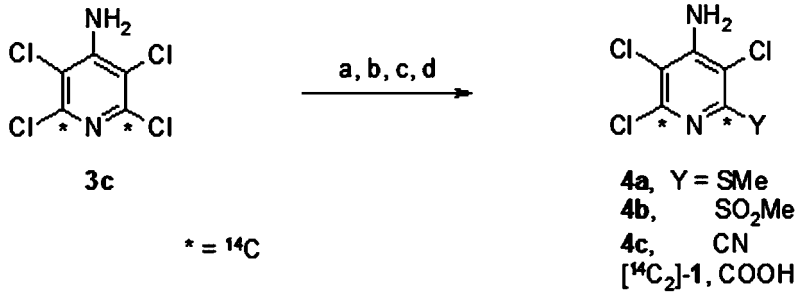

Scheme 3. (a) NaSMe, DMF; (b) NaOCl, CH_2Cl_2-H_2O/HCl; (c) NaCN, DMSO; (d) 75% H_2SO_4

Experimental

All chemicals were obtained from commercial suppliers and used without further purification unless otherwise noted. GC analyses were conducted on a HP 5890A GC using a 5 m × 0.53 mm (ID), 5 μm film, DB-1 capillary column. Analytical HPLC was conducted on a Beckman System Gold HPLC with a 126 solvent module and a 168 UV detector equipped with a PDA and a β-RAM radioactivity detector from INUS. HPLC analyses were run on a 4.6 × 250 mm YMC 5 μM ODS-AQ column, using gradient conditions with acetonitrile and water solvents containing 0.5% acetic acid (v/v) at a flow rate of 1 ml/min. Preparative HPLC purification of **4b** was carried out on a 20 × 250 mm YMC D-ODS-5 preparative column using an isocratic eluent consisting of 40% water and 60% acetonitrile at a flow rate of 10 ml/min. Mass spectral analyses were conducted by the DAS Regulatory Labs Analytical group in Indianapolis on a HP 1100 Series LC/MSD mass spectrometer with positive ESI and loop injection. Total radioactivity was measured by counting samples in Ultima Gold Scintillation Cocktail on a Packard 2500 TR Liquid Scintillation Analyzer. 2,6-[$^{14}C_2$]-Pentachloropyridine was obtained from American Radiolabeled Chemicals Inc. and used as received.

2,6-[$^{14}C_2$]-4-Methylthio-2,3,5,6-tetrachloropyridine, **3a**

A solution of 578 mg (2.30 mmol, 50.6 mCi) of 2,6-[$^{14}C_2$]-pentachloropyridine (22 mCi/mmol) in acetone was concentrated to dryness on a rotary evaporator and the neat [$^{14}C_2$]-**2** was dissolved in 8 ml of THF (Aldrich, anhydrous) and the resulting golden-yellow solution and several THF rinses (10 ml total) were transferred to a 50 ml, 2-neck reaction flask, equipped with a magnetic stir bar, using a stainless steel, double-ended needle. An aqueous solution of sodium thiomethoxide (Aldrich, 95%) was prepared by dissolving 195 mg (2.78 mmol)

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of it in 1 ml of water that had been previously purged with nitrogen. The aqueous solution of sodium thiomethoxide was added drop-wise, under nitrogen, to the rapidly stirred, THF solution of [$^{14}C_2$]-**2** at 0–5°C. After 6.5 h at 0–5°C, GC analysis indicated incomplete consumption of [$^{14}C_2$]-**2** so an additional 10.0 mg (0.142 mmol) of solid sodium thiomethoxide was added. After stirring for another $1\frac{1}{2}$ h, the excess sodium thiomethoxide in the reaction was quenched by the addition of 70 µl (1.2 mmol) of acetic acid. The THF was removed with a stream of nitrogen, 7 ml of water were added, and the resulting mixture was extracted with ether (5×4 ml). The combined ether extracts were washed with water (2×3.5 ml) and then passed through a column of anhydrous sodium sulfate. GC analysis of the crude reaction solution indicated $>95\%$ selective formation of **3a** and the presence of $<1.5\%$ of the isomeric 2-methiotetrachloropyridine and $<1\%$ of the bis(methylthiolated) side-products. Concentration of the ether solution to dryness on a rotary evaporator afforded 613 mg ($>99\%$ crude) of **3a** as a solid.

2,6-[$^{14}C_2$]-4-Methylsulfinyl-2,3,5,6-tetrachloropyridine, **3b**

To a 50-ml flask, equipped with a stir bar and containing 613 mg (2.32 mmol) of **3a**, was added 9 ml of CH_2Cl_2. The resulting solution was cooled in an ice-water bath and diluted with 2 ml of water and 0.5 ml of 12 N hydrochloric acid. The resulting mixture was vigorously stirred and treated with 4.8 ml (2.6 mmol) of sodium hypochlorite solution (Aldrich, $>4\%$ NaOCl) by drop-wise addition over 5–10 min. After 1.5 h at 0–5°C GC analysis indicated that no **3a** remained so the excess oxidant was quenched with 26.4 mg (0.254 mmol) of sodium bisulfite dissolved in 1 ml of water. The aqueous phase was extracted with CH_2Cl_2 (4×3 ml) and the combined organic phases were washed with water (2×7 ml) and then dried by passing through a column of anhydrous sodium sulfate. Analysis by HPLC with radiochemical detection indicated the sample to consist of 93% of **3b**. The CH_2Cl_2 solution was concentrated to dryness on a rotary evaporator to afford 644 mg ($>99\%$ crude) of **3b**.

2,6-[$^{14}C_2$]-4-Amino-2,3,5,6-tetrachloropyridine, **3c**

To a flask containing 644 mg (2.31 mmol) of **3b** was added 10 ml (5 mmol) of 0.5 M ammonia (Aldrich) in dioxane. The resulting solution was placed under an atmosphere of ammonia gas using a latex balloon to maintain a static pressure and heated at 45–50°C for 40 h. The balloon was recharged seven times with ammonia gas during the reaction. After cooling, the dioxane was removed using a rotary evaporator. The crude material was diluted with of 10 ml of water and extracted with EtOAc (4×7.5 ml). The combined EtOAc extracts were passed through a column of anhydrous sodium sulfate and analyzed by HPLC ($>88\%$ radiochemical purity of **3c**). The EtOAc solution

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was concentrated to dryness on a rotary evaporator to afford 557 mg (>99% crude) of **3c**.

2,6-[$^{14}C_2$]-4-Amino-2-methylthio-3,5,6-trichloropyridine, **4a**

To a flask containing 557 mg (2.40 mmol) of **3c** and a magnetic stir bar was added 10 ml of DMF (Aldrich). To the rapidly stirred solution of **3c** at room temperature was added, drop-wise, over 5 min, a solution of 305 mg (4.35 mmol) of sodium thiomethoxide dissolved in 2 ml of water. After 16 h at room temperature, HPLC analysis indicated <1.5% of residual **3c**, and ca. 3.6% of the bis(methylthiolated) side-product. The reaction mixture was acidified with 0.060 ml (1.05 mmol) of acetic acid and the DMF was removed on the rotary evaporator. The crude material obtained was diluted with 10 ml of water and extracted into CH_2Cl_2 (4 × 5–6 ml). Analysis by HPLC indicated the solution to contain >88% radiochemically pure **4a**. The solution was concentrated to dryness on a rotary evaporator to afford 612 mg (>99% crude) of **4a**.

2,6-[$^{14}C_2$]-4-Amino-2-methylsulfonyl-3,5,6-trichloropyridine, **4b**

To a flask containing 612 mg (2.51 mmol) of **4a** was added 13 ml of CH_2Cl_2 to obtain a solution. The flask was placed in an ice-water bath and treated with 7.5 ml (15 mmol) of 2 N HCl. The mixture was stirred vigorously and then treated, drop-wise with 4.7 ml (2.5 mmol) of sodium hypochlorite solution (Aldrich, >4% NaOCl). After 1 h, the reaction mixture was analyzed by tlc (silica gel, 80/20 ethyl acetate/hexane) and HPLC and found to be ca. 35% converted to **4b**. Over the next 3 h, an additional 5.7 mL (3.06 mmol) of NaOCl were added in several portions to drive the reaction to completion. After complete conversion to **4b**, the excess oxidant was quenched with 149 mg (1.43 mmol) of solid $NaHSO_3$. The aqueous phase was extracted with CH_2Cl_2 (5 × 5 ml) and the combined organic extracts were dried with anhydrous sodium sulfate. Analysis of the CH_2Cl_2 solution by HPLC found it to be composed radiochemically of >85% **4b**. The organic solution was concentrated to dryness on a rotary evaporator and further dried at high vacuum to afford 659 mg (95% crude) of **4b**. The crude material was purified by preparative HPLC using the conditions given at the beginning of the experimental section. The combined fractions containing **4b** were concentrated on a rotary evaporator to remove the acetonitrile and the remaining water mixture containing purified **4b** was extracted with CH_2Cl_2 (4 × 15 ml). The combined organic extracts were dried with anhydrous sodium sulfate and then analyzed by HPLC and found to contain >99% radiochemically pure **4b**. The CH_2Cl_2 solution was evaporated to dryness and further dried at high vacuum, to afford 490 mg (71%) of **4b**.

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2,6-[$^{14}C_2$]-4-Amino-2-cyano-3,5,6-trichloropyridine, **4c**

To a flask containing 490 mg (1.78 mmol) of **4b** was added 16 ml of DMSO (Fisher) and 262 mg (5.36 mmol) of NaCN (Fisher). The flask was heated at 45–55°C for 7 h, then placed in an ice-water bath and diluted with 50 ml of ice-chilled water. A fine precipitate began to form as the mixture was stirred and after 2 h, the mixture was filtered through a sintered glass funnel. The solid collected was washed with cold water and then transferred from the filter to a 50-ml flask as an acetone slurry. The acetone solution was concentrated to dryness on a rotary evaporator and further dried at high vacuum, to afford 342 mg (86%) of **4c** (found to be >98% radiochemically pure by HPLC analysis).

*2,6-[$^{14}C_2$]-4-Amino-3,5,6-trichloropicolinic Acid, [$^{14}C_2$]-***1**

A flask containing 342 mg (1.54 mmol) of **4c** and 7.6 ml of 75% aqueous sulfuric acid was heated at 135–145°C. After 1 h, HPLC analysis indicated >99% radiochemical conversion to [$^{14}C_2$]-**1**. After cooling to room temperature, the reaction flask was placed in an ice-water bath and ca. 15 g of ice and 25 ml of ice-chilled water were carefully added. The reaction mixture was stirred for 1 h in the ice-water bath and the solids formed were collected by filtration, washed with 20 ml of ice-chilled water and then were transferred as a slurry in acetone to a 50-ml flask. The acetone solution was concentrated to dryness on a rotary evaporator and further dried at high vacuum to afford 351 mg (94%) of [$^{14}C_2$]-**1** as an off-white solid. Analysis indicated 35.8 mCi of total radioactivity at a specific activity of 24.6 mCi/mmol and a radiochemical purity of >99%: MS (ESI+) 241 (M+1). The sample of [$^{14}C_2$]-**1** was found to match an unlabeled standard of **1** when compared by: HPLC retention times, normal and reverse phase thin layer chromatography R_f values, mass spectral analysis and UV analysis (peak shape comparison).

Conclusion

The preparative sequence described constitutes an efficient and highly selective preparation of [$^{14}C_2$]-**1**. The seven-step synthesis starting from [$^{14}C_2$]-**2** involved the use of standard laboratory glassware, normal chemical reagents and no high pressure or unusual reaction conditions. Only one formal purification was utilized throughout the sequence which indicates the highly efficient and selective nature of the chemistry employed.

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