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Research Article

An efficient large-scale synthesis of 1*H*-indazole-[3-^{14}C]carboxylic acid

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Abstract: An efficient large-scale carbon-14 synthesis of 1*H*-indazole-[3-^{14}C]carboxylic acid starting from [^{14}C]potassium cyanide is reported. Key transformations encountered during the synthesis include aromatic nucleophilic substitution of 2-nitrofluorobenzene by ethyl [^{14}C]cyanoacetate, a mild decarbethoxylation and an aniline nitrosation/cyclization. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: carbon-14; 1*H*-indazole-3-carboxylic acid; ethyl cyanoacetate

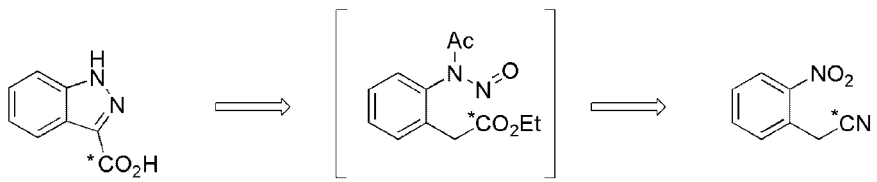
Introduction

We required ~200 mCi of 1*H*-indazole-[3-^{14}C]carboxylic acid (**1a**) for use as a labeled substructure of a Pfizer drug development candidate. Of numerous established non-isotope-labeled indazole synthesis strategies, we chose to exploit a nitrosation/cyclization approach to 1*H*-indazole-3-carboxylic acid reported by Yoshida *et al.*[1] for carbon-14 labeling because we anticipated it would support the introduction of the carboxylic acid function as a (labeled) nitrile. The ability of this approach to efficiently deliver 200 mCi of **1a** hinged on the development of a succinct and high yielding radiosynthesis of 2-(2-nitrophenyl)-[1-^{14}C]acetonitrile (**3a**) from K^{14}CN.

Results and Discussion

Our strategy for preparing **1a** (Scheme 1) necessitated the development of an efficient large-scale synthesis of **3a**. Our initial design for accessing **3** was to simply perform cyanide displacement on a benzyl bromide precursor. However, this chemistry proved largely inefficient for preparing **3b** when demonstrated with a single equivalent of KCN under a variety of reaction conditions. The benzylic carbon of **3b** is sufficiently acidic (facilitated by the *ortho* nitro function) to undergo further alkylation with available benzyl bromide and predominantly form over-alkylated byproducts.[2]

This result prompted us to investigate an alternative approach to **3a** that utilized ethyl [^{14}C]cyanoacetate to install the required carbon-14 nitrile as a labeled acetonitrile anion equivalent. Bromoacetic acid (**4**) was deprotonated with aqueous NaOH and the resulting anion was heated at reflux with 986 mCi (52 mCi/mmol, ~1 eq) of K^{14}CN (Scheme 2). After 3 h the reaction was cooled in an ice bath acidified with conc. HCl and concentrated to a residue. This residue was extracted with EtOH, treated with a catalytic



1a (* = ^{14}C)
1b (* = ^{12}C)

2a,b

3a,b

Scheme 1

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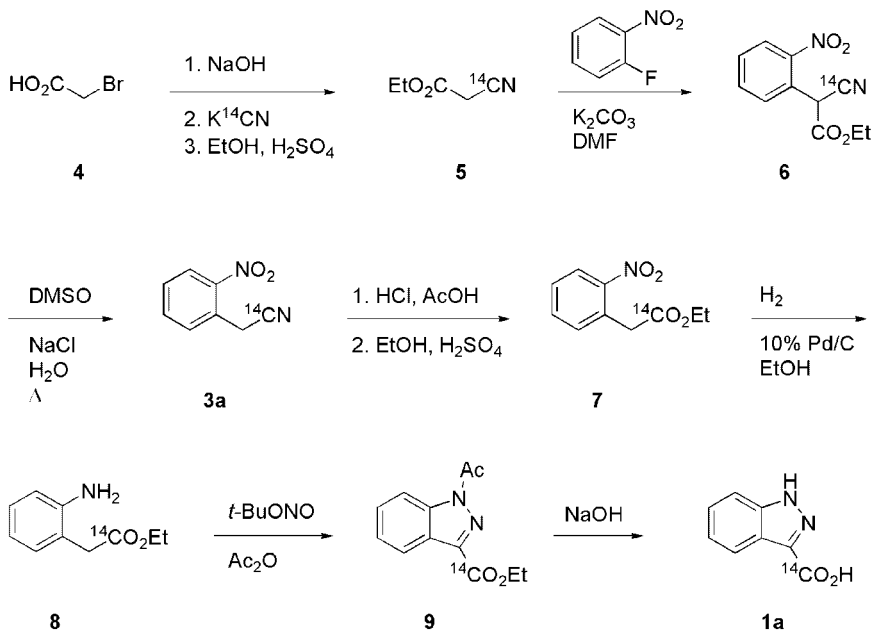

Scheme 2

amount of H_2SO_4 and heated at reflux. When the esterification was deemed complete by radio-thin larger chromatography (TLC) the product was isolated by extraction to afford 801 mCi (81%) of radiochemically pure **5**. Ethyl cyanoacetate has been shown to efficiently undergo aromatic nucleophilic substitutions on suitably functionalized aryl fluorides.[3] In the present case, **5** (801 mCi) was treated with K_2CO_3 and a slight excess of 2-fluoronitrobenzene in hot DMF to cleanly afford 783 mCi (98%) of nitrile ester **6**. To arrive at the desired substituted acetonitrile, decarbethoxylation was required without concurrent nitrile hydrolysis. Premature nitrile hydrolysis would result in a symmetrical $^{12}C/^{14}C$ diacid intermediate and subsequent loss of label and specific activity reduction. Krapcho decarboxylation conditions[4] effectively performed this operation, converting **6** to **3a** in good yield. Benzyl nitrile **3a** (759 mCi) was refluxed in concentrated HCl and acetic acid to effect nitrile hydrolysis, affording crude carboxylic acid which was esterified with ethanol to afford 710 mCi (94%) of 96% radiochemically pure nitroester **7** after extractive workup. Hydrogenation of **7** delivered aminoester **8** and this was treated with *t*-butylnitrite/acetic anhydride to form a transient nitroso intermediate **2a** that underwent rapid intramolecular cyclization to indazole **9** (539 mCi, 76% yield from **7**). Finally, 215 mCi of **9** was subjected to alkaline hydrolysis to yield 203 mCi (94%) of 1*H*-indazole-[3-^{14}C]carboxylic acid (**1a**) void of any chemical and radiochemical impurities.

Experimental

Materials and methods

$K^{14}CN$ (52 mCi/mmol) was purchased from PerkinElmer Life & Analytical Sciences. All remaining reagents and solvents were purchased from Sigma-Aldrich Corporation and used as received. Radioactivity measurements were performed on a Packard Tricarb 2900TR liquid scintillation analyzer using Beckman–Coulter Ready Safe liquid scintillation cocktail. TLC plates were analyzed on a Bioscan AR-2000 linear analyzer. Radio-high-performance liquid chromatography (HPLC) was performed using an Agilent 1100 Series HPLC system equipped with an Agilent 1100 Series diode array detector and an IN/US Systems B-RAM Model 2 radioactivity detector. Luna C18 3 μm, 50 × 4.6 mm, 210 nm, 100% 98:2 10 mM aqueous formic acid:AcN for 1 min, then linear gradient to 80% 2:98 10 mM aqueous formic acid AcN by 8 min, then return to 100% 98:2 10 mM aqueous formic acid:AcN by 8.10 min, 1 mL/min flow rate, HPLC solvent : Beckman–Coulter Ready Safe liquid scintillation cocktail (1:3).

Ethyl [^{14}C]cyanoacetate (5)

$K^{14}CN$ (986 mCi, 19.0 mmol, 52 mCi/mmol) was added to a solution of bromoacetic acid (2.90 g, 20.9 mmol) in 20 mL of 1 M aqueous NaOH and the resulting solution was heated at 100°C for 3 h. The reaction was then

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cooled in an ice bath, treated dropwise with 1.5 mL of concentrated aqueous HCl and evaporated to a solid residue. The residue was suspended in 50 mL of EtOH and filtered through a fritted glass funnel. The filtrate was treated with 1 mL of H_2SO_4 and heated at reflux for 7 h, at which time the esterification was shown to be complete by radio-TLC (EtOAc:hexanes 20:80) versus an authentic product standard. The reaction solution was concentrated to ~5 mL by rotary evaporation, treated with 50 mL of ice-cold saturated aqueous $NaHCO_3$ and extracted with 50 mL of EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to dryness to afford 801 mCi of **5** that was >99% radiochemically pure by radio-TLC (R_f = 0.2, EtOAc:hexanes 20:80).

Ethyl [2-^{14}C]cyano-2-(2-nitrophenyl)acetate (6)

2-Nitrofluorobenzene (2.40 g, 17.0 mmol) and K_2CO_3 (3.19 g, 23.0 mmol) were added to a solution of ethyl [^{14}C]cyanoacetate (**5**) (801 mCi, 15.4 mmol, 52 mCi/mmol) in 22 mL of DMSO. The resulting suspension was heated at 100°C for 4 h, at which time the reaction was deemed complete by radio-TLC (EtOAc:hexanes 20:80) versus an authentic product standard. The reaction was cooled to room temperature, diluted with 75 mL of EtOAc and washed with 50 mL of 1 N aqueous HCl, followed by brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness to afford 783 mCi of **6** that was 95% radiochemically pure by radio-TLC (R_f = 0.3, EtOAc: hexanes 20:80).

2-(2-Nitrophenyl)-[1-^{14}C]acetonitrile (3a)

NaCl (0.23 g, 3.9 mmol) was added to a solution of ethyl 2-[^{14}C]cyano-2-(2-nitrophenyl)acetate (**6**) (783 mCi, 15.1 mmol, 52 mCi/mmol) and water (2.3 g, 41.4 mmol) in 23 mL of DMSO. The reaction was heated at 140°C for 6 h and then cooled to room temperature and partitioned between 50 mL of EtOAc and 50 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness to yield 759 mCi of **3a** that was 93% radiochemically pure by radio-HPLC and co-eluted with an authentic product standard.

Ethyl 2-(2-nitrophenyl)-[1-^{14}C]acetate (7)

A solution of 2-(2-nitrophenyl)-[1-^{14}C]acetonitrile (**3a**) (759 mCi, 14.6 mmol, 52 mCi/mmol) in 6 mL of concentrated HCl, 1 mL of acetic acid and 1 mL of water was heated at reflux for 6 h and then concentrated to a residue. The residue was dissolved in 45 mL of ethanol and 1 mL of H_2SO_4 and heated at reflux for 36 h. The reaction solution was then concentrated to approximately 5 mL total volume by rotary evaporation and then partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness to yield 710 mCi of **7** that was 96% radiochemically pure by radio-TLC (R_f = 0.7, EtOAc:hexanes 40:60) versus an authentic product standard.

Ethyl 2-(2-aminophenyl)-[1-^{14}C]acetate (8)

A nitrogen purged solution of ethyl 2-(2-nitrophenyl)-[1-^{14}C]acetate (**7**) (710 mCi, 13.6 mmol, 52 mCi/mmol) in 20 mL of EtOH and 3 mL of IPA was treated with ~30 mg of 10% Pd/C and stirred under a balloon of hydrogen for 14 h. The reaction mixture was filtered and the filtrate was concentrated to dryness to yield 642 mCi of **8** that was 94% radiochemically pure by radio-HPLC and co-eluted with an authentic product standard.

Ethyl *N*-acetyl-1*H*-indazole-[3-^{14}C]carboxylate (9)

A solution of ethyl 2-(2-aminophenyl)-[1-^{14}C]acetate (**8**) (642 mCi, 12.3 mmol, 52 mCi/mmol) in 14 mL of toluene was treated with 4 mL of acetic anhydride and heated to 90°C. *t*-Butyl nitrite (1.9 mL) was added dropwise to the solution over 90 min. The reaction was then cooled to room temperature, concentrated to dryness and the resulting residue was dissolved in CH_2Cl_2 and washed with 5% aqueous K_2CO_3. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to yield 539 mCi of **9** that was 94% radiochemically pure by radio-HPLC and co-eluted with an authentic product standard.

1*H*-Indazole-[3-^{14}C]carboxylic acid (1a)

Ethyl *N*-acetyl-1*H*-indazole-3-[^{14}C]carboxylate (**9**) (215 mCi, 4.1 mmol, 52 mCi/mmol) was taken up in 10 mL of water and treated with 0.50 g of NaOH pellets. The resulting mixture was heated at reflux for 3 h at which time HPLC indicated that the hydrolysis was complete versus an authentic product standard. The reaction was cooled to room temperature and acidified with 20% aqueous HCl, causing solids to precipitate. The mixture was cooled to 0°C and product solids were collected by filtration, rinsing with water, to afford 0.64 g (203 mCi) of **1a** that was of >99% chemical and radiochemical purity by radio-HPLC and co-eluted with an authentic product standard.

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