

Synthesis of [¹⁴C]Azodicarbonamide

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SUMMARY

[¹⁴C]Azodicarbonamide, a potent inhibitor of HIV-1 and HIV-2 was prepared by reaction of hydrazine sulfate with potassium [¹⁴C]cyanate, followed by oxidation of the resulting biurea. The overall radiochemical yield was 57% and the specific activity of the product was 1.48 GBq/mmol (39.9 mCi/mmol).

Key Words: [¹⁴C]Azodicarbonamide (ADA), HIV-1, HIV-2, inhibitor

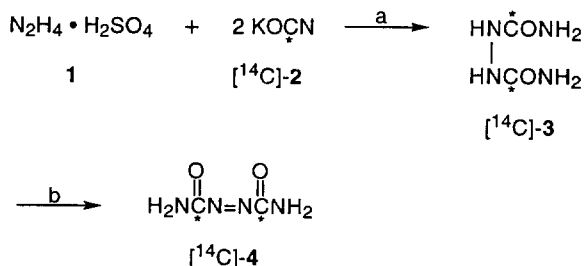
INTRODUCTION

Azodicarbonamide (ADA, **4**), in phase II clinical trials in Europe against the human immunodeficiency virus, specifically targets the zinc fingers of nucleocapsid p7 protein (NCp7) of HIV-1 and HIV-2.¹ These zinc fingers contain the highly conserved sequence Cys-X₂-Cys-X₄-His-X₄-Cys (CCHC). ADA electrophilically interacts with zinc-coordinating cysteines in CCHC, resulting in zinc ejection from the zinc finger and interruption of critical functions of NCp7 during virus assembly and the early phase of infection. The anticipated lack of viral resistance due to the conserved nature of the CCHC sequence makes these zinc fingers prime intervention points. Rice, et al.² also recently disclosed ADA as the first known compound to progress to human trials that inhibits HIV-1 and a broad range of retroviruses by targeting the nucleocapsid CCHC domains. We describe here the synthesis of carbon-14 labeled ADA for use in further pharmacological evaluation of this potential drug.

RESULTS AND DISCUSSION

The synthesis of [^{14}C]-ADA was adapted from the literature procedure³ used for preparing the nonlabeled compound. The reaction scheme is shown in Chart 1.

Chart 1



a) CH_3COOH , RT, 12 h

b) NH_4NO_3 , $\text{Cu}(\text{OAc})_2$, 115 °C, 10 min

The reported³ procedure for preparing biurea (3) involves reaction of hydrazine sulfate (1) with an excess of potassium cyanate (2) in the presence of a molar equivalent of acetic acid. Since 2 was the intended source of the carbon-14 label, we attempted the synthesis of 3 using a stoichiometric amount of 2 rather than the excess reported. Several experiments were run, but the yield of 3 was only 67% under the best conditions.

We next investigated whether or not potassium cyanate could be added in portions with the idea that less than an equivalent of labeled potassium cyanate could be added to the reaction mixture first followed by an excess of nonlabeled potassium cyanate to complete the reaction. In the first experiment 5.5 mmol of 2 was added to 5 mmol of 1; after 12 h an additional 5.5 mmol of 2 was added along with 5 mmol of acetic acid. This gave an 88% yield of 3. A second experiment was done exactly as the first except that the 5 mmol of acetic acid was added with the first portion of 2. This experiment afforded a 76% yield of 3. Based on these results, a tracer run was done on a 3.5 mmol scale using the conditions of the first experiment with the first addition being tracer labeled [^{14}C]-2 and the second addition being nonlabeled 2. This resulted in ~70% chemical yield but only 54% radiochemical yield of [^{14}C]-2. It appeared that a considerable portion of the first addition of 2 is lost due to decomposition. To overcome the problem

of low radiochemical yield, a second tracer run was done in which 1.8 mmol of non-labeled **2** was added to 3.5 mmol of **1**. After 1 h, 3.5 mmol of tracer [¹⁴C]-**2** was added, and after 4 h an additional 1.8 mmol of nonlabeled **2** and 3.5 mmol of acetic acid were added. Work up of this reaction afforded an 82% chemical and 90% radiochemical yield of **3**.

Two literature⁴ methods of oxidation of **3** to **4** were investigated. The most convenient, and the method that gave the best yields involved the copper(II) catalyzed ammonium nitrate oxidation^{4a} of **3**, which afforded an 86% yield of [¹⁴C]-**4** in the tracer run.

The final synthesis was carried out as described for the tracer runs. Stepwise reaction of [¹⁴C]-**2** with **1** afforded an 82% chemical yield of [¹⁴C]-**3**. This product was used without purification in the oxidation step to afford [¹⁴C]-**4** in 73% chemical yield. The overall chemical yield was 62%, and the overall radiochemical yield was 57%. The product was 98% radiochemically pure by HPLC⁵, and had IR and ¹H NMR spectra identical to an authentic sample.⁶ The specific activity was 39.9 mCi/mmol (1.48 GBq/mmol).

EXPERIMENTAL

Reagent grade chemicals were purchased from commercial suppliers and were used without further purification. ¹H NMR spectra were recorded on a Bruker AM500 spectrometer in DMSO d₆. Radioactive samples were counted on a Packard Tricarb 4000 liquid scintillation counter. Analytical TLC were performed on E. Merck silica gel 60F-254 (plates). Developed TLC plates were scanned on a Bioscan System 200 Imaging Scanner. HPLC-RAM was done using a Waters Associates Model 510 dual pump system with a Waters U6K septumless injector, and IN/US Systems, Inc. Model 2075 β RAM Flow-Through Radioactivity Monitor.

[¹⁴C]Biurea (**3**)

A solution of hydrazine sulfate (312 mg, 2.4 mmol) and nonlabeled potassium cyanate (113 mg, 1.4 mmol) in 2 mL of distilled water was stirred for 1 h at ambient temperature. A solution of potassium [¹⁴C]cyanate (2.4 mmol, 97 mCi) in 7 mL of water was added via syringe over a 15 min period. The reaction was allowed to stir for 4 h at ambient temperature. A solution of nonlabeled potassium cyanate (112 mg, 1.4 mmol) in 0.5 mL of water was added via syringe with constant stirring. Glacial acetic acid

(144 mg, 2.4 mmol) was added to the reaction mixture. The evolved carbon dioxide was collected in a barium carbonate trap. The mixture was allowed to stir overnight at ambient temperature. The precipitated biurea (**3**) was collected by centrifugation and washed with cold (0 °C) water, ethanol and ethyl ether. The **3** was dried under vacuum to afford 232 mg (1.9 mmol, 82% chemical yield) of material suitable for use in the next reaction.

[¹⁴C]Azidodicarbonamide (**4**)

A solution of **3** (230 mg, 1.9 mmol) and ammonium nitrate (230 mg, 2.8 mmol) in 5 mL of glacial acetic acid and copper(II) acetate (5 mg) was brought to reflux and stirred for 10 min. The reaction was cooled to 45 °C. The product was collected by centrifugation and washed with 2 x 2 mL of cold (0 °C) water and dried under vacuum overnight to afford 164 mg (73% chemical yield) of **4** as an orange powder. The radiochemical yield for the two steps was 57%. Analysis of **4** by HPLC (Waters Assoc. radial pak silica gel 10 μ, 8 x 100 mm, methanol-ether 2:98, 1.0 mL/min, UV-275 nm, *t_R* 14 min:42 sec) showed it to be 98% radiochemically pure, mp 223d °C and ¹H NMR (identical with a standard sample⁶). The specific activity was 39.9 mCi/mmol (1.48 GBq/mmol).

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