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Synthesis of an acromelic acid A analog-based ^{11}C-labeled PET tracer for exploration of the site of action of acromelic acid A in allodynia induction

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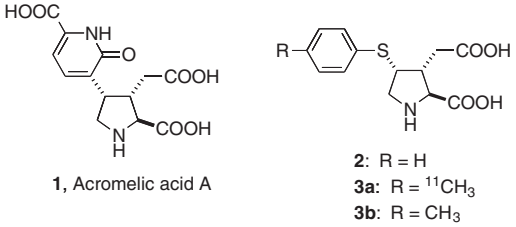
ABSTRACT

A novel ^{11}C-labeled PET (positron emission tomography) tracer, which was designed based on the (phenylthio)pyrrolidine derivative that can competitively block the acromelic acid A-induced allodynia, was synthesized. A protocol in which methylation by palladium-mediated coupling of the boronate derivative with [^{11}C]CH_3I and deprotection of the protected amino acid moiety are successively performed in one-pot within 5 min was established for the synthesis of the tracer. The tracer is potentially useful as a tool to investigate the mode of action of acromelic acid A in the induction of allodynia.

Food poisoning due to a toadstool, *Clitocybe acromelalga*, causes severe allodynia accompanied by erythema and burning pain at one's hands and feet.[1] Allodynia refers to painful paresthesia in response to tactile stimuli, which are normally perceived as innocuous. The responsible toxin for the poisoning has not been identified for a long time. However, Minami and Ito recently reported that acromelic acid A[2,3] (Fig. 1), a minor constituent isolated from *C. acromelalga*, induces allodynia in mice by intrathecal (i.t.) administration.[4] This finding raises the possibility that acromelic acid A is at least one of the key substances for the *C. acromelalga* poisoning in humans.[5]

Allodynia, together with spontaneous pain and hyperalgesia, is the cardinal symptom of intractable neuropathic pain, such as postherpetic neuralgia and complex regional pain syndrome. Although the pathogenic mechanism of allodynia is not fully understood, the involvement of prostaglandin, glutamate and ATP receptors in the induction and maintenance of allodynia is reported.[6–8] Thus, it has been shown that i.t. administration of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and NMDA (*N*-methyl-D-aspartate), each being a specific agonist for the AMPA and NMDA receptor subtypes among the ionotropic glutamate receptors, respectively, cause allodynia in mice.[7] Acromelic acid A belongs to the class of kainoids, which include a structure like that of glutamic acid, and is known to cause the strong depolarization of neurons.[9,10] Accumulated studies have shown that kainoids bind to and activate kainate and AMPA receptors, but not the NMDA subtype.[11,12] Furthermore, acromelic acid A has a higher affinity for AMPA receptor than kainate receptor,[11] and its in vivo behavioral and pathological effects are different from those of kainic acid.[13,14] Based on these findings, acromelic acid A is considered to evoke allodynia through AMPA receptor. However, extensive evidence from pharmacological studies including the glutamate receptor antagonism suggests the existence of an acromelic acid A-specific receptor distinct from the already known ionotropic glutamate receptors including AMPA receptor.[4,15,16] If we can identify such a receptor involved in the induction of allodynia, it may provide a novel target to develop promising analgesic drugs for use in the treatment of neuropathic pain. Thus, our objective is to prepare



1, Acromelic acid A

2: R = H
3a: R = $^{11}CH_3$
3b: R = CH_3

Figure 1. Chemical structures of acromelic acid A, its analogs and a PET tracer.

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a molecular probe for the identification and functional analysis of the putative receptor for acromelic acid A.

The allodynia-inducing effect of acromelic acid A can be evaluated only by behavioral pharmacological studies in animals. Particularly, it should be emphasized that i.t. injected acromelic acid A exerts its effect at extremely low doses (1 ag to 10 fg/mouse),[4] suggesting a lower level of expression of the receptor responsible for allodynia induction. There is currently no information on what kind of cells express the receptor that is activated by acromelic acid A to induce allodynia. Thus, determining the target tissues and types of cells in which acromelic acid A exerts its effect in vivo is the first step to identify the receptor. Based on these circumstances, we intended to address this issue by using positron emission tomography (PET), which enables the in vivo kinetic analysis of biologically active substances with an ultra-high sensitivity. In this paper, we describe the design and synthesis of a PET probe targeting the site of action of acromelic acid A.

The labeling of acromelic acid A itself by a positron-emitting radionuclide is not an easy task because of its definitive molecular structure. However, we previously reported that the simplified acromelic acid A analog **2**,[17,18] with a phenylthio group instead of pyridone ring at C-4 of the pyrrolidine skeleton (Fig. 1), potently suppresses the allodynia induced by acromelic acid A.[19] Thus the acromelic acid A-induced allodynia was blocked in a dose-dependent manner with an ID_{50} value of 2.19 fg/mouse, when **2** was i.t. injected simultaneously with acromelic acid A (1 fg/mouse).[19] The effect of **2** is specific to allodynia, not affecting thermal or mechanical nociception or inflammatory pain. These facts strongly suggest that both **2** and acromelic acid A possibly share the target receptor with almost equal affinity. Additionally, the simplified structure of **2** makes the structural modification as biochemical probes easy. Consequently, we considered **2** to be an appropriate compound for the basis of a PET probe design.

In general, ^{11}C and ^{18}F are utilized as positron-emitting radionuclides for the synthesis of PET tracers, and a variety of methods introducing such nuclides into chemical agents have been established.[20–23] Judging from the structure of **2** and applicability of such labeling reactions, we designed **3a** as a candidate for a PET probe, in which the structural change from **2** is slight (only substitution by a methyl) assuming not affecting the biological activity. For the introduction of $[^{11}C]CH_3$, we planned to adopt our rapid methylation of organoboron compounds with $[^{11}C]CH_3I$ under palladium catalysis.[24] The corresponding boron precursor, however, is favorable to be protected at the amino acid moiety in its structure because the free secondary amino group and carboxylate are nucleophilic and may affect the efficiency of the methylation at the boron moiety with $[^{11}C]CH_3I$. Based on this circumstance, an additional deprotection process after rapid methylation is required to obtain the desired PET tracer.

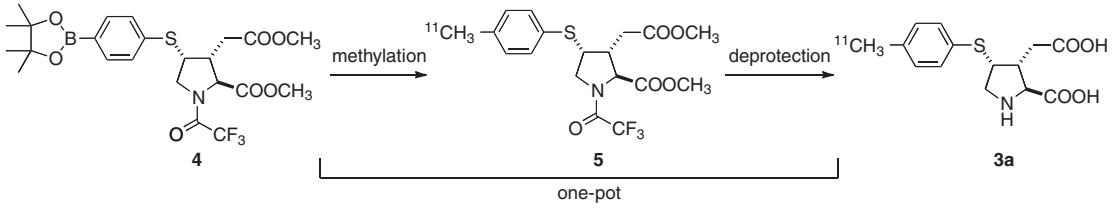
For preparation of a PET tracer with high radioactivity, it is generally assumed that the total synthesis time including labeling reactions, HPLC purification and radiopharmaceutical formulation, should be within about two times that of the half-life of a positron-emitting nuclide.[25] Since the half-life of ^{11}C is 20.4 min, the total synthesis time of 40 min meets the criteria for the ^{11}C-labeled PET tracers. Thus, approximately 5–6 min can be allocated to the sequence of methylation–deprotection in the present case when securing time for the post-reaction operations. This means that the deprotection must be accomplished in one or fewer minutes because the rapid methylation reportedly proceeds in about 5 min.[24] In this context, we have devised a route sequentially conducting the methylation and deprotection in one-pot in which the organoboron compound **4** is utilized as the precursor of the PET probe **3a** (Scheme 1). The amino and two carboxy groups of **4** are protected with trifluoroacetyl and methyl (as esters) groups, respectively. Trifluoroacetamide and methyl ester groups have a similar reactivity toward hydroxide,[26] and therefore, both protecting groups can easily be removed by treating with an aqueous alkaline solution in a single operation. Furthermore, since the standard protocol of the rapid methylation uses *N*,*N*-dimethylformamide (DMF) as the solvent, we consider that the deprotection can be achieved in one-pot by direct addition of an alkaline solution to the rapid methylation reaction mixture without workup.

Before synthesizing the PET tracer **3a**, we decided to prepare and evaluate the biological activity of the nonradioactive molecule **3b**. Thus, the intermediary compound **6** was synthesized from *trans*-4-hydroxy-L-proline according to the reported procedure.[18,27,28] The reaction of **6** with di-*p*-tolyl disulfide in the presence of PBu_3 afforded the coupling product **7** in 97% yield.[29,30] Subsequent hydrolysis of **7** was achieved by heating in hydrochloric acid to give the desired compound **3b** as a white solid in 95% yield after ion-exchange chromatography and lyophylization (Scheme 2).

We next assessed the effect of **3b** on the acromelic acid A-induced allodynia in mice to determine whether the designed molecule is eligible for the PET tracer targeting the receptor in question. First, we confirmed that **3b** alone, like **2**, did not induce allodynia by the i.t. administration at 1–100 fg/mouse (data not shown). The allodynic response of mice to tactile stimuli was then assessed when **3b** was i.t. injected simultaneously with acromelic acid A (1 fg/mouse).[4,19] As expected, **3b** blocked the acromelic acid A-induced allodynia in a dose-dependent manner with a potency equivalent as **2** (Fig. 2),[19] providing the validity of the design of **3a** as the PET tracer.

Following this promising result, we next intended to establish the one-pot, two-step procedure for preparing the PET tracer **3a**. To start with, the precursor organoboron derivative **4** was prepared according to the pathway illustrated in Scheme 3. The phosphine-mediated coupling of the preceding intermediary compound **6** with bis(4-bromophenyl) disulfide gave the (4-bromophenyl)thio derivative **8** in 96% yield.[29,30] Acidic hydrolysis of the amide and ester moieties of **8** followed by re-esterification of the resulting acids with methanol and re-amidation with trifluoroacetic anhydride resulted in the formation of the protected (4-bromophenyl)thio derivative **11** with the overall yield of 75% from **8** in three steps. Finally, the palladium-catalyzed coupling of **8** with bis(pinacolato)diboron afforded the desired precursor **4** in 70% yield.[31]

By using the precursor **4**, we examined the one-pot methylation-deprotection protocol for preparing the PET tracer under cold conditions (Scheme 4). The rapid methylation of **4** was conducted



Scheme 1. Paradigm for one-pot, two-step synthesis of the acromelic acid A analog-based PET tracer.