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# Synthesis and preliminary biological evaluation of [$^{11}$C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate for the fractalkine receptor ($CX_3CR1$)

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Competitive binding assay
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ABSTRACT

The reference standard methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate (**5**) and its precursor 2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucine (**6**) were synthesized from 6-amino-2-mercaptopyrimidin-4-ol and BnBr with overall chemical yield 7% in five steps and 4% in six steps, respectively. The target tracer [$^{11}$C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate ([$^{11}$C]**5**) was prepared from the acid precursor with [$^{11}$C]$CH_3OTf$ through *O*-[$^{11}$C] methylation and isolated by HPLC combined with SPE in 40–50% radiochemical yield, based on [$^{11}$C] $CO_2$ and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the specific activity (SA) at EOB was 370–1110 GBq/μmol with a total synthesis time of ~40-min from EOB. The radioligand depletion experiment of [$^{11}$C]**5** did not display specific binding to $CX_3CR1$, and the competitive binding assay of ligand **5** found much lower $CX_3CR1$ binding affinity.



$CX_3C$ chemokine receptor 1 ($CX_3CR1$), also known as fractalkine receptor or G-protein coupled receptor 13 (GPR13), is a protein in humans.[1] $CX_3CR1$ binds the chemokine $CX_3CL1$, also called fractalkine ligand or neurotactin.[2] $CX_3CR1$ is expressed in the brain, spleen, and in subpopulations of leukocytes, cells of monocytic lineage, and neutrophils but also in lymphocytes, and associated with various cancer, cardiovascular and neurological diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD).[3] $CX_3CR1$ is an interesting therapeutic target, and many selective $CX_3CR1$ antagonists have been developed.[4,5] Methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate (**5**) recently developed by AstraZeneca is a potent and selective $CX_3CR1$ antagonist with $K_i$ 8.3 and 1940 nM for $CX_3CR1$ and CXCR2, respectively, and selectivity index (SI) 230.[6] $CX_3CR1$ has also become a promising target for molecular imaging of $CX_3CR1$-mediated diseases and image-guided therapy using positron emission tomography (PET) modality. However, radionuclides including carbon-11 and fluorine-18 labeled $CX_3CR1$ antagonists are still not reported. In our previous work, we have developed carbon-11-labeled naphthalene-sulfonamides as potential radioligands for PET imaging of chemokine receptor 8 (CCR8), as indicated in Fig. 1.[7] In this ongoing study, we first target $CX_3CR1$ and develop radiolabeled $CX_3CR1$ antagonists. Here we report the synthesis and preliminary biological evaluation of [$^{11}$C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate ([$^{11}$C]**5**) as a new candidate PET agent for imaging of CX3CR1.

The reference standard **5** and its desmethylated acid precursor 2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucine (**6**) were synthesized as depicted in Scheme 1, according to the literature method with modifications.[6,8] The alkylation of commercially available starting material 2-amino-6-hydroxy-2-mercaptopyrimidine with benzyl bromide in 1 M NaOH gave compound **1** in 92% yield, which was collected by filtration and was sufficiently pure to be used in next step without further purification. Compound **1** was reacted with potassium thiocyanate, pyridine, and bromine in *N*,*N*-dimethylformamide (DMF) to provide intermediate **2** in 83% yield, which was followed by condensation at elevated temperature to afford the thiazole **3** in 95% yield. The 7-hydroxy group of compound **3** was then converted to the corresponding chloride **4** to introduce a better leaving group chloro via a Vilsmeier reaction in 91% yield. In this reaction, the organic base *N*, *N*-dimethylanline was removed, consequently the reaction process and workup procedure were simplified, and the yield was increased from 60% to 91%.[6] The chloro group of compound **4**

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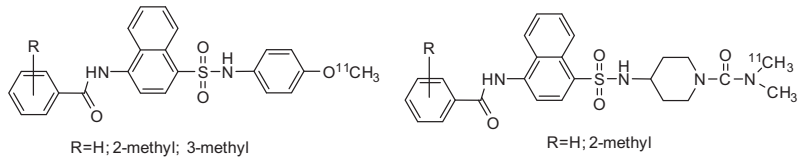
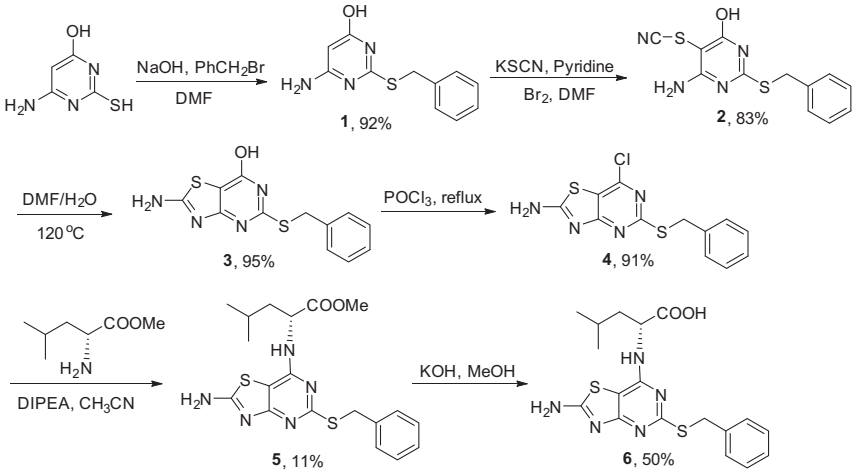



**Fig. 1.** PET radioligands for imaging of CCR8.

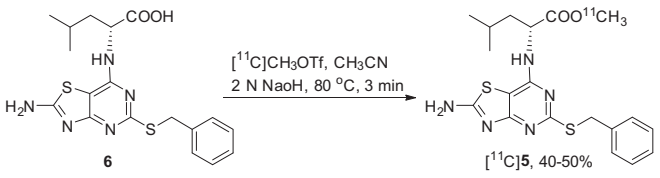



**Scheme 1.** Synthesis of methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate (**5**) and 2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucine (**6**).

was subsequently displaced in a nucleophilic aromatic substitution reaction with excess methyl D-leucinate in the solvent anhydrous $CH_3CN$ or *N*-methylpyrrolidone and *N*,*N*-diisopropylethylamine (DIPEA) as a catalyst to give the standard compound **5** in only 11% yield. The poor yield is due to that it is difficult to displace aromatic chloro with methyl D-leucinate. The hydrolysis of compound **5** in KOH/methanol at room temperature (RT) for 21 h provided the acid precursor **6** in 50% yield.

Synthesis of the target tracer ([$^{11}$C]**5**) is shown in Scheme 2. The acid precursor **6** underwent *O*-[$^{11}$C]methylation[9–11] using the reactive [$^{11}$C]methylating agent [$^{11}$C]methyl triflate ([$^{11}$C]$CH_3OTf$)[12,13] in acetonitrile at 80 °C under basic condition (2 N NaOH). The product was isolated by semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column, and then concentrated by solid-phase extraction (SPE)[14,15] with a disposable C-18 Light Sep-Pak cartridge to produce the corresponding pure radiolabeled compound [$^{11}$C]**5** in 40–50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [$^{11}$C]$CO_2$.

The radiosynthesis process included three stages: 1) labeling reaction; 2) purification; and 3) formulation. The radiolabeled precursor we used is more reactive [$^{11}$C]$CH_3OTf$, instead of commonly used [$^{11}$C]methyl iodide ([$^{11}$C]$CH_3I$),[16] in *O*-[$^{11}$C]methylation to improve radiochemical yield of [$^{11}$C]**5**. An Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module is employed to produce




**Scheme 2.** Synthesis of [$^{11}$C]methyl (2-amino-5-(benzylthio)thiazolo[4,5-*d*]pyrimidin-7-yl)-D-leucinate ([$^{11}$C]**5**).

[$^{11}$C]methylating agent either [$^{11}$C]$CH_3OTf$ or [$^{11}$C]$CH_3I$ ([$^{11}$C]$CH_3Br$ passed through a NaI column). The direct comparison between [$^{11}$C]$CH_3OTf$ and [$^{11}$C]$CH_3I$ confirmed the aforementioned result. The labeling reaction was conducted using a V-vial method. Addition of aqueous $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of [$^{11}$C]**5** from its acid precursor **6**. We used Sep-Pak trap/release method instead of rotatory evaporation for formulation to improve the chemical purity of radiolabeled product [$^{11}$C]**5**. In addition, a C18 Light Sep-Pak to replace a C18 Plus Sep-Pak allowed final product formulation with ≤5% ethanol.[17] Overall, it took ~40 min for synthesis, purification and dose formulation.

The radiosynthesis was performed in a self-designed automated multi-purpose [$^{11}$C]-radiosynthesis module.[18–20] This radiosynthesis module facilitated the overall design of the reaction, purification and reformulation capabilities in a fashion suitable for adaptation to preparation of human doses. In addition, the module is designed to allow in-process measurement of [$^{11}$C]-tracer specific activity (SA, GBq/μmol at EOB) using a radiation detector at the outlet of the HPLC-portion of the system. For the reported syntheses, the product SA was in a range of 370–1110 GBq/μmol at EOB. The major factors including [$^{11}$C]-target and [$^{11}$C]-radiosynthesis unit that affect the EOB SA significantly to lead to such a wide range from 370 to 1110 GBq/μmol have been discussed in our previous works.[21] The general methods to increase SA have been described as well, and the SA of our [$^{11}$C]-tracers is significantly improved.[21] The 'wide range' of SA we reported is for the same [$^{11}$C]-tracer produced in different days, because very different [$^{11}$C]-target and [$^{11}$C]-radiosynthesis unit situations would make SA in a wide range. Likewise, the methods to minimize such wide range of SA from practice perspective have been provided in our previous works.[21] At the end of synthesis (EOS), the SA of [$^{11}$C]-tracer was determined again by analytical HPLC,[22] calculated, decay