# A New Efficient Synthetic Method for 3-Iodothyronamine and Its Potent Hypothermic Efficacy

Kim, Joong-Gon[a] Song, Young-Kyu[b] Jeon, Su-Yeon[b] Lim, Ye-Ji[b]
Ju, Hyunwoo[c] Choi, Inho*[,c] Chung, Chan-Moon*[,b]

[a] *Biotechnology Division, Hanwha Chemical R&D Center, Daejeon 305-345, Korea*
[b] *Department of Chemistry and Medical Chemistry, Yonsei University, Wonju, Gangwon-do 220-710, Korea*
[c] *Department of Biological Science and Technology, Yonsei University, Wonju, Gangwon-do 220-710, Korea*

We developed a new efficient synthetic method for a 3-iodothyronamine ($T_1$AM) that has advantages of less synthetic steps and much higher overall yield compared to those in the conventional method. Our animal study showed that $T_1$AM synthesized by the method exerted a potent hypothermic effect in non-hibernator mice.

**Keywords** 3-iodothyronamine, hibernation-like state, ICR mouse

## Introduction

Mammalian hibernation has been considered as a fascinating physiological trait that enables them to overcome harsh conditions (*e.g.*, coldness and food shortage).[1] Hibernators actively suppress metabolism and body temperature ($T_b$) and survive for four to six months of winter without feeding. Because of the hypometabolic and hypothermic features, hibernation mechanism is considered to offer significant medical benefits in emergency medical care, organ transplantation, and extended spaceflights.[2]

Metabolic rate and $T_b$ are principally controlled by thyroid hormones (TH) such as triiodothyronine ($T_3$) and thyroxine ($T_4$). Since these hormones are thermogenic, their levels in blood circulation are found to increase in winter.[3] Induction of hibernation is the opposite of the TH effect. Until recently, however, little was known about a molecular mechanism underlying the hibernation triggering process. One clue might be seen from the action of 3-iodothyronamine ($T_1$AM, **10**), a natural derivative of $T_4$.[4,5] When a single-dose was injected to laboratory mice, it induced rapid hypometabolism and hypothermia, with peak effect in 1—2 h. The $T_b$ was then recovered gradually in about 6 h after injection.

The first synthesis of $T_1$AM (**10**) was reported by Scanlan *et al*. who developed a seven-step synthetic process.[6,7] The method involves the synthesis of *N*-*t*-Boc-3-iodotyramine from tyramine and 4-(triisopropyl)-silyloxyphenylboronic acid from *p*-bromophenol, coupling of the two compounds, and removal of the protecting groups. However, the overall yield of the conventional method was fairly low (3%), so it is necessary to synthesize the compound in a more efficient manner for a broad range of the possible medical applications. Herein, we describe a new efficient synthetic method for $T_1$AM (**10**) having reduced synthetic steps and significantly enhanced overall yield. Hypothermic efficacy of the compound was also studied using laboratory mice.

As illustrated in Scheme 1, our new method employs 4-chloro-3-nitrobenzyl alcohol (**1**), 1-chloro-4-methyl-2-nitrobenzene (**2**), or 2-(4-chlorophenyl)acetonitrile (**3**) as starting materials. 2-(4-Chloro-3-nitrophenyl)acetonitrile (**4**) could be prepared by four synthetic routes. First, **4** was synthesized via 4-chloro-2-nitrobenzyl chloride intermediate that formed by treating **1** with aqueous HCl. The intermediate then afforded **4** by reaction with NaCN in the presence of a catalytic amount of NaI. Second, **4** was also synthesized via 4-chloro-2-nitrobenzyl bromide intermediate that was formed by reaction of **1** with $CBr_4$. Third, **4** could also be synthesized by benzyl bromination of **2** using *N*-bromosuccinimide (NBS) and benzoyl peroxide (BPO) followed by substitution of bromide with cyanide.[8] Fourth, regioselective mono nitration of **3** directly gave **4** in 74% yield.

As shown in Scheme 2, the coupling reaction of **4** with 4-methoxyphenol (**5**) was performed to obtain **6** in the presence of NaH.[9,10] In the conventional method,[6,7] the coupling reaction of *N*-*t*-Boc-3-iodotyramine with 4-(triisopropyl)silyloxyphenylboronic acid gave *N*-*t*-Boc-4'-triisopropylsilyloxy-3-iodothyronamine in a low yield (36%). Compared to this, a high yield (89%) was achieved under our coupling reaction condition.

**7** was prepared from **6** in 88% yield via diazotization followed by iodination of corresponding aniline.[11,12] In contrast, the conventional iodination method[6,7] had

* E-mail: cmchung@yonsei.ac.kr(C.-M. Chung), ichoi@yonsei.ac.kr(I. Choi); Tel.: +82 337602266; Fax: +82 337602182
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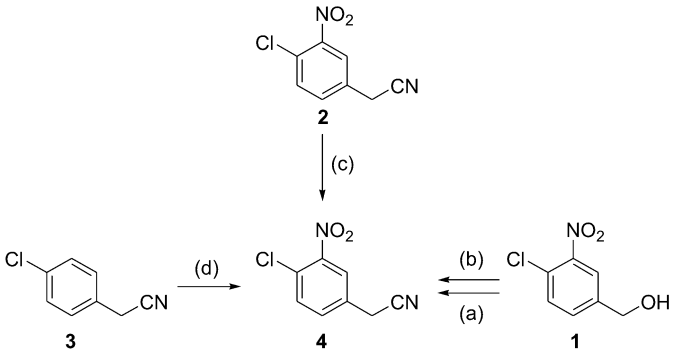
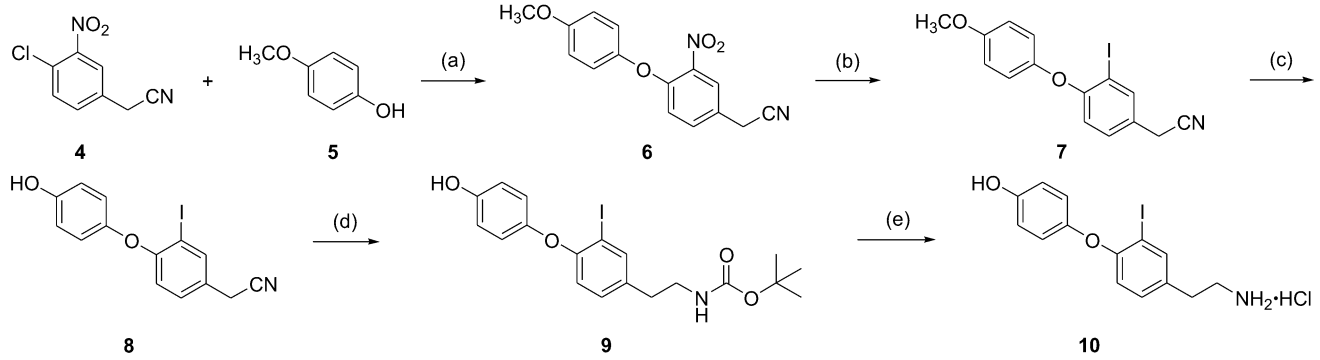
**Scheme 1** Synthesis of **4**




**Reagents and conditions**: (a) 35% HCl, r.t., 2 h, then NaCN, NaI, anhy acetone, reflux, 12 h, 83%; (b) $CBr_4$, TPP, $CH_2Cl_2$, 0 ℃ to r.t., 2 h, then NaCN, NaI, anhy acetone, reflux, 12 h, 73%; (c) NBS, dibenzoylperoxide, chlorobenzene, reflux, overnight, then NaCN, NaI, anhy acetone, reflux, 12 h, 62%; (d) fuming $HNO_3$, 95—100 ℃, 2.5 h, 74%.

**Scheme 2** Synthesis of $T_1AM$ (**10**)




**Reagents and conditions**: (a) NaH, DMF, r.t., 2 h, 89%; (b) Pd/C, $H_2$, EA, r.t., 6 h, then $NaNO_2$, $H_2SO_4$, 0—5 ℃, 30 min, then KI, $H_2O$, r.t., overnight, 88%; (c) $BBr_3$, $CH_2Cl_2$, −78 ℃ to r.t., 15 h, 89%; (d) $(Boc)_2O$, $NiCl_2$, $NaBH_4$, anhy MeOH, 0 ℃ to r.t., 1.5 h, 84%; (e) 2 mol•$L^{-1}$ HCl/EA, r.t., overnight, 92%.

some problems such as a very low yield (19%) and difficulty in purification due to the formation of a considerable amount of *N*-*t*-Boc-3,5-diiodotyramine byproduct. In this work the preparation of iodo compound **7** proceeds regioselectively because nitro group is replaced with iodine. **8** was obtained by demethylation of **7** using $BBr_3$, and **9** was synthesized by catalytic reduction of the cyano group and subsequent Boc protection of **8**.[13] $T_1AM$ (**10**)[14] was prepared by Boc deprotection of **9** with HCl, and its $^1H$ and $^{13}C$ NMR spectra are in good agreement with those previously reported.[7]

Next, to investigate the hypothermic efficacy of $T_1AM$ (**10**) synthesized in this work, it was treated to laboratory mice.[15] As shown in Figure 1A, the control mouse maintained $T_b$ in a range of 36.4—37.3 ℃. This thermoregulatory activity was contrasted by the observation that $T_b$ was immediately decreased in the $T_1AM$-injected subjects (Figure 1B). The extent of the change was dose-dependent,[4] with the lowest $T_b$ of 32.2 ℃ by 25 mg•$kg^{-1}$ and 28.7 ℃ by 50 mg•$kg^{-1}$ at an ambient temperature ($T_a$) of 23 ℃. The $T_b$ was then gradually recovered for the following hours. It is worth recognizing that the treated subjects (50 mg•$kg^{-1}$) were unable to escape from an extremely low $T_b$ condition, although a sort of brief spasm was noticed at $T_b$ of about 15 ℃ (Figure 1C). This indicates that the hibernation-like state of mice is fundamentally different from deep torpor of true hibernators. In the latter case, like squirrels and bats, $T_b$ can decrease down to near 0 ℃.[1] Notably, the hypothermic effect was additive within a limited low range, because the $T_b$ decreased by the first injection (50 mg•$kg^{-1}$) was further lowered by the second treatment of the same dosage (Figure 1D). At this $T_a$ level, the treated subjects were likely to keep $T_b$ at >28 ℃ (Figures 1B and 1D) and even by a doubled dosage (100 mg•$kg^{-1}$).[4] Together, these results imply that non-hibernator mammals may remain torpid for an extended period at a certain $T_b$ (*e.g.*, 30 ℃) by repeated administration of the compound.

In conclusion, a new efficient synthetic method was developed for $T_1AM$ (**10**). Our synthetic method has advantages of less synthetic steps and much higher

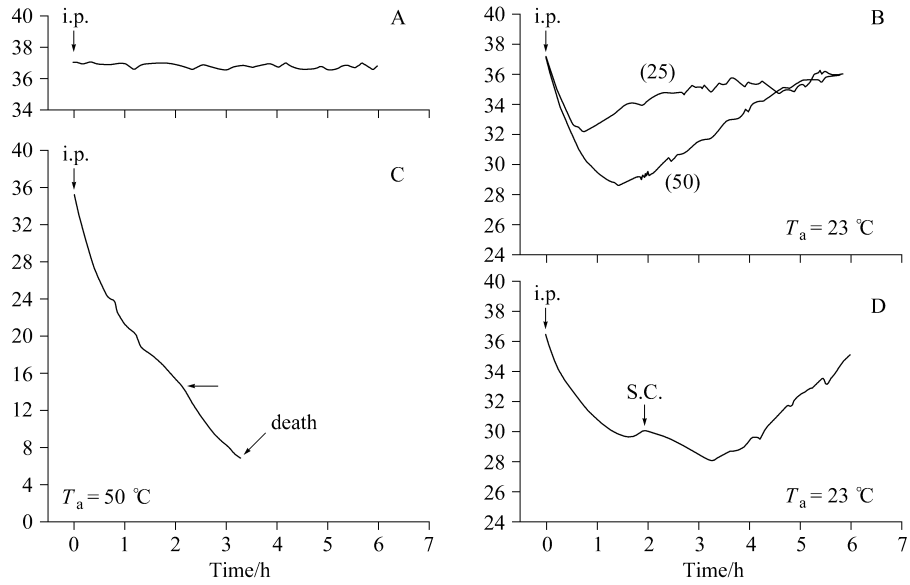



**Figure 1** $T_b$ traces of ICR male mice after vehicle (A) or $T_1$AM injection (B—D). Note that ambient temperature ($T_a$) was (23±0.5) ℃ for A, B and D, and (5±1.0) ℃ for C. (A) The control mouse displayed constant $T_b$ (*ca.* 37 ℃), whereas (B) the subjects showed dose-dependent hypothermic responses to either 25 or 50 mg•kg$^{-1}$. (C) At $T_a$ of 5 ℃, the treated mouse (50 mg•kg$^{-1}$) succumbed to death at $T_b$ below 9 ℃. The horizontal arrow indicates the $T_b$ point (*ca.* 15 ℃) at which a brief spasm was observed. (D) $T_1$AM treatment exerted an additive effect as $T_b$ at the rising phase following the first treatment (50 mg•kg$^{-1}$) was further decreased by the second trial of the same dosage. i.p.: intraperitoneal; s. c.: subcutaneous.

overall yields (33%—45%) compared to those in the conventional method (7-step process, 3% overall yield[6,7]). $T_1$AM (**10**) synthesized by this new method showed a potent hypothermic effect. Because of these hypometabolic and hypothermic efficacies, it is expected that $T_1$AM has possible pharmaceutical merits for emergency medical care, organ transplantation, long-term space mission, as well as remedies for diabetes and extended life span.[2,16,17]

## References and notes

1 Carey, H. V.; Andrews, M. T.; Martin, S. L. *Physiol. Rev.* **2003**, *83*, 1153.
2 Lee, C. C. *Annu. Rev. Med.* **2008**, *59*, 177.
3 Acheson, K.; Jequier, E.; Burger, A.; Danforth, E., Jr. *Metabolism* **1984**, *33*, 262.
4 Scanlan, T. S.; Suchland, K. L.; Hart, M. E.; Chiellini, G.; Huang, Y.; Kruzich, P. J.; Frascarelli, S.; Crossley, D. A.; Bunzow, J. R.; Ronca-Testoni, S.; Lin, E. T.; Hatton, D.; Zucchi, R.; Grandy, D. K. *Nat. Med.* **2004**, *10*, 638.
5 Piehl, S.; Hoefig, C. S.; Scanlan, T. S.; Kohrle, J. *Endocr. Rev.* **2011**, *32*, 64.
6 Hart, M. E.; Suchland, K. L.; Miyakawa, M.; Bunzow, J. R.; Grandy, D. K.; Scanlan, T. S. *J. Med. Chem.* **2006**, *49*, 1101.
7 Scanlan, T. S.; Hart, M. E.; Grandy, D. K.; Bunzow, J. R. *US 6979750*, **2005**.
8 Kwark, Y.-J. *Macromol. Res.* **2008**, *16*, 238.
9 Eicher, T.; Fey, S.; Puhl, W.; Biichel, E.; Speicher, A. *Eur. J. Org. Chem.* **1998**, 877.
10 Theil, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 2345.
11 In, J.-K.; Lee, M.-S.; Yang, J.-E.; Kwak, J.-H.; Lee, H.; Boovanahalli, S. K.; Lee, K.; Kim, S. J.; Moon, S. K.; Lee, S.; Choi, N, S.; Ahn, S. K.; Jung, J.-K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1799.
12 Xiang, Y.; Caron, P.-Y.; Lillie, B. M.; Vaidyanathan, R. *Org. Process Res. Dev.* **2008**, *12*, 116.
13 Caddick, S.; Judd, D. B.; Lewis, A. K. K.; Reich, M. T.; Williams, M. R. V. *Tetrahedron* **2003**, *59*, 5417.
14 m. p. 214—215 ℃; $^1$H NMR (DMSO-$d_6$, 400 MHz) $\delta$: 9.36 (s, 1H), 7.77 (s, 1H), 7.74 (brs, 3H), 7.23—7.20 (m, 1H), 6.82—6.76 (m, 4H), 6.71 (d, $J$=8.4 Hz, 1H), 3.03 (t, $J$=7.6 Hz, 2H), 2.80 (t, $J$=7.6 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz) $\delta$: 155.9, 153.8, 148.1, 139.3, 133.9, 130.2, 119.9, 117.5, 116.2, 88.3, 38.8, 31.5; IR (KBr) $v$: 3383, 3120, 3017, 1503, 1484, 1266, 1221, 1195, 1039 cm$^{-1}$.
15 Animal experiment. To examine efficacy of the synthesized $T_1$AM (**10**), $T_b$ of individual mice was monitored for 6 h after a single injection. Institute of Cancer Research (ICR) male mice at ten weeks of age (*ca.* 35 g) were purchased from a local supplier and housed at (23±0.5) ℃ in a 12 h∶12 h light-dark cycle with the light on at 06: 00. Mice had *ad libitum* access to standard Purina chow and water. Each mouse was injected intraperitoneally with 25 or 50 mg•kg$^{-1}$ $T_1$AM dissolved in 60% dimethyl sulfoxide (DMSO) and 40% saline (pH 7.4), or vehicle (60% DMSO and 40% saline) for the control.[4] $T_b$ of the subjects was continuously recorded with a 0.025 mm diameter duplex cop-

per-constantan thermocouple (California Fine Wire, Grover City, CA) and an Omega 91100—20 thermocouple thermometer (Cole-Palmer Instrument, Vernon Hills, IL). The sensing tip of the thermocouple was inserted *ca*. 7 mm into the pectoral muscle. In another set of experiment, a couple of mice (treated with 50 mg•kg$^{-1}$ $T_1$AM) were examined at $T_a$ of (5±1.0) ℃ to test whether they were able to alert and arouse as their $T_b$ decreased to an extremely low level. The experimental procedure of the study was approved by the Yonsei University Animal Care and Use Committee.

16 Don, C. W.; Longstreth, W. T. Jr.; Maynard, C.; Olsufka, M., Nichol, G.; Ray, T.; Kupchik, N.; Deem, S.; Copass, M. K.; Cobb, L. A.; Kim, F. *Crit. Care Med*. **2009**, *37*, 3062.

17 Storey, K. B. *Gerontology* **2010**, *56*, 220.

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