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Synthesis of a labeled inhibitor of HIV-1 attachment: 1-(4-benzoylpiperazin-1-yl)-2-(4,7-dimethoxy-1H-pyrrolo[2,3-c]pyridinyl-3-yl-[U-^{14}C]ethane-1,2-dione, BMS-488043-^{14}C

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BMS-488043 is a potent inhibitor of the interaction between HIV-1 gp120 and the host cell receptor CD4. We prepared the carbon-14-labeled version to support preclinical studies of the compound. It was prepared from ethyl [U-^{14}C]oxalyl chloride by a sequence using Friedel–Crafts acylation reaction. The overall radiochemical yield was 82% with a purity of $>$99.9%.

Keywords: HIV-1; attachment inhibitor; gp120-CD4 interaction inhibitor

Introduction

The emergence of resistance, mutant strains, adverse drug side-effects and drug toxicities from long-term use, are all major challenges to the sustained efficacy of current antiretroviral therapies and the goal of eradicating HIV.[1] Although combination drug therapies, referred to as HAART, and other treatment modalities aimed at these challenges have undeniably reduced mortality and extended the lives of patients,[2] the inability to sustain the control of viral replication[3] eventually results in treatment failure. There is therefore need for new classes of antiretroviral drugs with distinct mechanisms of action that will improve the options available to these patients. BMS-488043 is a novel HIV-CD4 attachment inhibitor.[4] It acts by interfering in the gp120 binding to cellular CD4 receptors.[5] The conformational change induced by the pre-CD4 bound envelope prevents gp120-CD4 interactions and the downstream events leading to HIV-1 infection and viral replication. BMS-488043, in Figure 1, has demonstrated proof of concept antiviral efficacy for this mechanism in the clinic and the safety profile in HIV-infected subjects was favorable.[6] Further preclinical characterization of BMS-488043 required the carbon-14 isotopomer and we have synthesized it in a short high-yielding sequence from ethyl [U-^{14}C]oxalyl chloride. Herein, we are discussing the details of the preparation (Scheme 1).

Experimental

All reactions were carried out under an atmosphere of argon unless otherwise specified. Solvents were commercial grade and used without purification or drying. Column chromatography was carried out on Merck Kiesegel 60 (230 μ) silica gel. Flash chromatographic separations were performed on a Biotage Flash System using a pre-packed silica gel cartridge. TLC visualization reagents included (10% iodine plus 10% AcOH) in 40% aqueous KI and Cerium sulfate in 10% Sulfuric acid. ^{1}H NMR spectra were recorded at 300, 400 or 500 MHz. Chemical shifts are given in ppm relative to tetramethylsilane (TMS). Agilent 1100 HPLC System including solvent degasser, pump, automated injector, and a variable UV detector connected to IN/US BetaRam model 4 Flow Detector with a 0.25 mL detector cell was used in the analyses of compounds. The HPLC column was Waters Xterra Ms C8, 5 μ, 4.6 × 250 mm. Elution Solvent System A = 10 mM Ammonium acetate, and B = Acetonitrile under Gradient Condition of 0–8 min, 30–60% B; 8–12 min, 60–30% B, and a Flow rate of 1.0 mL/min, with detection by UV at 254 and 280 nm. Radiochemical Counting was performed on a Packard 574 Liquid Scintillation Analyzer using Beckman Readi-Solv MP cocktail. LC/MS analysis was performed on a Finnigan LXQ Mass Spectrometer System, LC/MS method: Generic-B20 (+p ESI full mass). Ethyl [U-^{14}C]oxalyl chloride (3.70 GBq, 100 mCi) was purchased from Amersham Biosciences UK Limited.

2-(4,7-Dimethoxy-1H-pyrrolo[2,3-c]pyridine-3-yl)-2-[U-^{14}C] oxoacetic acid 3

4,7-Dimethoxy-*1H*-pyrrolo[*2,3-c*]pyridine hydrochloride (1.22 g, 5.65 mmol) was added to a stirred suspension of anhydrous aluminum chloride (2.26 g, 16.95 mmol) in nitromethane (25 mL) under nitrogen atmosphere. The mixture was stirred for 8 min and ethyl [U-^{14}C]oxalyl chloride (100 mCi, Sp. Act. 115 mCi/mmol, diluted with 'cold' to 653 mg, (532.2 μL) 5.65 mmol)in dry dichloromethane (2 mL) was added in one portion. After the reaction was stirred for 30 min at room temperature, it was quenched by pouring carefully onto 20% ammonium acetate

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solution in water. The mixture was stirred for additional 10 min and extracted with methylene chloride (3 × 20 mL). Evaporation of the organic extract gave a solid **2**, indicated by HPLC to be >98% radiochemically pure and determined by counting to be ~quantitative (>99.6 mCi) yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (tr, 3H), 3.90 (s, 3H), 4.02 (s, 3H), 4.36 (q, 2H, J = 7.1 Hz), 7.45 (s, 1H), 8.15 (d, 1H, J = 3.4 Hz), 9.74 (brs, 1H). HPLC (Waters Xterra MS C8, 5 micron 4.6 × 250 mm column, Solvent system A = 10 mM Ammonium acetate, B = Acetonitrile, Gradient Run 0–8 min 30–60% B, 8–12 min 60–30% B, Flow rate 1 mL/min at UV detection 254 nm, elution at Retention time of 7 min, 98.6% chemical purity. The material **2** was dissolved in methanol (50 mL) and while stirring 0.1 M NaOH (12 mL) was added. After the hydrolysis was completed, as indicated by HPLC, the organic solvent was evaporated completely under reduced pressure, the remaining aqueous phase was washed with ethyl acetate (2 × 30 mL) and the organic portion was discarded. Acidification of the aqueous portion with 1N HCl (12 mL) yielded solid that was collected and dried under high vacuum to give **3** (2.27 g, Sp. Act. 43.19 μCi/mg, 98.17 mCi, 98.17%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 3.82 (s, 3H), 3.99 (s, 3H), 7.45 (s, 1H), 8.17 (d, 1H, J = 3.4 Hz), 13.05 (brs, 1H).

1-(4-Benzoylpiperazin-1-yl)-2-(4,7-dimethoxy-1H-pyrrolo [2,3-c]pyridin-3-yl)[U-^{14}C]ethane-1,2-dion 4, [^{14}C]-BMS-488043

To a suspension of **3** (780 mg, 2.16 mmol, 33.69 mCi, Sp. Act. 43.19 μC/mg) and HATU (1.232 g, 3.24 mmol, 1.5 eq) in dry DMF: DCM (1:1 v/v), (20 mL) was added a mixture of DMAP (791.6 mg, 6.48 mmol, 3.0 equiv), and **5**, BMS-19848, (734.5 mg, 3.24 mmol). The mixture was stirred for 4 h under nitrogen at room temperature and concentrated to a residue. This material was applied to a pre-column packed with C_{18} silica gel and it was connected to C_{18} separation column. After equilibration with water (1000 mL) the compound was eluted by step-gradient method with 15, 30, 45 and 60% acetonitrile in water (500 mL) each time. The radioactive fractions (at 45% solvent effluent) were checked by HPLC method and the pure fractions were combined. Methanol and toluene were added to aid the evaporation of acetonitrile under high vacuum at room temperature. A white solid separated after the organic solvent was evaporated. It was collected by filtration, dried under high vacuum overnight to give **4** [^{14}C]-BMS-488043 (661 mg, 28.15 mCi, Sp.Act. 42.6 μCi/mg, 18.0 mCi/mmol, 83.5%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 1.56 (s), 3.51(br), 3.76 (br), 3.93 (s, 3H), 4.04 (s, 3H), 7.42, 7.46, 8.01 (d, 1H), 9.25 (brs, 1H). By HPLC (conditions as in foregoing experiment) compound co-eluted with authentic reference at Retention time of 6 min, Purity was determined to be >99.9%.

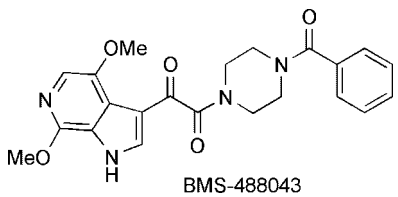
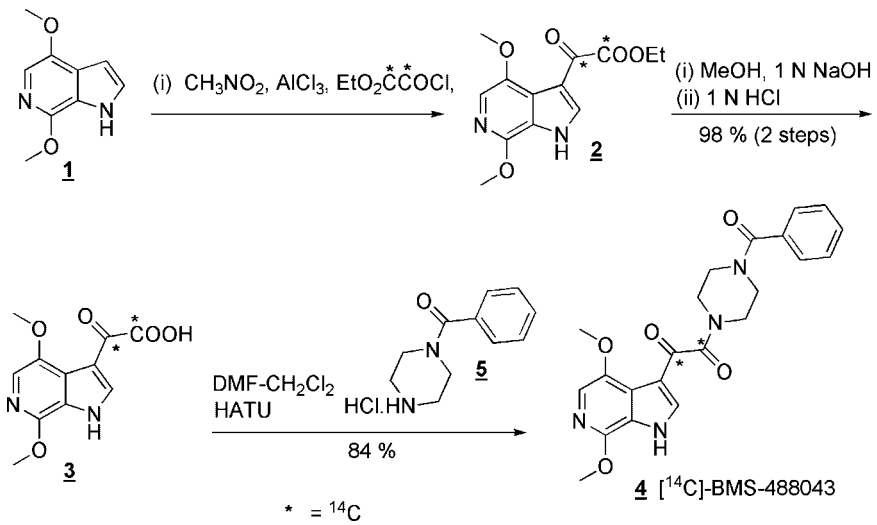


Figure 1. HIV-CD4 attachment inhibitor.

Results and discussion

In designing the current synthesis we wanted to be able to take advantage of known intermediates from the existing work in the HIV attachment program.[5] From this perspective, the bridging of the two halves of BMS-488043 with a label from elements of oxalyl chloride was considered favorable. It is known that 4,7-dimethoxy-*1H*-pyrrolo[*2,3-c*]pyridine BMS-544347 **1** could be C-acylated with ethyl oxalyl chloride to produce ethyl 2-(4,7-dimethoxy-*1H*-pyrrolo[*2,3-c*]pyridine-3-yl)-2-oxoacetate **2**. The free acid **3** from this product could then be coupled to phenyl(piperazin-1-yl)methanone to afford BMS-488043. While developing the reaction sequence outlined above we encountered conditions that would not give acceptable yields of labeled product. A more practical sequence that includes changes to the isolation protocol had to be developed. The Friedel–Crafts reaction of ethyl oxalyl chloride with 4,7-dimethoxy-*1H*-pyrrolo-[*2,3-c*]pyridine **1** to make the ethyl pyrrolo-oxoacetic ester according to the process reported in a patent application[5] produced product yields less than ideal for the preparation of labeled material. The reaction of these compounds in dichloromethane resulted in extensive polymerization which complicated product recovery. However, with nitromethane as the reaction medium, the reaction proceeded smoothly to provide the desired product in excellent yield. It was ascertained in our studies that about 5% v/v



Scheme 1. Preparation of [^{14}C]-BMS-488043.

dichloromethane, which we would need to transfer the labeled reagent, did not adversely affect the yield of the desired product. The optimized reaction conditions involved mixing compound **1** and aluminum chloride in anhydrous nitromethane and, reacting with ethyl [U-^{14}C]oxalyl chloride, added at room temperature as a solution in DCM, to furnish the -pyrrolo[*2,3-c*]pyridine 3-yl)-2-[U-^{14}C]oxoacetic acid ethyl ester **2** in 99.6% radiochemical yield. The ester grouping was removed by hydrolysis and acidification to provide the free acid **3** in a total yield of 98% for the two steps. The free acid **3** was activated with HATU and treated with **5**, BMS-196848, in the presence of 3 eq of DMAP in a reaction which proceeded quantitatively to the desired product. However, the isolation of product was burdensome and recovery was poor. It seemed that the product might not be amenable to the extraction method used for isolation and therefore we examined the use of a C_{18}-column to separate the reaction debris and isolate the pure product in a few fractions. In the event, the product separated as a solid upon the concentration of pure fractions under reduced pressure, allowing us to collect the product by filtration. The foregoing procedure afforded 84% yield of **4** [^{14}C]-BMS-488043. The overall reaction yield from ethyl [U-^{14}C]oxalyl chloride was found to be 82%. All of the compounds were identified by a combination of chromatographic comparison with authentic material where available, or with the appropriate products from pre-labeling studies as reference, and by matching the spectroscopic data of references with those of the labeled versions.

Conclusion

We have synthesized [^{14}C]-BMS-488043 from commercially obtained uniformly labeled ethyl oxalyl-[$^{14}C_2$] acid chloride in 82% overall yield. By switching to nitromethane we avoided the troublesome polymerization observed in dichloromethane as the solvent for the Friedel–Crafts reaction. Along with the foregoing the reverse phase isolation of product was advantageous in obtaining high yield of [^{14}C]-BMS488043.

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