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A New Efficient Synthetic Method for 3-Iodothyronamine Involving Sonication and its Potent Hypothermic Efficacy

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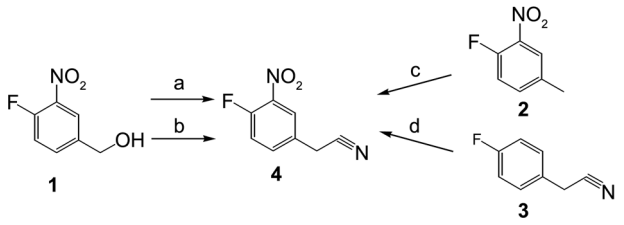
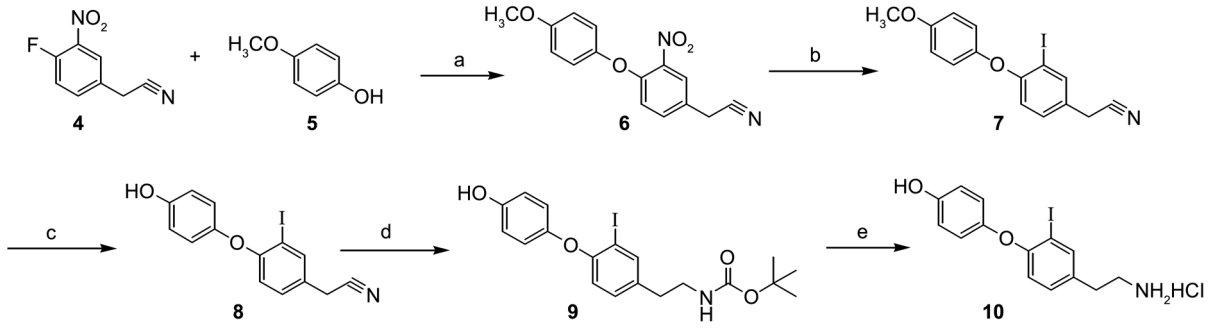
Over recent years, chemically-induced hibernation has attracted much attention in fields of hibernation biology, medical science and space biology.[1,2] Several compounds such as thyronamine and gaseous H_2S are shown to trigger hibernation-like state (HS) in non-hibernator mice.[3,4] For instance, a single-dose administration of thyronamine elicited rapid suppression of metabolic rate, body temperature (T_b) and heart rate, and elevated utilization of lipid over carbohydrate for metabolism in the laboratory mice.[3,5] From the perspective of HS induction, 3-iodothyronamine (T_1AM) has drawn a particular attention because it is an endogenous compound derived from deiodination and decarboxylation of thyroid hormone T_4 (thyroxine). The compound is shown to cause little side effect or damage on neural and other critical organs even for a long-term, repeated administration.[3,6a,7] These findings render T_1AM to have pharmaceutical benefits that can be applied to emergency medical care (e.g., stroke, hypoxic assault), organ transplantation, and a long-duration spaceflight.[2]

For such wide utilization to be realized, it is necessary to synthesize the compound in a more efficient manner. The synthetic process of T_1AM, however, is limited to a method that has been developed by Hart *et al.* in which the overall yield was fairly low (7-step process, 3% overall yield).[6a] In this paper, we describe a new synthetic method for T_1AM that has advantages of reduced synthetic steps and significantly enhanced overall yield. In addition, the hypothermic efficacy of T_1AM was investigated using laboratory mice.

(4-Fluoro-3-nitrophenyl)acetonitrile (**4**) was synthesized from **1** *via* 4-fluoro-3-nitrobenzyl chloride intermediate or 4-fluoro-3-nitrobenzyl bromide intermediate (Scheme 1). The compound **4** could also be synthesized by benzyl bromination of 1-fluoro-4-methyl-2-nitrobenzene (**2**) followed by substitution of bromide with cyanide.[8] In addition, regioselective mono nitration of 4-fluorophenylacetonitrile (**3**) directly gave **4**. Among these methods the fourth one would be the most advantageous because it proceeds without intermediate formation. In organic synthesis ultrasound irradiation usually provides improved yields, shorten reaction time,



Scheme 1. Synthesis of **4**. Reagents and conditions: (a) 35% HCl, rt, 2 h, then NaCN, NaI, anhyd. acetone, reflux, 12 h, 84%; (b) CBr_4, TPP, CH_2Cl_2, 0 °C to rt, 2 h, then NaCN, NaI, anhyd. acetone, reflux, 12 h, 75%; (c) NBS, dibenzoylperoxide, chlorobenzene, reflux, overnight, then NaCN, NaI, anhyd. acetone, reflux, 12 h, 59%; (d) fuming HNO_3, 95-100 °C, 2.5 h, 76%.



Scheme 2. Synthesis of T_1AM (**10**). Reagents and conditions: (a) Cs_2CO_3, DMSO, sonication, 58 °C, 5 h, NaH, DMF, rt, 2 h, 78%; (b) Pd/C, H_2, EA, rt, 6 h, then $NaNO_2$, H_2SO_4, 0-5 °C, 30 min, then KI, H_2O, rt, overnight, 88%; (c) BBr_3, CH_2Cl_2, −78 °C to rt, 15 h, 89%; (d) $(Boc)_2O$, $NiCl_2$, $NaBH_4$, anhyd. MeOH, 0 °C to rt, 1.5 h, 84%; (e) 2 M HCl/EA, rt, overnight, 92%.

and increase selectivity.[9] Our coupling reaction was conducted under ultrasound irradiation for 5 h using almost equimolar amounts of **4** and **5**, and a relatively good yield (78%) was achieved (Scheme 2). In the conventional method,[6a] the coupling reaction of *N*-*t*-Boc-3-iodotyramine and 4-(triisopropyl)silyloxyphenylboronic acid was performed overnight at a mole ratio of iodotyramine/boronic acid = 1:2, and the yield was low (36%). It should be noted that our coupling reaction has advantages of no need of excess reagent, shorter reaction time, and much improved yield compared with the conventional method. Iodo compound **7** was prepared from **6** *via* diazotization followed by iodination of corresponding aniline in a good yield of 88% (Scheme 2).[10] In contrast, the traditional iodination method had some problems such as a very low yield (19%) and difficulty in purification because of the formation of a considerable amount of diiodotyramine byproduct.[6a] In this work the iodination proceeds regioselectively because nitro group is replaced with iodine. Compound **8** was obtained by demethylation of **7**, and **9** was synthesized by catalytic reduction of the cyano group and subsequent Boc protection of **8**. Finally, T_1AM (**10**) was obtained by Boc deprotection of **9**, and its ^1H and ^{13}C NMR spectra are in good agreement with those previously reported.[6a]

The hypothermic efficacy was investigated by treating laboratory mice with T_1AM synthesized in this work. Figure 1A shows an overall trace of T_b before and after i.p., injection of T_1AM in a mouse. Before injection, the subject maintained an average T_b (mean ± SD) at 36.6 ± 0.18 °C. After treatment (50 mg kg^{-1}), the hypothermia was induced instantaneously and rapidly. The pattern of T_b change, including the extent and rate of decrease and time for recovery, was almost the same that reported previously.[3] The lowest T_b (28.6 °C) was reached at the time of 1.4 h after injection. It is thus apparent that T_1AM produced by a new synthetic method showed the hypothermic potency as similar as that of Hart *et al.* (2006).[6a]

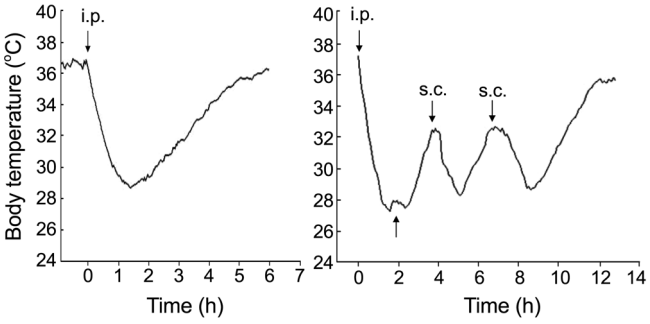


Figure 1. (A) A trace of T_b of a male ICR mouse (40.2 g) before and after a single i.p. injection of T_1AM (50 mg kg^{-1}). Note that T_b was rapidly declined to 28.6 °C after ~1.4 h of treatment, and returned to the normothermic level after 5-6 h. (B) A T_b trace of a male mouse (37.5 g) monitored across ~13 h of experiment. The first hypothermia was induced by an i.p. injection, and subsequent hypothermia was induced by two consecutive s.c. injections when T_b returned to ~32 °C. The time points of these s.c. injections were 3.7 h and 6.5 h, respectively, after the i.p. injection. A small bump at the lowest T_b (indicated by an upward arrow) reflects leg and postural adjustments of the subject, indicating that the mouse was not anesthetic even in the deep hypothermia.

Our next attempt was to investigate whether the subject would sustain the hypothermic state for a much longer time by multi-dose administration of T_1AM. For this purpose, we tried three injections of the same dosage to the subject. Figure 1B illustrates that the mouse, again, decreased T_b immediately after the i.p. injection and exhibited the lowest T_b of 27.4 °C. In the following treatments, the s.c. injections were applied to minimize disturbance of the torpid animal that was in a crouched posture. The injections were given whenever T_b returned to ~32 °C after hypothermia. This repeated administration resulted in an oscillation of the subject's T_b. In these s.c. trials, it is notable that a time delay was observed between the injection and the actual decline of T_b. Such delay was not seen in the i.p. treatment. This slow response might result from a relatively low distribution of blood flow in the skin (< 10%) compared to that in the abdominal region (~30%) of the mouse.[11] The following rapid decrease in T_b indicates that the subcutaneously injected T_1AM exerted the same hypothermic efficacy once it might flow into blood circulation.

In conclusion, our new synthetic method for T_1AM has advantages of less synthetic steps and much higher overall yield (28-40%) compared to those in the conventional method.[6b] In particular, our coupling reaction provides several advantages of no need of excess reagent, shorter reaction time, and much improved yield. Treatment of T_1AM significantly lowered T_b of the mice, and multi-dose treatments of the compound would be able to keep the subject in the prolonged hypothermic state.

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