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A concise and efficient synthesis of flumazenil and its precursor for radiolabeling with fluorine-18

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ABSTRACT

Presently there is a strong interest in developing radioligands for in vivo imaging the $GABA_A$-Bz site with positron emission tomography (PET). Flumazenil (**1**), a high-affinity $GABA_A$-Bz site inverse agonist, is amenable for ^{11}C and ^{18}F-labeling. The current methods for synthesis of **1** and its precursor for ^{18}F-labeling are not ideal and restrict structure–activity relationship (SAR) development. Herein we present a novel and less troublesome synthesis of **1** and its cognates to aid in the development of improved radioligands for PET imaging of $GABA_A$-Bz site.

1. Introduction

The brain's γ-aminobutyric acid sub-type A ($GABA_A$) receptor is a membrane-bound heteromeric protein comprising α, β, and γ sub-units.[1] It is one of the principal binding sites for GABA, a major inhibitory neurotransmitter. A distinct ligand class (i.e., benzodiazepines) binds to a specific region on the $GABA_A$ receptor, known as the benzodiazepine (Bz) site.[2] This site is located between the α and the γ sub-units, whereas the binding site for GABA is located between the α and the β sub-units. The $GABA_A$ is a ligand-gated ion channel and ionotropic receptor. Agonist modulation of the Bz site enhances GABA's efficacy at the $GABA_A$ receptors, resulting in reduced activity of the neuron.[3] The $GABA_A$-Bz site is likely involved in several neurophysiologic processes, such as anxiety,[4] learning,[5] and memory.[5] Imaging the $GABA_A$-Bz site non-invasively with positron emission tomography (PET) under control and diseased conditions will help clarify its neurophysiologic role.

The imidazobenzodiazepine, Flumazenil (**1**, Fig. 1)[6], is a high-affinity $GABA_A$-Bz site antagonist that has been used clinically to treat benzodiazepine intoxication.[7] **1** is capable of being labeled with positron emitters (Fig. 1), carbon-11 ($t_{1/2}$ = 20.4 min), and fluorine-18 ($t_{1/2}$ = 109.8 min).[8] Over the past 20 years, [^{11}C]**1** (Fig. 1) has been used to image and quantify the $GABA_A$-Bz site with PET. More recently, [^{18}F]**1** (Fig. 1) has been developed as a suitable and in some cases a superior alternative.[9,10] However radiolabeling of **1** with fluorine-18 requires a difficult to obtain and costly precursor, Nitromazenil (**2**, Fig. 1).

The current methods for the syntheses of **1** and its cognates are not ideal and restrict certain structure–activity relationship (SAR) development within the imidazobenzodiazepine platform. For example, little is known about the effects of certain functionalities at the amide nitrogen. The prototypical synthetic method[11–14] of **1** and its cognates (Scheme 1) starts by reacting the appropriate isatoic anhydride with sarcosine in dimethyl sulfoxide yielding the amide (**3**). In the next step, **3** is converted to a iminophosphate (**4**) or iminochloride (**5**). Iminophosphates and iminochlorides are unstable, and as a result, they could be troublesome in large-scale reactions.[14] Treatment of **4** or **5** with ethyl isocyanoacetate under strongly basic conditions gives **1** or one of its cognates.

Herein we sought to develop a more efficient and less troublesome synthetic method of **1** and **2** for use in molecular imaging.

2. Results and discussion

To start, ethyl 4-methyl-5-imidazolecarboxylate (**6**) and methyl 2-fluoro-5-nitrobenzoate (**7**) were identified as attractive precursors for generating imidazobenzodiazapine backbone (**8**), while allowing next-step reactivity. It was reasonable to expect that under basic conditions, the anion of **6** would react with **7** by S_NAr mechanism that is facilitated by Meisenheimer complex (Scheme 2). Although **6** is commercially available and relatively cheap, **7** requires a one-step synthesis by esterification of commercially available 2-fluoro-5-nitrobenzoic acid.[15] The reaction was carried out in

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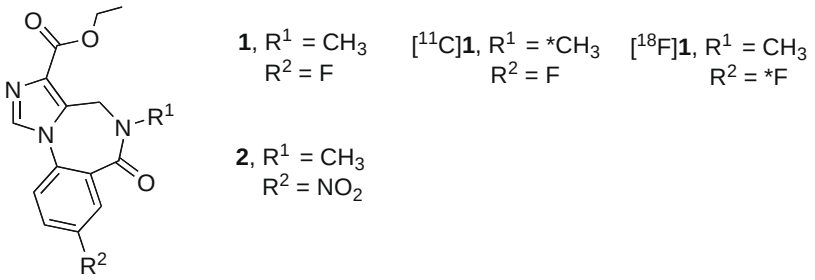
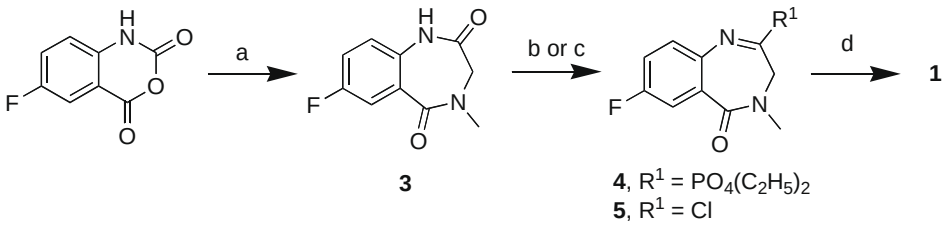
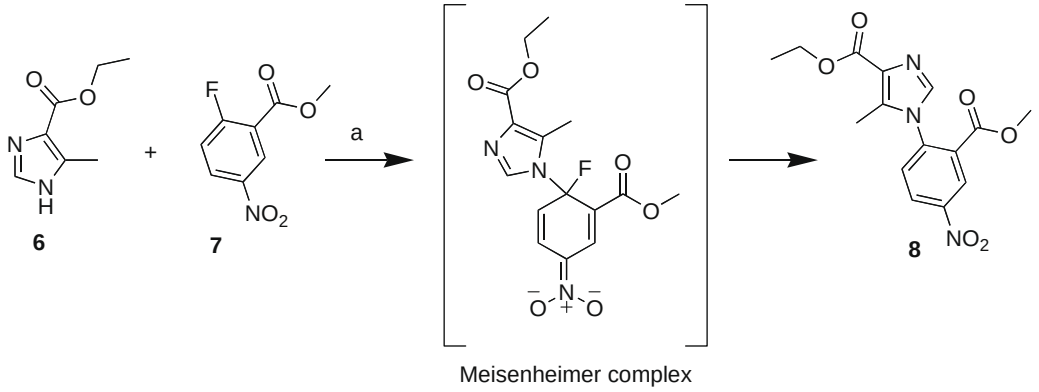


Figure 1. Structures of **1** (Flumazenil), [^{11}C]**1**, [^{18}F]**1** and **2** (Nitromazenil). Asterisks denote position of radiolabel.



Scheme 1. Prototypical synthesis of **1**. Reagents and conditions: (a) sarcosine, DMSO, 140 °C; (b) $POCl_3$, toluene, *N*,*N*-dimethyl-*p*-toluidine, 100 °C; (c) NaH, THF, DMF, $(EtO)_2PCl$; (d) NaH, DMF, $CNCH_2CO_2Et$.



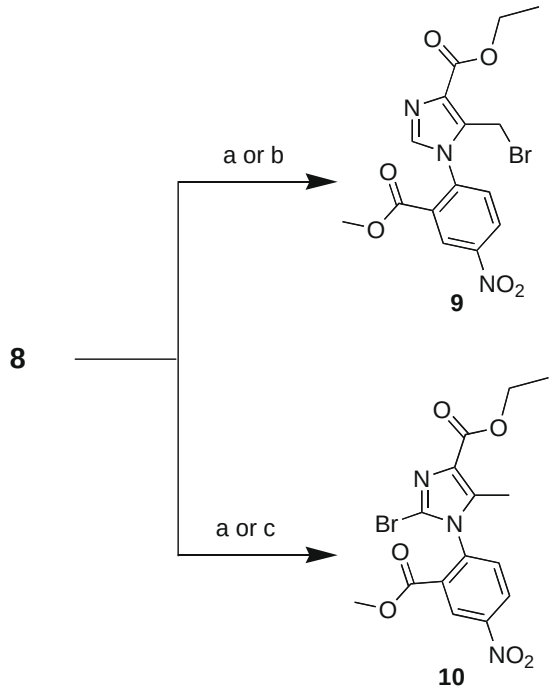
Scheme 2. Synthesis of **8**. Reagents, condition and yield: (a) DMSO, Cs_2CO_3, rt, 58%.

polar-aprotic solvent, DMSO, to increase the nucleophilicity of anionic-**7** and simplify the work-up procedure. DMSO is completely miscible in water. Hence, addition of reaction mixture to excess water allowed for simple vacuum filtration of precipitated product (**8**). ^{1}H NMR and TLC analysis of the crude detected only one out of two possible regioisomeric products, **8** and ethyl 1-(2-(methoxycarbonyl)-4-nitrophenyl)-4-methyl-1*H*-imidazole-5-carboxylate. Since **2** can only be formed by **8**, regioselectivity would be established in the subsequent reaction steps.

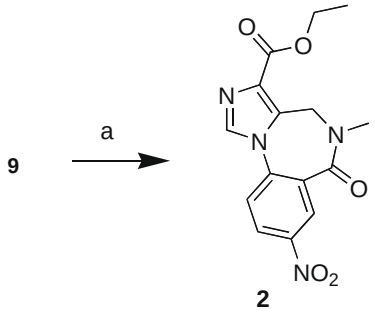
In the next step, the methyl imidazole functionality of **8** was exploited due to its susceptibility for oxidation. By the application of Wohl–Ziegler reaction, various allylic and aryl methyl groups may undergo selective bromination by addition of *N*-bromosuccinamide (NBS) and radical initiator (e.g., benzoyl peroxide).[16] In most cases, this reaction needs to be carried out using a non-polar solvent (e.g., benzene) in order to limit the amount of formed bromine. The Wohl–Ziegler reaction may be catalyzed by increased temperature or light irradiation. Under conditions in which **8** was dissolved in benzene and refluxed with NBS-benzoyl peroxide, the amount of formed **9** (Scheme 3) was variable and in some cases yielded a by-product (**10**, Scheme 3). It was reasoned that **10** was formed by electrophilic aromatic substitution (EAS) with bromine. To test this hypothesis an analytical sample of **10** was prepared by refluxing **8** with NBS-benzoyl peroxide in acetonitrile, as polar solvent that favors the formation of bromine. More consistent yields of **9** were achieved by using light irradiation as a catalyst.

Previous studies have shown that seven-membered ring lactam formation can occur with intramolecular methylene bromide and methyl ester functionalities.[17] Here it was reasoned that the formation of **2** would be achieved under similar reaction conditions. Indeed by heating the isolated **9** and methylamine dissolved in methanol to reflux with *N*,*N*-diisopropylethylamine (DIEPA) the product (**2**) was formed in adequate yield (Scheme 4). In an effort to streamline the total synthesis of **1**, isolation of reactive intermediate (**9**) was not attempted. Instead crude **9** was immediately dissolved in methanol and reacted with methylamine and DIEPA. By doing this the overall yield from **8** was improved (Scheme 4).

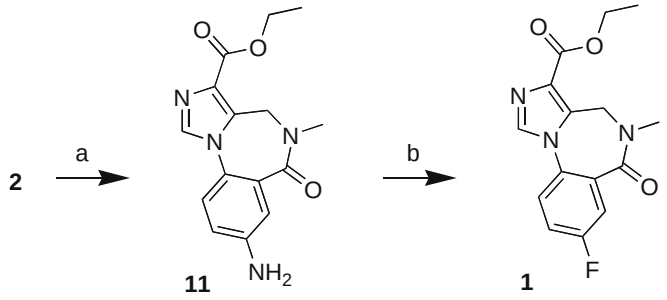
To complete the synthesis of **1**, the nitro functionality in **2** was readily reduced to the corresponding amine **11** with an acidic solution of $TiCl_3$ in methanol (Scheme 5). In contrast to a previous report, isolated **11** was a solid with a high melting point (see 'Experimental' section).[18] Generally, there are two methods for the synthesis of aryl fluorine from an aryl amine, which are variations of the Balz-Schiemann reaction. In the first method, the aryl



Scheme 3. Syntheses of **9** and **10**. Reagents, conditions, and yields: (a) NBS, benzene, benzoyl peroxide(cat), reflux; (b) NBS, benzene, benzoyl peroxide(cat), *hv* (60 W), 86%; (d) NBS, MeCN, benzoyl peroxide$_{(cat)}$, reflux, 41%.



Scheme 4. Synthesis of **2**. Reagents, conditions, and yields: (a) MeOH, DIEPA, $MeNH_2$, reflux, 64% (from **9**), 61% (from **8** without isolating **9**).



Scheme 5. Syntheses of **11** and **1**. Reagents, conditions and yields: (a) aq-$TiCl_3$, MeOH, rt, 83%; (b) $NaNO_2$, 70% HF–pyridine, rt for 1 h, 100 °C for 1 h, 51%.

amine is diazotized with sodium nitrite in the presence of fluoroboric acid. The resulting diazonium fluoroborate precipitate is isolated and then thermally decomposed at high temperature to give aryl fluoride.[19] A variation of this method has been reported, in which, the decomposition to aryl fluoride is carried out photochemically.[20] In the second method, the aryl amine is converted to the aryl fluoride in one-pot by diazotization in 70% HF–pyridine in high-yield.[21] This was the method of choice for the final step (Scheme 5). Recently, a method for the preparation of truly anhydrous fluoride ion has been established.[22] Since **2** is susceptible to S_NAr reaction by activated fluoride ion, as in radiolabeling conditions, this represents an attractive pathway for the synthesis **1** without use of Balz-Schiemann reaction.

3. Conclusion

In conclusion, we have developed a unique synthesis of **1** and its cognates that allows for increased SAR development within the imidazobenzodiazapine platform. Furthermore this novel pathway allows for the synthesis of precursor for ^{18}F-labeling and reference compound in the same process. This newly developed synthetic pathway should aid in the development of ^{18}F-labeled $GABA_A$-Bz site radioligands with enhanced in vivo properties.

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Supplementary data

Supplementary data (experimental details of chemistry and compound characterization) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.10.029.

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