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Synthesis of [$^{13}\text{C}_6$]3,4-diaminobenzoic acid as a precursor for stable isotope labeled benzimidazoles

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ABSTRACT

The synthesis of [$^{13}\text{C}_6$]3,4-diaminobenzoic acid from commercially available [$^{13}\text{C}_6$]aniline is described in six steps. Salient features of this route include the preparation of a differentially protected 3,4-diaminobenzonitrile, hydrogen gas free aromatic nitro group reduction with ammonium formate and facile benzimidazole ring closure of the ortho-arylenediamine with triethylorthoformate. This stable-labeled 3,4-diaminobenzoic acid is an ideal [M+6]isotopomer to synthesize complex benzimidazole fragments for mass spectrometry internal assays.

Keywords:

Benzimidazole
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Introduction

The synthesis of stable isotope labeled active pharmaceutical ingredients is a vital area of research for drug development.¹ Stable isotopomers are generally employed as LC-MS/MS internal standards to quantify parent API and metabolites in biological matrices.² Additionally, isotope-labeling experiments are often employed to determine metabolic reaction rates via measurement of kinetic isotope effects.³ Aside from pharmaceutical research, stable isotopomers are key research tools for the analysis of metabolic flux in complex plants and microorganisms.⁴

Benzimidazoles are a common building block for medicinal chemistry.⁵ The scaffold has been utilized in a variety of therapeutic areas such as the angiotensin II receptor antagonist Micardis (telmisartan), the proton pump inhibitor Prilosec (omeprazole), and the anthelmintic agents such as mebendazole and albendazole.⁶ Other benzimidazole containing drugs include: afobazole, bendamustine, bilastine, bisdisulizole, ciclobendazole, dabigatran etexilate, fenbendazole, etonitazene, flubendazole, nocodazole, pimobendan, rivoglitazone, vetmedin.

Given the prevalence of the benzimidazole scaffold in many therapeutic areas and the lack of commercially available carbon-13 labeled benzimidazole, we envisioned a straightforward synthetic route to produce an [M+6]isotopomer starting material from which other benzimidazoles could be prepared. When considering the general synthetic problem of benzimidazole synthesis the 1,2-diamine relationship is a necessity as well as plausible substitution of N, C2, C4, C5, C6, and C7.⁷ Due to

symmetry of the aromatic ring, introduction of a third substituent can yield either the 1,2,3- or 1,2,4-trisubstituted benzene ring. The synthesis of trisubstituted benzene rings is particularly challenging due to the regioselectivity issues encountered dictated by the electronic effects of ring substituents. Herein, a general solution for the preparation of a 1,2,4-trisubstituted benzene ring is presented for stable-labeled benzimidazole synthesis.

Figure 1. Representative medicinal agents containing the benzimidazole scaffold

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For the particular case of **1**, direct hydrogen to deuterium exchange at C2, C4, C6, and C7 could give at most the [M+4]isotopomer. While such chemistry rapidly produces a labeled compound, achieving uniform fidelity of exchange labeling is often difficult with mixtures observed in the parent manifold.⁸ To overcome this limitation and achieve strict isotopomer homogeneity, the commercially available uniformly ring labeled aniline **2** was employed. The retrosynthetic strategy is ultimately reduced to synthesizing a 3,4-diaminobenzoic acid **7**. The strategy relies on the amino group in aniline **2** to selectively direct iodination to the *para*-position. Nucleophilic aromatic substitution of iodine for cyanide using copper(I) cyanide then installs the acid functional group equivalent. Now, with the amino and cyano groups reinforcing direction to the 3-position, the second ring nitrogen is installed via nitration.

Figure 2. Retrosynthetic Analysis of **1**

Results and Discussion

As depicted in Figure 2, the synthesis commenced with the *para*-iodination of [¹³C₆]aniline **2** in aqueous sodium bicarbonate with molecular iodine affording [¹³C₆]4-iodoaniline **3** in quantitative yield.⁹ The symmetry of the ¹H and ¹³C NMRs confirmed selective formation of the *para*-substituted isomer with the C4 shift at 75.7 ppm. In order to attenuate the electron rich aniline for mononitration, the aromatic amine was protected as the acetamide with acetyl chloride in 87% yield.¹⁰

Scheme 1. Reagents and conditions: (a) I₂, NaHCO₃, H₂O, 0°C, 99%; (b) AcCl, Et₃N, CH₂Cl₂, 87%; (c) CuCN, DMF, 150°C, μ-wave, 30 min, 93%; (d) HNO₃, H₂SO₄, 5°C, 95%; (e) H₂SO₄, H₂O, Δ, 71%; (f) HCO₂NH₄, Pd/C, CH₃OH, Δ, 97%.

Continuing on with the synthesis, substitution of the iodide **3** with two equivalents of copper (I) cyanide in refluxing DMF using microwave heating provided the [¹³C₆]benzonitrile **4** in 93% yield. The infrared spectrum showed a strong nitrile absorption at 2220 cm⁻¹. The ¹³C signal of C4 shifted downfield to 104.6 ppm indicating substitution of the iodide for the electron-withdrawing cyano group.¹¹ The cooperative nature of the *o/p* directing acetamide and the *m*-directing nitrile set the stage for nitration adjacent to the acetamido group. To that end, nitration of **4** proceeded smoothly to [¹³C₆]nitro aromatic **5** in 95% yield with 1.8 equivalents of nitric acid in sulfuric acid by strictly maintaining the internal temperature below 10 °C. The ¹³C NMR at this stage showed six distinct signals with the symmetry of the aromatic ring now broken. In the subsequent step, concomitant acid mediated hydrolysis of the amide and nitrile groups with concentrated sulfuric acid at 140°C liberated the [¹³C₆]aminoacid **6** in 71% yield.

The nitro group of **6** was reduced with ammonium formate (5 equivalents) in methanol catalyzed by 10% palladium on carbon furnishing the [¹³C₆]diamine **7** in 97% yield.¹² The ease of this reaction and simple workup (filtration of catalyst and removal of solvent) makes it preferential to either high pressure hydrogenation (special equipment)¹³ or tin(II) chloride reduction (difficult workup protocol).¹⁴ The ¹³C NMR recorded two downfield signals at 133.5 ppm and 140.6 ppm thereby confirming the *ortho* relationship between the amines. Cyclodehydration of the *ortho*-arylenediamine **7** was conducted in refluxing toluene in the presence of triethylorthoformate (2 equivalents) to afford **1** in 92% yield. Due to near identical HPLC retention times of the diamine **7** and benzimidazole **1**, the reaction was assayed by mass spectrometry. After 2 hours, the diamine (*m/z* 158.97) was completely converted to the benzimidazole (*m/z* 168.98). Under the described conditions, none of the benzimidazole dimer **8** (¹²C₂¹³C₁₂H₁₂N₄O₂) resulting from intermolecular condensation of the diamine **7** was observed by mass spectrometry (*m/z* 280.13).

Scheme 2. Reagents and conditions: (a) (EtO)₃CH, PhCH₃, Δ.

Conclusion

A 6-step synthesis of uniformly ring labeled ¹³C ring labeled [M+6]3,4-diaminobenzoic acid **7** has been carried out on gram scale in 52% overall yield. It was shown that the diamine **7** can easily be transformed into benzimidazole **1** in 92% yield. This versatile route offers entry into 3,4-disubstituted benzoic acid derivatives proceeding through a differentially protected diamine **5**. The acid functionality at C5 imparts a functional handle for installation of oxygen (Baeyer-Villiger oxidation), nitrogen (Beckmann rearrangement) and sulfur (Baeyer-Villiger then Newman-Kwart *O* → *S* rearrangement).¹⁵ Additionally, the acidic benzimidazole NH can be readily alkylated or acylated. This route readily lends itself to ¹⁴C labeling at three junctures from commercially available radioisotopic building blocks:¹⁶ (1) Total

synthesis beginning with uniformly ring labeled [^{14}C -U]aniline, (2) Intercepting intermediate **3** by aryl cyanation with K^{14}CN , and (3) Cyclization of diamine **7** with [^{14}C]triethylorthoformate or synthetic equivalent.¹⁷ Each intermediate isolated in Scheme 2 is a solid, nearly eliminating volatility and exposure concerns, as well as avoiding chromatography, thus reducing the potential for disposing of expensive radioactive contaminated solvent waste.

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Supplementary Material

Supplementary material consisting of experimental procedures and spectral data associated with article can be found, in the online version at [http//](http://).

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