Journal of Fluorine Chemistry 155 (2013) 110–117

Contents lists available at SciVerse ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Improved synthesis of the hypoxia probe 5-deutero-5-fluoro-5-deoxy-azomycin arabinoside (FAZA) as a model process for tritium radiolabeling

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ARTICLE INFO

Article history:
Received 22 April 2013
Accepted 20 June 2013
Available online 29 June 2013

Keywords:
Hypoxia
PET imaging
Deuterium
Tritium
FAZA

ABSTRACT

Tritium-labelled fluoroazomycin arabinoside, [^{3}H]-FAZA, is a useful probe for the investigation of hypoxia, furthermore it is safer and easier to handle than the PET tracer [^{18}F]-FAZA when used in cell based assays. The only known synthesis of deuterium- and tritium-labelled FAZA was re-investigated and optimized. Then, a new and improved synthesis of [^{2}H]-FAZA was developed as a model process for tritium radiolabelling. This novel synthesis is expected to greatly facilitate access to [^{3}H]-FAZA.

1. Introduction

Most tumour types feature hypoxic (low oxygen) regions, and in some neoplastic pathology hypoxia may be present in up to 60% of patients [1]. Generally, tumours with high hypoxic volumes have a poor prognosis because they are associated with an aggressive phenotype and increased risk of metastasis [2]. Furthermore these tumours tend to respond poorly to radiotherapy and/or chemotherapy [3]. Accordingly, tumour hypoxia is a key driver of tumour growth, proliferation, maintenance and resistance to therapy. For the reasons above, a robust and reliable method to identify hypoxia in tumours would have significant value as a predictive biomarker to identify patients and tumours likely to be non-responsive to chemotherapy and radiotherapy. PET imaging is a particularly attractive option to study hypoxia, as it is non-invasive, quantitative, does not require biopsy tissue, and can provide information across the entire tumour [4]. In the last decades, several PET and SPECT hypoxia markers have been developed, many of which are based on the use of nitroimidazole derivatives as first proposed by Chapman in 1979 [5]. Nitroimidazole tracers are able to detect tumour hypoxia since they accumulate in hypoxic tissues by a bio reductive linkage mechanism. The nitroso and hydroxylamino-imidazole metabolites produced in hypoxic tissues covalently bind to cellular molecules therefore trace quantities of labelled compounds are chemically bound to hypoxic cells [6].

Among the fluorinated hypoxia PET tracers, [^{18}F]-FAZA, (fluoroazomycin arabinoside **1**, Fig. 1) developed by Kumar in 1998 [7], was found to be useful for imaging hypoxia in various tumours such as glioblastoma, with a remarkably high tumour-to-background and it can be considered the "gold standard" for the measurement of tissue hypoxia [8].

Although ^{18}F-FAZA is nowadays produced in many clinical PET facilities and the ^{18}F radiosynthesis has been widely investigated, optimized, and automated [9], the deuterium (**2**) and tritium labelled (**3**) parent compounds are not commercially available. [^{3}H]-FAZA is an attractive molecular tool for *in vitro* studies and comparison of different and novel hypoxia tracers. Moreover it is safer and much easier to handle than [^{18}F]-FAZA when used in cell based assays. Last but not least, [^{3}H]-FAZA can be conveniently stored for many years (tritium half-life is 12.32 years).

Here we report our studies towards the synthesis of the deuterium labelled [^{2}H]-FAZA **2**, which was obtained by two different synthetic strategies, as a model process for [^{3}H]-FAZA synthesis. The first route is based on a modification of the Kumar method [7], the only one reported to date for the synthesis of **2** and **3**, which unfortunately in our hands could not be reproduced as originally described by the authors. The second is an optimized alternative route to **2** based on the use of different protective

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http://dx.doi.org/10.1016/j.jfluchem.2013.06.013

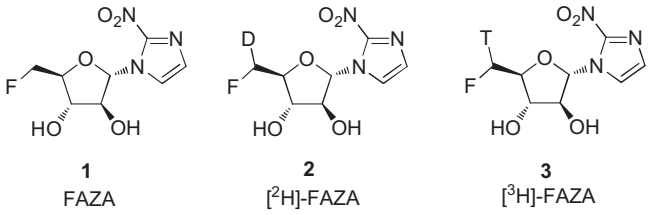


Fig. 1. FAZA and its labelled derivatives.

groups, which should represent a more efficient access to [^{3}H]-FAZA **3**.

2. Results and discussion

2.1. Synthesis of FAZA *1*

Our synthesis of FAZA (**1**) is based on the coupling of 2-nitro-1-(triethylsilyl)imidazole **4** with the protected arabinose derivative **5** (Scheme 1), according to the strategy published by Schweifer and Hammerschmidt [10]. The key intermediate **6** was then converted into FAZA (**1**) following the pathway proposed by Kumar and co-workers [7].

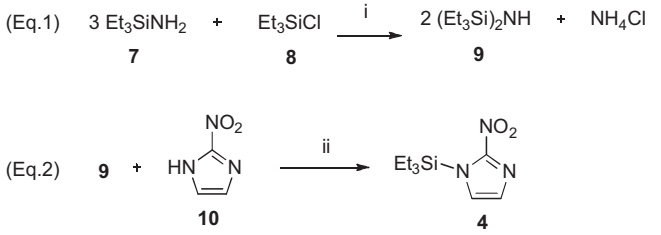
Both starting substrates **4** (Scheme 2) and **5** (Scheme 3) had to be prepared. Hexaethyldisilazane **9** (Scheme 2, Eq. 1) was prepared from (triethylsilyl)amine **7** and chlorotriethylsilane **8** in the presence of a catalytic amount of triethylsilyl triflate in dry toluene. A refluxing mixture of 2-nitroimidazole (**10**) and hexaethyldisilazane **9** in pyridine afforded quantitatively the desired substrate **4** (Scheme 2, Eq. 2) [10].

The synthesis of FAZA (**1**) (Scheme 3) started from D-arabinose (**11**) which was fully protected obtaining a mixture of anomeric acetates **5** (α:β = 60:40) [10]. The coupling reaction between 2-nitro-1-(triethylsilyl)imidazole **4** and the anomers **5** afforded the nitroimidazole D-arabinofuranose derivative **6** that was subjected to selective deprotection of the primary hydroxyl group in order to obtain the compound **12** [10]. The subsequent deoxyfluorination was achieved using diethylaminosulfur trifluoride (DAST) in DCM, which gave the protected fluorinated precursor **13** that was then fully deprotected with ammonia in methanol giving FAZA (**1**) [7].

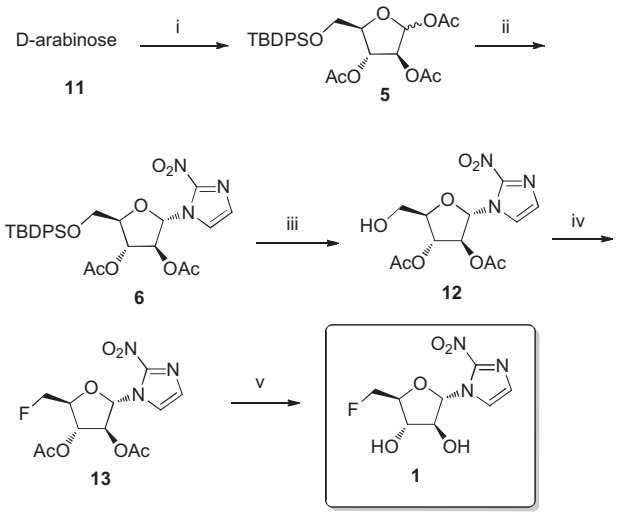
2.2. Synthesis of [^{2}H]-FAZA *2*

Once the synthesis of FAZA **1** was optimized, we switched to the synthesis of the deuterated analogue **2**, initially following the procedure reported by Kumar and co-workers [7]. The retro-synthetic approach (Scheme 4) involved the oxidation of the alcohol **12** to the corresponding aldehyde **14a**, followed by reduction with a deuterating agent to provide the deuterated alcohol **15a**. Subsequently, using the same reactions used for the synthesis of FAZA, we planned to obtain our target molecule **2**.

However, in our hands the oxidation step from **12** to **14a** (Scheme 5) could not be reproduced following the conditions (Pfitzner–Moffatt oxidation) described by Kumar et al. [7]. It is worth noting that the Kumar's protocol involved the treatment of **12** "dissolved in anhydrous dimethyl sulfoxide" with DCC followed



Scheme 2. *Reagent and conditions*: (i) TfOTES, toluene (79%) and (ii) Pyridine, reflux (quantit.).



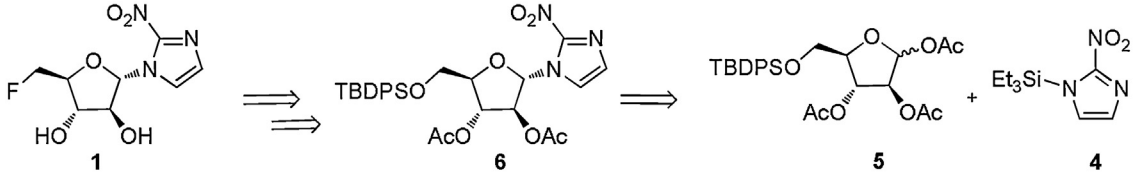
Scheme 3. Synthesis of FAZA. *Reagent and conditions*: (i) (1) TBDPSCl, Py, −20 °C; (2) Ac_2O, rt (71% over 2 steps); (ii) 4, TfOTES, CH_3CN, −8 °C (72%); (iii) KF, benzoic acid, CH_3CN, reflux (80%); and (iv) DAST, DCM, rt (50%); v: 2 N NH_3 in MeOH, 4 °C (60%).

by cooling on an ice bath, which not surprisingly in our hands resulted in a solid mixture and no reaction. Upon heating until melting of the mixture, we observed the formation of a complex mixture of unidentified products, in which we could not identify the desired aldehyde **14a**.

Stimulated by the need of a more effective and reliable procedure, we first modified the conditions of the Pfitzner–Moffatt oxidation as shown in Scheme 6 and Table 1, entry 1. We obtained the desired aldehyde **14a** but also a consistent quantity of the by-product **14b**, arising from elimination of the acetate in position 3 of the arabinose ring. Unfortunately these two aldehydes could be separated neither by flash chromatography nor by HPLC.

At that point we investigated different oxidation conditions with the aim of suppressing the formation of the by-product **14b** (Table 1, entries 2–6). Eventually, the best conditions we were able to identify afforded a more acceptable 30% of the undesired aldehyde **14b** (entry 3).

The inseparable mixture of the two aldehydes **14a** and **14b** (Scheme 7) was then reduced with deuterated sodium cyanoborohydride ($NaBD_3CN$) affording the two deuterated alcohols **15a** and



Scheme 1. Retro-synthesis of FAZA **1**.