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An efficient synthesis of [$^{13}C_6$]-3,5-dichloroaniline

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3,5-Dichloroaniline is commonly found in many compounds with pharmacological and other biological activities. [$^{13}C_6$]-Aniline or its hydrochloride salt was converted in three steps to [$^{13}C_6$]-3,5-dichloroaniline, which can be incorporated in compounds of interest and used as internal standards in drug metabolism and pharmacokinetics (DMPK) studies.

Keywords: 3, 5-dichloroaniline; 1, 3-diamino-5-chlorobenzene; carbon-13; internal standards

Introduction

3,5-Dichloroaniline (3,5-DCA) is used in the production of a number of products, including agricultural chemicals, azodyes and pigments and in pharmaceuticals.

For example, dicarboximidic fungicides Vinclozolin and Iprione, which are used on large scale on grapes, contain the 3,5-DCA moiety.[1] Their common metabolite 3,5-DCA has been used as a biomarker for exposure to these fungicides.[2] It has even been suggested that low-level exposure to 3,5-DCA may be associated with adverse health effects such as endocrine disruption and nephrotoxicity.[3]

In the pharmaceutical industry, the 3,5-DCA moiety is present in several drug candidates, like the potent LFA-1-mediated cell adhesion antagonists, which are being developed as potential drugs to treat autoimmune diseases.[4]

Herein we report the synthesis of [$^{13}C_6$]-3,5-DCA starting from the commercially available [$^{13}C_6$]-aniline. [$^{13}C_6$]-3,5-DCA is incorporated in the synthesis of drug candidates and used as an internal standard in the drug metabolism and pharmocokinetics (DMPK) studies, or it can be used as a biomarker for biologically active compounds that contain this motif.

Results and discussion

Direct chlorination of aniline with freshly crystallized *N*-chlorosuccinimide (NCS) in either methanol or trifluroroacetic acid gave 2,4,6-trichloroaniline in yields up to 80% after flash chromatography purification. In order to improve the isolation, aniline was protected as the acetanilide and treated with NCS.[5] To our surprise and despite the large access of NCS over extended periods of time up to 2 weeks, the only product isolated was the 2,4-dichloroacetanilide in 98% yield. Deprotection of this acetanilide and bromination with freshly crystallized NBS gave 2-bromo-4,6-dichloroaniline in 99% yield.[6] Deamination via the diazonium salt using either hypophosphorous acid, or ferrous sulfate in dimethyl formamide gave 1-bromo-3,5-dichlorobenzene.[6,7] Amination according to Hartwig or Buchwald procedures gave 3,5-dichloroanilne in 85% yield.[8,9]

2,4-Dichloroacetanilide was also subjected to nitration by nitric acid:sulfuric acid mixture.[10] In our hands the major product was 2,4-dicholoro-5-nitroacetanilide (78%). The desired 2,4-dichloro-6-nitroacetanilide was obtained in only 18% yield and it was difficult to separate it from the above isomer.

Another attempt that failed to give the desired product included the chlorination of nitrobenzene. We reasoned that the nitro group would direct the chlorination to the *meta* positions. However, several chlorinating reagents and different conditions failed to give the desired product.

A simple protection of the aniline as the hydrochloride salt is actually sufficient to obtain 2,4,6-tricholoroaniline in very high yields and without byproducts.[11]

Thus, the aniline was protected as the salt by treatment with concentrated HCl in carbon tetrachloride. The salt can be easily handled as a white solid in open air. Chlorination in carbon tetrachloride with chlorine gas at 0°C gave 2,4,6-trichloroaniline in 98% yield. The chlorine should be bubbled slowly to the suspension of the aniline hydrochloride salt. Deamination as seen above and amino-dehalogenation gave the desired 3,5-dichloroaniline in quantitative yield (Scheme 1). We found when excess lithium hexamethyldisilazane (LiHMDS) is used, 1,3-diamino-5-chlorobenzene is isolated with yields up to 60%. The triaminobenzene was not produced even with a large excess of LiHMDS and heating for longer periods of time. Amination of 1,3,5-trichlorobenzene to 3,5-dichloroaniline was reported as well using copper acetate and ammonia at 120°C in an autoclave.[12]

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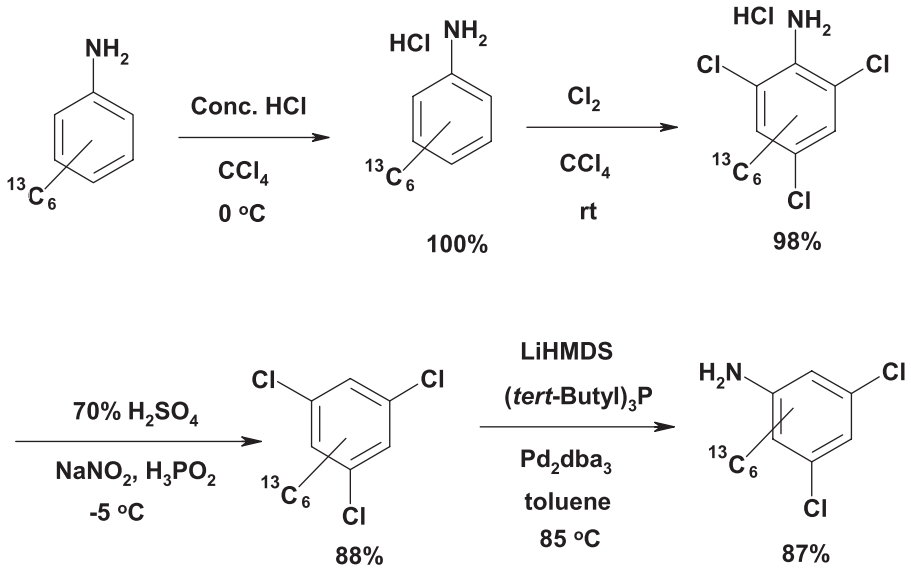

Scheme 1. Synthesis of [$^{13}C_6$]-3,5-DCA from [$^{13}C_6$]-aniline.

Experimental procedures

Materials and methods

LC-MS spectra were acquired by a Hewlett-Packard auto sampler Series 1100, connected to a Micromass Platform LCZ in the electron spray positive ion mode using 95% to 5% water/acetonitrile gradient (0.1% formic acid) for 3 min, and a SunFire™ C_{18}, 3.5 µm (4.6 × 30 mm) column. NMR spectra were recorded with a Bruker-Biospin DPX-400 spectrometer operating at 400.13 MHz ^{1}H frequency and at 100.66 MHz for ^{13}C using deuterated chloroform as a solvent and tetramethyl silane as the internal standard unless stated otherwise. Silica gel TLC was performed on pre-coated aluminum sheets with fluorescent indicator (EM Separating, Gibbstown, NJ). All reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI). Aniline-$^{13}C_6$ 99 atom % ^{13}C was purchased from *Isotec* (Miamisburg, OH). Solvents were all of HPLC grade.

Synthesis

[$^{13}C_6$]-2,4,6-Trichloroaniline

A solution of aniline-$^{13}C_6$ (1.0 g, 10.1 mmol) in CCl_4 (5 ml) was cooled to 0°C in an ice-bath. Concentrated HCl (12 N, 1.5 ml) was added dropwise to give a white solid. After stirring for one hour at 0°C, the solid was filtered, washed with CCl_4 (2 × 5 ml) and dried under reduced pressure to give 1.42 g of a white solid. This solid was suspended in carbon tetrachloride (12 ml) and absolute ethanol (0.3 ml). Chlorine gas was bubbled slowly through the suspension at room temperature to give a fluffy yellow solid. After 30 min no starting material was detected by LC. Water (25 ml) was added to the mixture and stirred for 15 min. The solid was filtered, washed with water (25 ml), and dried under reduced pressure for 12 h to give 1.98 g of an off-white solid with 98% purity as judged from LC-MS and in 98% yield. ^{1}H NMR ($CDCl_3$): δ7.12 (dm, J^1H-^{13}C = 172.22 Hz, 2H), 4.43 (brs, NH_2). ^{13}C NMR ($CDCl_3$): δ139.02(dt, J = 3.3, 69.96 Hz, C_1), 127.60(dt, J = 8.01, 66.64 Hz, C_3), 121.81(dt, J = 5.15, 66.64 Hz, C_4), 119.64 (dt, J = 4.08, 67.20 Hz, C_2). LCMS: ESI: MH^+ = 207 (100%).

This product can be also prepared directly from aniline. Thus, a solution of the aniline-$^{13}C_6$ (2.0 g, 20.2 mmol) was dissolved in CH_3OH. Freshly crystallized NCS (12 g, 9.0 mmol) was added to one portion at room temperature. The resulting dark mixture was stirred in the dark at room temperature until no starting material was detected (20 h). The dark mixture was concentrated under reduced pressure and the residue was dissolved in chloroform (50 ml) and washed with saturated $NaHCO_3$ (200 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 6.7 g of a brown solid. Purification by flash chromatography using CH_2Cl_2 gave 3.3 g of a solid material in 80% yield. ^{1}HNMR was identical to the product isolated from chlorine gas.

[$^{13}C_6$]-1,3,5-Tricholorobenzene

The above compound (1.6 g, 7.9 mmol) was dissolved slowly in a solution of 70% H_2SO_4 (8 ml). The solution was then cooled in an ice-bath to about −5°C and a solution of $NaNO_2$ (1.2 g, 17.4 mmol) in water (2 ml) was added slowly over 30 min. After stirring at −5 to 0°C for 30 min, a solution of H_3PO_2 (30% in water, 12 ml) was added dropwise in 20 min period and the resulting mixture was warmed slowly to room temperature and stirred for 12 h. The dark mixture was poured into ice-cold water and the resulting solid was filtered to give 1.4 g of a pink solid. Purification by flash chromatography using CH_2Cl_2 as eluant gave 1.3 g of a white fluffy solid in 88% yield. ^{1}H NMR ($CDCl_3$): δ7.27 (dm, J^1H-^{13}C = 172.01 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ135.54 (dt, J = 6.65, 67.83 Hz, C_1), 127.18 (dt, J = 8.71, 65.01 Hz, C_2). LCMS: ESI: MH^+ = 189 (100%).

[$^{13}C_6$]-3,5-Dichloroaniline

A mixture of the above trichlorobenzene (0.19 g, 1.01 mmol) $Pd_2(dba)_3$ (27 mg, 0.03 mmol), P(*t*-Bu)$_3$ (10 µl, 0.033 mmol, 90% tech.) in toluene (5 ml) was degassed and nitrogen was introduced. A solution of LiHMDS (1.0 M in THF, 1.1 ml) was added over 10 min and the mixture was heated to 85°C and stirred for 12 h. After cooling to room temperature, aqueous HCl

(1.0 N, 1.1 ml) was added slowly and the mixture was stirred for a few minutes. The mixture was then extracted with ether and the combined extracts were washed with aqueous NaOH (1.0 M, 50 ml), dried over $MgSO_4$, filtered and concentrated *in vacuo* to give 0.24 g of a dark solid. Purification by flash chromatography using 1–10% CH_3OH/CH_2Cl_2 gave 150 mg of an off-white solid in 87% yield. For larger quantities the product can be purified by sublimation. ^1H NMR ($CDCl_3$): δ6.72 (dm, J^1H-^{13}C = 172.87 Hz, 1H), 6.54 (dm, J^1H-^{13}C = 172.87 Hz, 2H), 3.78 (brs, NH_2). ^{13}C NMR ($CDCl_3$): δ148.19 (dt, J = 4.10, 62.60 Hz, C_1), 135.40 (dt, J = 4.10, 66.27 Hz, C_3), 118.26 (dt, J = 4.26, 66.27 Hz, C_4), 113.20 (dt, J = 4.26, 62.60 Hz, C_2). LCMS: ESI: MH^+ = 168.35 and 209.42 (MeCN adduct, 100%).

[$^{13}C_6$]-1,3-Diamino-5-chlorobenzene

[$^{13}C_6$]-1,3,5-Trichlorobenzene (195 mg, 1.04 mmol), $Pd_2(dba)_3$ (55 mg, 0.06 mmol), P(*t*-Bu)$_3$ (20 µl, 0.066 mmol, 90% tech.) in toluene (5 ml) was degassed before nitrogen was introduced. A solution of LiHMDS (1.0 M in THF, 2.2 ml) was added over 20 min and the mixture was heated to 85°C and stirred for 14 h. Work up as seen before and purification by flash chromatography using 1–10% CH_3OH/CH_2Cl_2 gave 90 mg of a yellow solid in 60% yield of the desired product. ^1H NMR ($CDCl_3$): δ6.09 (dm, J^1H-^{13}C = 167.87 Hz, 2H), 5.86 (dm, J^1H-^{13}C = 154.40 Hz, 1H), 3.61 (brs, 4H, $2NH_2$). ^{13}C NMR ($CDCl_3$): δ148.32 (dt, J = 3.85, 66.12 Hz, C_1 and C_3), 135.50 (dt, J = 5.16, 69.60 Hz, C_5), 105.85 (t, J = 68.00 Hz, C_4 and C_6), 99.64 (t, J = 66.00 Hz, C_2). LCMS: ESI: MH^+ = 149.46 (100%).

Conclusion

[$^{13}C_6$]-3,5-Dichloroaniline is prepared from the commercially available [$^{13}C_6$]-aniline or its hydrochloride salt in a three step synthesis and in 75% overall yield. This moiety can be introduced in the synthesis of drug candidates and used as an internal standard in DMPK studies or as a biomarker for compounds containing this motif.

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