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Research Article

Synthesis of [$^{13}C_7$]3,5-dichlorobenzylamine hydrochloride

Simon J. Harwood*
Isotope Chemistry, GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK

Summary

Commercially available [$^{13}C_6$]aniline (2) was readily converted into [$^{13}C_6$]1-bromo-3,5-dichlorobenzene (5). A further stable label was introduced by cyanation of aryl bromide (5), using K^{13}CN and cuprous iodide. Borane reduction of benzonitrile (6), followed by an acid work up, furnished [$^{13}C_7$]3,5-dichlorobenzylamine hydrochloride (1), which was further elaborated to provide an internal standard for use in LC-MS assays. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: carbon-13; chlorination; bromination; deamination; cyanation; borane reduction

Introduction

The 3,5-dichlorobenzylamine *motif* is commonly found in pharmacologically important molecules[1] and is a useful building block in organic synthesis. As stable labelled drug substances are routinely used as internal standards in LC-MS assays, simple preparation of stable labelled 3,5-dichlorobenzylamine would be synthetically useful. This is particularly true if there are limited labelling opportunities in the rest of the molecule as, due to the presence of the 2 chlorine atoms, a mass increase of at least M + 7 is normally required so that the parent ion cluster is well separated from that of the unlabelled drug.

1,3,5-Trisubstituted benzene rings can be difficult to access, particularly in the absence of strongly electron withdrawing groups to *meta* direct electrophilic substitution. The challenge in this project was to prepare [M + 7] 3,5-dichlorobenzylamine hydrochloride, a compound with no *meta* directing groups, from commercially available labelled precursors. Herein we describe the 5-step synthesis of [$^{13}C_7$]3,5-dichlorobenzylamine hydrochloride (1).

*Correspondence to: S. J. Harwood, Isotope Chemistry, GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK. E-mail: simon.j.harwood@gsk.com

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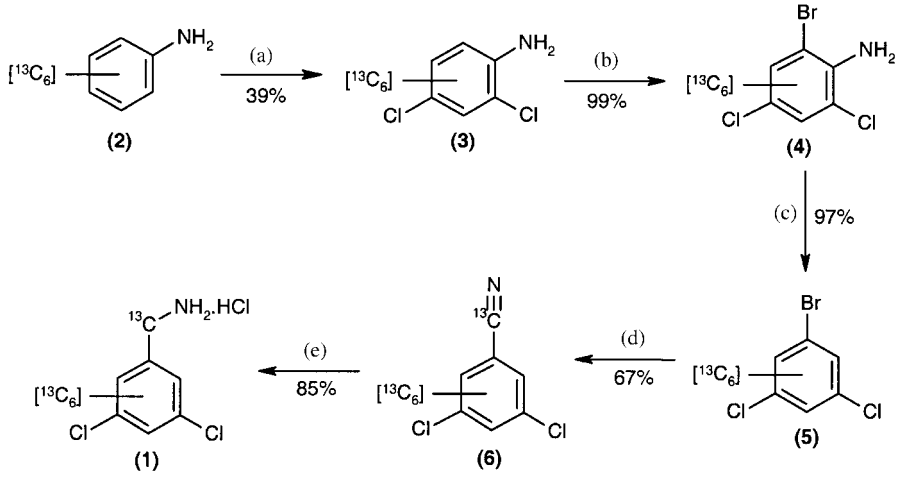
Results and discussion

Although monochlorination of aniline can be achieved in good regioselectivity and yield,[2] dichlorination of aniline is usually carried out indirectly via the acetanilide.[3] During our research we have found that direct dichlorination of aniline can be achieved using *N*-chlorosuccinimide. Although this reaction gives a mixture of regioisomers, 2,4-dichloroaniline can be easily isolated from the crude mixture by column chromatography. Treatment of [$^{13}C_6$]aniline (2) with 2 equivalents of freshly recrystallised (Recrystallised from boiling water (approx. 30 g of material in 300 ml of water) and dried overnight under high vacuum.) *N*-chlorosuccinimide gave [$^{13}C_6$]2,4-dichloroaniline (3) in 39% yield (Scheme 1). Bromination of (3), using 1 equivalent of freshly recrystallised (Recrystallised from boiling water (approx. 30 g of material in 300 ml of water) and dried overnight under high vacuum.) *N*-bromosuccinimide, gave [$^{13}C_6$]2-bromo-4,6-dichloroaniline (4) in 99% yield. Deamination of (4) was achieved by conversion of the aniline nitrogen to the corresponding diazonium salt, using 70% w/w sulfuric acid and sodium nitrite, and then reduction with hypophosphorus acid. This gave [$^{13}C_6$]1-bromo-3,5-dichlorobenzene (5) in 97% yield. The reaction conditions were based on work by Agrawal[4] who found that diazonium salt formation in similar systems worked best when sulfuric acid was used. Furthermore, he reported that the formation was sensitive to both acid concentration and reaction temperature.

The reaction conditions for successful cyanation of 1-bromo-3,5-dichlorobenzene were critical; the best conditions found were potassium cyanide and cuprous iodide in refluxing NMP, and gave unlabelled 3,5-dichlorobenzoni-



Scheme 1. (a) NCS, $CHCl_3$, rt; (b) NBS, $CHCl_3$, rt; (c)(i) 70% w/w H_2SO_4, $NaNO_2$, H_2O. (ii) H_3PO_2. (d) K^{13}CN, CuI, NMP, reflux; (e)(i) BH_3-THF, THF, reflux. (ii) HCl, rt. (iii) MeOH

trile in 83% isolated yield (78% HPLC conversion yield; a/a). Interestingly, treatment of 1-bromo-3,5-dichlorobenzene with potassium cyanide and cuprous iodide in refluxing DMF gave unlabelled 3,5-dichlorobenzonitrile in only 29% HPLC conversion yield (a/a) together with a number of unidentifiable components. Cyanation of the aryl bromide with cuprous cyanide in either refluxing DMF or refluxing NMP gave unlabelled 3,5-dichlorobenzonitrile in 40–45% HPLC conversion yield (a/a), and with zinc cyanide and a palladium (0) catalyst gave the unlabelled desired product in 45% HPLC conversion yield (a/a). It is noteworthy that 3,5-dichlorobenzonitrile is volatile under vacuum, making it difficult to obtain an accurate isolated yield of the desired product. Cyanation of [$^{13}C_6$]1-bromo-3,5-dichlorobenzene (5), using [^{13}C]potassium cyanide and cuprous iodide in refluxing NMP, gave [$^{13}C_7$]3,5-dichlorobenzonitrile (6) in 67% isolated yield. Reduction of [$^{13}C_7$]3,5-dichlorobenzonitrile (6) with borane-THF, followed by an acid work up, gave [$^{13}C_7$]3,5-dichlorobenzylamine hydrochloride (1) in 85% yield. [$^{13}C_7$] Benzylamine (1) was further elaborated to provide an internal standard for use in LC-MS assays.

Experimental

1 H NMR spectra were recorded on a Bruker spectrometer at 400 MHz. Mass spectra were recorded on a Micromass Quattro Ultima instrument and a Polaris GC/MS. All column chromatography was carried out over Merck Kieselgel 60 (9385) silica gel. HPLC analyses were performed on an HP Agilent system using a Luna C18 column (3 µm, 50×2 mm) with water/acetonitrile as the mobile phase (gradient = 0–100% acetonitrile over 8 min).

N-chlorosuccinimide and *N*-bromosuccinimide were both recrystallised from boiling water (approx. 30 g of material in 300 ml water) and dried overnight under high vacuum. Cuprous iodide was ground with a pestle and mortar and stored overnight under high vacuum.

2, 4-Dichloroaniline (3)

To a stirred solution of [$^{13}C_6$]aniline (2) (5.21 g; 52.6 mmol) in dry chloroform (100 ml), at room temperature under nitrogen, was added freshly recrystallised (Recrystallised from boiling water (approx. 30 g of material in 300 ml of water) and dried overnight under high vacuum.) *N*-chlorosuccinimide (14.04 g; 105.2 mmol). The reaction mixture was stirred at room temperature for 18 h and the solvent was evaporated. The dark purple residue was diluted with saturated sodium bicarbonate (aq):water/1:1 (200 ml) and extracted thoroughly with diethyl ether. The combined organic layers were washed with water and saturated brine, then dried (Na_2SO_4). Evaporation of the solvent gave a dark purple solid which was purified by column

chromatography over silica gel, eluted with isohexane:ethyl acetate/ 99:1 → 97:3 → 95:5. [$^{13}C_6$]2,4-dichloroaniline (3) (3.43 g; 39%) was obtained as a dark purple solid.

HPLC analysis: 4.61 min, 97.7% (co-runs with authentic unlabelled material).

GCMS analysis (EI): 7.66 min, M^+ 167.

[$^{13}C_6$]2-Bromo-4,6-dichloroaniline (4)

To a stirred solution of [$^{13}C_6$]2,4-dichloroaniline (3) (6.53 g; 38.9 mmol) in dry chloroform (60 ml), at room temperature under nitrogen, was added freshly recrystallised (Recrystallised from boiling water (approx. 30 g of material in 300 ml of water) and dried overnight under high vacuum.) *N*-bromosuccinimide (6.92 g; 38.9 mmol). The reaction mixture was stirred at room temperature for 1 h, diluted with water (100 ml) and extracted thoroughly with chloroform. The combined organic layers were washed with water and saturated brine, dried (Na_2SO_4) and the solvent was evaporated. Crude [$^{13}C_6$]2-bromo-4,6-dichloroaniline (4) (9.51 g; 99%) was obtained as a red/ brown solid.

HPLC analysis: 5.72 min, 97.4% (co-runs with authentic unlabelled material).

GCMS analysis (EI): 9.18 min, M^+ 247 (authentic unlabelled material = M^+ 241).

[$^{13}C_6$]1-Bromo-3,5-dichlorobenzene (5)

A suspension of [$^{13}C_6$]2-bromo-4,6-dichloroaniline (4) (9.51 g; 38.5 mmol) in sulfuric acid (56 ml of a 70% w/w solution in water), at room temperature under nitrogen, was stirred for 20 min. The mixture was then cooled to −10°C and a solution of sodium nitrite (3.99 g; 57.8 mmol) in water (7.5 ml) was added dropwise over 15 min. The reaction mixture remained below 0°C during the addition. The mixture was stirred at −10°C for 30 min and hypophosphorus acid (34 ml of a 50% solution in water, 0.33 mol) was added dropwise over 30 min. The reaction mixture remained below 0°C during the addition. The reaction mixture was stirred at −10°C for 3 h, allowed to warm up to room temperature and stirred for a further 18 h. Water (200 ml) was added and the aqueous layer was extracted thoroughly with dichloromethane. The combined organic layers were dried (Na_2SO_4) and the solvent was evaporated. Crude [$^{13}C_6$]1-bromo-3,5-dichlorobenzene (5) (8.67 g; 97%) was obtained as a red/brown solid.

HPLC analysis: 6.51 min, 96.6% (co-runs with authentic unlabelled material).

MS analysis (EI): M^+ 230 (authentic unlabelled material = M^+ 224).

[$^{13}C_7$]3,5-Dichlorobenzonitrile (6)

A suspension of [$^{13}C_6$]1-bromo-3,5-dichlorobenzene (5) (8.67 g; 37.4 mmol), [^{13}C]potassium cyanide (2.55 g; 38.6 mmol) and freshly ground cuprous iodide (Cuprous iodide was ground with a pestle and mortar and stored overnight under high vacuum.) (3.56 g; 18.7 mmol) in dry NMP (120 ml), under nitrogen, was heated under reflux for 18 h. The mixture was allowed to cool to room temperature, added to water (900 ml) and saturated brine (100 ml), and thoroughly extracted with diethyl ether. The organic layers were carefully filtered under low vacuum to remove any sediment, and the sediment was washed with diethyl ether (200 ml). The combined organic layers were washed with water and saturated brine, then dried (Na_2SO_4). Evaporation of the solvent gave a brown solid which was purified by column chromatography over silica gel, eluted with isohexane:ethyl acetate/99:1 → 97:3 → 95:5. [$^{13}C_7$]3,5-dichlorobenzonitrile (6) (4.51 g; 67%) was obtained as an off-white solid.

HPLC analysis: 5.11 min, 79.6% (co-runs with authentic unlabelled material).

GCMS analysis (EI): 6.77 min, M^+ 178 (authentic unlabelled material = M^+ 171).

[$^{13}C_7$]3,5-Dichlorobenzylamine hydrochloride (1)

To a solution of [$^{13}C_7$]3,5-dichlorobenzonitrile (6) (3.48 g; 19.4 mmol) in dry THF (80 ml), at room temperature under nitrogen, was added dropwise, over 20 min, a solution of borane-THF (120 ml of a 1 M solution in THF; 120.0 mmol). The reaction mixture was heated under reflux for 3.5 h, allowed to cool to room temperature and left to stand overnight. The mixture was carefully acidified to pH 2 by dropwise addition of 5 M hydrochloric acid, and stirred for 30 min. The solvent was evaporated and the product was co-evaporated with methanol several times to remove any boron impurities. Sonication of the pale yellow solid in diethyl ether, followed by filtration, gave [$^{13}C_7$]3,5-dichlorobenzylamine hydrochloride (1) (3.62 g; 85%) as a white solid.

HPLC analysis: 2.66 min, 85.7% (co-runs with authentic unlabelled material).

MS analysis (EI): M^+ 183 (authentic unlabelled material = M^+ 176).

NMR analysis (DMSO): Concordant with structure (broad signals).

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