Efficient Synthesis of [^{11}C]Ramelteon as a Positron Emission Tomography Probe for Imaging Melatonin Receptors Involved in Circadian Rhythms

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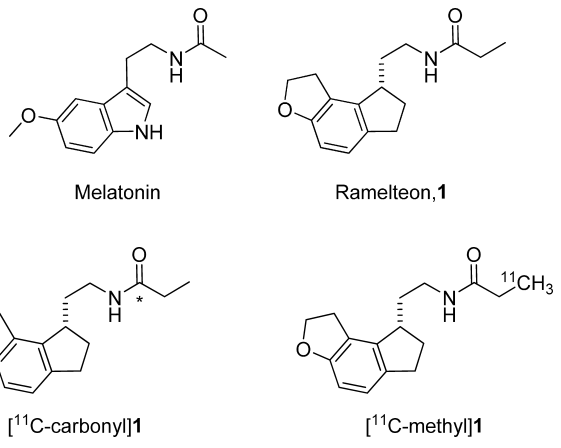
Ramelteon (TAK-375) is a novel melatonin receptor agonist that is used for clinical treatment of insomnia. The present report describes radiolabeling of ramelteon with the short-lived positron-emitter ^{11}C ($T_{1/2}$=20.4 min) by 2 methods. One method was [^{11}C]methylation of an acetoamide precursor and the other was [^{11}C]acylation of the corresponding amine precursor. First, [^{11}C]methylation method showed the low reproducibility together with the production of many kinds of side products from which the [^{11}C-methyl]Ramelteon was separated with chemical purity of <28% and radiochemical purity of >98%. Whereas, the [^{11}C]acylation method showed high efficiency and reproducibility with a good radiochemical yield (22—43%, decay corrected), high chemical and radiochemical purities (>99% each), and high specific activity (43—162 GBq/μmol) (n=5) after HPLC purification. [^{11}C]Ramelteon is a potential positron emission tomography (PET) probe for imaging the melatonin receptor.

Key words positron emission tomography; radiochemistry; melatonin; ramelteon

Melatonin, *N*-acetyl-5-methoxytryptamine, is the pineal hormone that has an important role in the regulation of mammalian circadian rhythms. The melatonin receptors are classified as MT_1, MT_2, and MT_3 based on pharmacological profiles. Among of them, both MT_1 and MT_2 receptors are thought to be involved in the maintenance of circadian rhythms.[1] Ramelteon (TAK-375), [(*S*)-*N*-[2-(1,6,7,8-tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethyl]propionamide] (**1**), is a highly selective MT_1/MT_2 receptor agonist.[2—4] The distinctive tricylic structure of ramelteon shows not only increased metabolic stability but also higher potency and selectivity for the MT_1/MT_2 receptor and it is used for the clinical treatment of insomnia.

Positron emission tomography (PET) is a powerful noninvasive molecular imaging technique for *in vivo* investigation of the distribution and dynamics of bioactive molecules.[5] The development of a suitable radiotracer for melatonin receptors is expected to increase the understanding of the action of melatonin at the molecular level in the human body. Since the discovery of ^{125}I-labeled 2-iodomelatonin,[6—8] a number of PET probes have been synthesized.[9—11] However, a specific PET probe for melatonin receptors has not yet been elaborated.

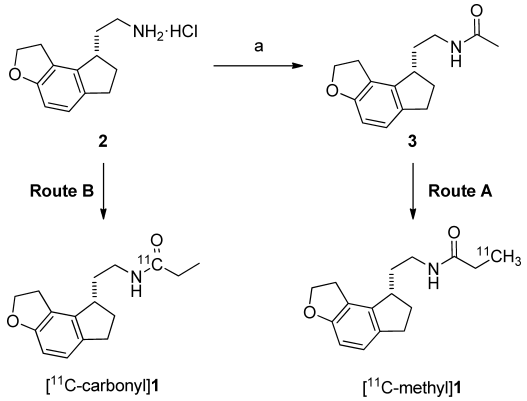
Described herein is ^{11}C-labeling of ramelteon by expecting the synthesis of a PET probe with a high preference for melatonin receptors in terms of affinity, selectivity, and *in vivo* stability.



Results and Discussion

^{11}C-Labeling of ramelteon was investigated *via* methylation as well as acylation using [^{11}C]methyl iodide (Route A) and [^{11}C]propionyl chloride (Route B), respectively (Chart 1).

Precursor **3**, *N*-[2-(1,6,7,8-tetrahydro-2*H*-indeno[5,4-*b*]-furan-8-yl)-ethyl]acetamide was prepared by Schotten–Baumann acetylation of (*S*)-2-1,6,7,8-tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethylamine hydrochloride (**2**).[12] We previously reported that the reaction of an enolate with [^{11}C]CH_3I proceeds very effectively and rapidly.[13] The enolates were sometimes generated without a protecting group for carbonyl compounds such as carboxylic acid ester and amide[14,15]; therefore, the lithiation of **3** was conducted without any protecting group. Thus, amide enolate was formed with ^{n}BuLi in tetrahydrofuran (THF) at −15 °C and then [^{11}C]CH_3I was



Reagents and conditions: a) AcCl, NaOH, THF–H_2O, rt, 1 h; Route A: $^{11}CH_3I$, ^{n}BuLi, THF, −10 °C, 1 min; Route B: (i) $^{11}CO_2$, EtMgBr, diethylether, −10 °C; (ii) phthaloyl dichloride, 2,6-di-*tert*-butylpyridine, 130 °C; (iii) Et_3N, CH_3CN, 0 °C, 1 min.

Chart 1. Synthesis of ^{11}C-Labeled **1**

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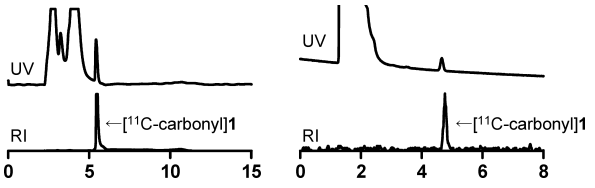

Fig. 1. Representative Chromatogram of [^{11}C-Carbonyl] **1**

UV absorption: 288 nm. Left: HPLC for purification of reaction mixture. Right: Analytical HPLC after preparative HPLC purification.

added at the same temperature. After 1 min at 30 °C, the reaction gave a complex mixture in which the desired [^{11}C-methyl]ramelteon accompanied by many kinds of side products was observed. Consequently, the chemical purity of isolated [^{11}C-methyl]ramelteon was extremely low (<28% at UV absorbance, λ=288 nm). The introduction of a protecting group to improve this synthetic route requires additional work for the deprotection to elongate the time which could be disadvantageous for the synthesis of a short-lived PET probe. Therefore, such trial was thus abandoned.

On the contrary, the synthesis of [^{11}C-methyl]ramelteon by the route B was quite successful. Thus, according to the method reported by Luthra and co-workers,[16] [^{11}C]propionyl chloride was prepared by reacting [^{11}C]CO_2 with ethyl magnesium bromide and then with phthaloyl chloride in the existence of di-*tert*-butylpyridine. The resulting [^{11}C]propionyl chloride was distilled into an acetonitrile solution of **2** and triethylamine at −10 °C. The acylation reaction was completed as soon as the [^{11}C]propionyl chloride was introduced in the reaction mixture. The total synthesis time was 22—26 min from the end of bombardment (EOB) to the pharmaceutical formulation. A decay-corrected radiochemical yield based on [^{11}C]CO_2 was 22—43% (n=5) and the isolated radioactivity was 4.8—9.0 GBq (n=5, at end of synthesis) with >99% radiochemical and chemical purities. Specific radioactivity at the end of synthesis was 43—162 GBq/μmol (n=5) (Fig. 1). The chemical identity of [^{11}C]ramelteon was confirmed by co-injection with ramelteon on an analytical HPLC. Thus, efficient synthesis of [^{11}C-carbonyl]ramelteon from the corresponding primary amine precursor **2** by [^{11}C]propionylation was achieved.

Conclusion

In summary, ramelteon, a selective melatonin receptor agonist ramelteon was efficiently labeled with ^{11}C. Comprising two labeling methods, [^{11}C]methylation and [^{11}C]acylation, the latter was much superior, giving [^{11}C]ramelteon in 22—43% decay-corrected radiochemical yield from [^{11}C]CO_2 with >99% chemical and radiochemical purities. Isolated radioactivity was 4.8—9.0 GBq sufficient for both animal and human PET studies. The total synthesis time was 22—26 min and the specific radioactivity at the end of synthesis was 43—162 GBq/μmol. The resulting [^{11}C]ramelteon could be used as a sharper PET probe for *in vivo* studies on melatonin receptors involved in circadian rhythms.

Experimental

All chemicals and solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan), Tokyo Kasei Kogyo (Tokyo, Japan), Nacalai Tesque (Kyoto, Japan), and ABX (Radeberg, Germany), and were used without further purification. Ramelteon for cold standards was purchased from Takeda Pharmaceutical Co. (Osaka, Japan). Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECX400P spectrometer (Tokyo, Japan) at ambient temperature. Signal patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad signal. Coupling constants (J values) are given in hertz (Hz). Carbon-11 was produced by an ^{14}N(p, α)^{11}C nuclear reaction using a CYPRIS HM-12S Cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). An automated radiolabeling system was used for heating the reaction mixture, dilution, HPLC injection, fraction collection, evaporation, and sterile filtration. Purification with semipreparative HPLC was performed on a JASCO system (Tokyo, Japan). Analytical HPLC was performed on a Shimadzu system (Kyoto, Japan) equipped with pumps and a UV detector, and effluent radioactivity was measured with an RLC700 radio analyzer (Aloka, Tokyo, Japan). The columns used for analytical and semipreparative HPLC were COSMOSIL C_{18} AR-II (Nacalai Tesque).

(*S*)-*N*-[2-(1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)ethyl]-acetamide (3) (*S*)-2-1,6,7,8-Tetrahydro-2*H*-indeno[5,4-*b*]furan-8-yl)-ethylamine hydrochloride (**2**)[17] (1.0 g, 4.2 mmol) was dissolved in 2 M NaOH (10 ml) and THF (10 ml). Acetyl chloride (330 mg, 4.2 mmol) was added to the solution. It was stirred at room temperature for 30 min and then extracted with ethyl acetate (10 ml, 3 times). Combined organic layers were dried over sodium sulfate and evaporated to dryness under vacuum. The crude product was purified by flash chromatography eluting with a ratio of dichloromethane : ethyl acetate of 2 : 1 to give **3** (932 mg, 90%) as a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.94 (d, J=8.0 Hz, 1H), 6.60 (d, J=8.0 Hz, 1H), 5.72 (br s, 1H), 4.67—4.47 (m, 2H), 3.35—3.07 (m, 5H), 2.90—2.74 (m, 5H), 2.31—2.22 (m, 1H), 2.05—1.98 (m, 1H), 1.96 (s, 3H), 1.83—1.88 (m, 1H), and 1.66—1.59 (m, 1H). $[\alpha]_D^{24}$ −85.1° (c=0.52, ethanol). MS (electrospray ionization (ESI)) m/z: 246 $[M+H]^+$, 244 $[M-H]^-$.

[^{11}C-Carbonyl]ramelteon ([^{11}C-Carbonyl] 1) [^{11}C]CO_2 was trapped from the gas target in a column containing molecular sieves (300 mg) at 30—50 °C. After trapping, the column was warmed to 180 °C, the released [^{11}C]CO_2 was bubbled into a diethyl ether solution of 0.1 M ethyl magnesium bromide (1.0 ml) at −10 °C with a flow of He of 20 ml/min. When transfer of radioactivity was complete, the He flow was stopped and a solution of phthaloyl dichloride (200 μl) and 2,5-di-*tert*-butylpyridine (100 μl) in dry diethyl ether (100 μl) was added. The mixture was heated to 130 °C, and the formed [^{11}C]propionyl chloride was distilled into a solution of amine hydrochloride **2** (0.5—0.7 mg) and triethylamine (50 μl) in acetonitrile (500 μl) at −10 °C with a flow of He of 50 ml/min. The mixture was placed at 0 °C for 1 min and then diluted with HPLC eluent (1 ml). The reaction mixture was purified by reverse-phase HPLC (COSMOSIL C_{18} AR-II 10 mm (i.d.)×250 mm, 5 ml/min, with a 1 : 1 ratio of CH_3CN : 10 mM $HCOONH_4$). The desired fraction (retention time=5—6 min) was collected into a flask containing ascorbic acid (200 μl), evaporated to dryness under vacuum, and the residue was taken up in polysorbate 80 : propylene glycol : saline with a 0.1 : 1 : 10 volume ratio (4 ml). The formulated solution was used to establish the chemical and radiochemical purity and specific radioactivity by analytical HPLC (COSMOSIL C_{18} AR-II, 4.6 mm (i.d.)×150 mm, 1 ml/min, and CH_3CN : 100 mM $HCOONH_4$ at a 50 : 50 ratio). The total synthesis time was 22—26 min and 5.0—9.0 GBq of [^{11}C]ramelteon was constantly isolated with 43—162 GBq/μmol of the specific radioactivity (n=5) with a good radiochemical yield (22—43%, decay corrected based on [^{11}C]CO_2). Chemical purity (UV; λ=288 nm) and radiochemical purity were always greater than 99%.

[^{11}C-Methyl]ramelteon ([^{11}C-Methyl] 2) [^{11}C]CO_2 was converted to [^{11}C]CH_3I *via* a reaction with 0.1 M lithium aluminum hydride in THF and subsequent reaction with hydroiodic acid.[18] 1.6 M ^{n}BuLi (0.1 ml) was added to a solution of **3** (5 mg) in anhydrous THF (0.4 ml) at −15 °C and it was left for 10 min. Then, [^{11}C]CH_3I was introduced to the mixture at the same temperature. The mixture was left at 30 °C for 1 min and then it was quenched with 10% HCOOH in acetonitrile (1.0 ml). The mixture was purified by the HPLC condition described above. The total synthesis time was 24—27 min to give [^{11}C]ramelteon with <28% chemical purity (UV; λ=288 nm) and >98% radiochemical purity (n=2).

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