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Synthesis of ^{13}C-Labeled 5,6,11-Trideoxytetrodotoxin

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The synthesis of ^{13}C-labeled 5,6,11-trideoxytetrodotoxin has been successfully achieved. This is the first example of the ^{13}C-labeled analogs in tetrodotoxin congeners.

5,6,11-Trideoxytetrodotoxin (trideoxyTTX) (**1**) and its 4-epimer **2** were originally isolated from the ovaries of the puffer fish, *Fugu poecilonotus*, by Yamashita and Yasumoto (Figure 1).[1] These are the less toxic natural congeners of tetrodotoxin (TTX) which is known as a potent blocker of the voltage-gated Na^+ channel.[2–7] TTX and its congeners have been isolated from not only puffer fish, but also other organisms. Recent analyses of TTX and its congeners in several samples revealed that a larger amount of **1** was accumulated in samples, and in some cases, the amount of **1** was greater than that of TTX (**3**).[8–11] The presence of **1** has attracted much attention as a clue to the biosynthetic and metabolic pathways of TTX that remain to be elucidated. Recently, Botana and co-workers proposed an interesting biological role of **1**. Based on the discrepanicies in the analytical data between an in vitro assay and LC/MS analysis of TTX and its congeners in the digestive glands of a European trumpet shell, the competitive ligand effect of **1** for the binding of TTX to Na^+ channels was proposed.[8] These analytical and biological studies prompted us to develop a synthetic route of ^{13}C-labeled **1** and **2** as a tool for bioorganic studies in this research area. We now report the first synthesis of the ^{13}C-labeled **1** and **2**.

We have reported the first total syntheses of trideoxyTTX (**1**) and its 4-epimer **2** via two key reactions: i) the asymmetric Strecker synthesis of **4** to create the concomitant stereogenic centers including the quaternary amino carbon center of **5**, and ii) the intramolecular cyclization via the cyanohydrins **7** to complete the total synthesis (Scheme 1). Taking advantage of this synthetic route, we planned the ^{13}C-labeling by the addition of TMS^{13}CN or Na^{13}CN to the aldehyde **6**. In a previous study, the cyanohydrin **7** was quantitatively synthesized by the addition of TMSCN to **6** in the presence of Et_3N.[12,13] However, the product ratio was moderate, producing a 3:2 mixture of (9*S*)-**7** and (9*R*)-**7** (Table 1, Entry 1). Therefore, we initially focused our research efforts on improving the stereoselectivity to give the desired (9*S*)-isomer prior to the incorporation of the ^{13}CN.

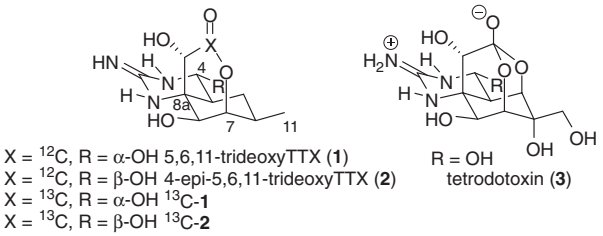
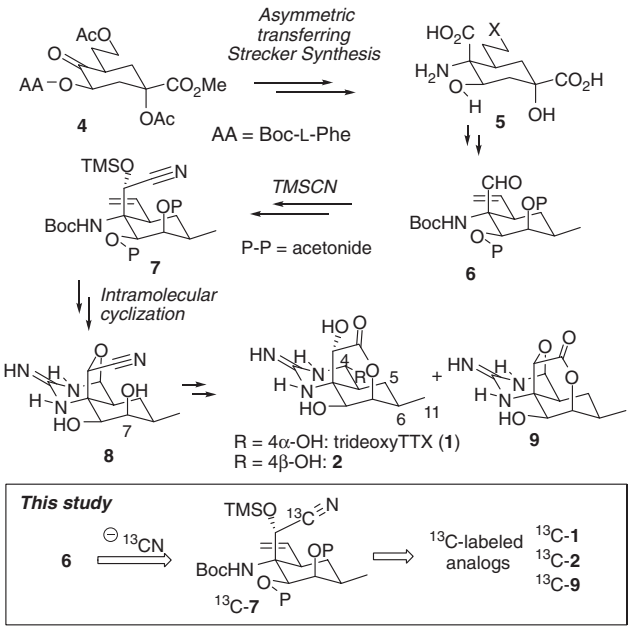


Figure 1.

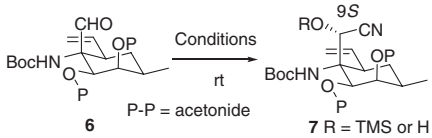
The addition reaction of TMSCN without any additives did not proceed at all (Entry 2). The ZnI_2[14] and $BiCl_3$[15] promoted addition reactions to produce a complex mixture (Entries 3 and 4). On the other hand, the use of NaCN as an alternative to TMSCN gave the desired (9*S*)-**7** as the major product [9*S*:9*R* = 64:36 (Entry 5)].

During the course of the first total synthesis of 11-deoxyTTX, Nishikawa et al. found that the addition reaction of magnesium trimethylsilyl acetylide to **10** produced the 2-propynyl alcohol **11** with high stereoselectivity (Scheme 2, eq 1).[16] We



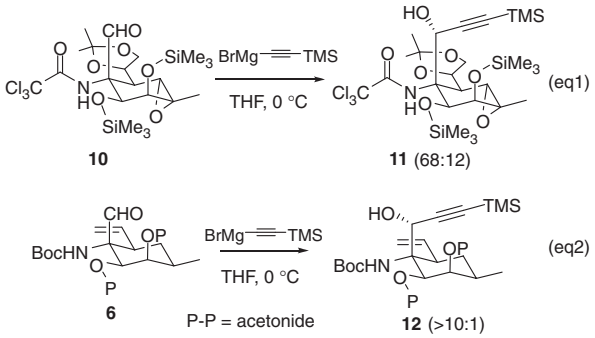
Scheme 1.

Table 1.

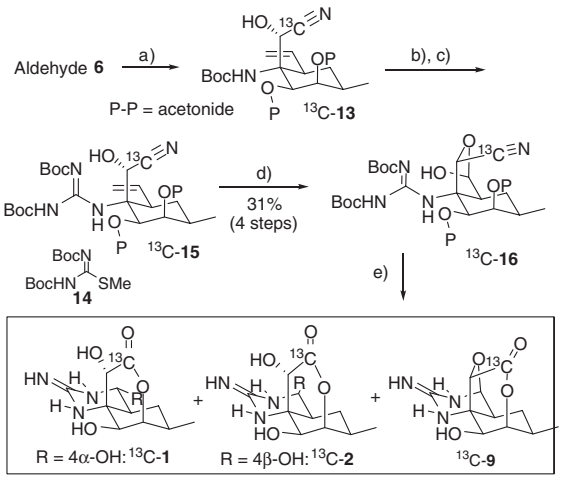


Entry	MCN	Solvent	Additive	Yield of **7**/%	9*S*:9*R*
1	TMSCN	CH_2Cl_2	Et_3N	98	60:40[a]
2	TMSCN	CH_2Cl_2	none	No reaction	—
3	TMSCN	CH_2Cl_2	ZnI_2	complex mixture	—
4	TMSCN	CH_2Cl_2	$BiCl_3$	complex mixture	—
5	NaCN	MeOH	Et_3N	58	64:36
6	NaCN	MeOH	$MgCl_2$	48	85:15[b]

[a]Product ratio was determined by comparison of the authentic data of **7**.[12] [b]Product ratio was determined after the conversion to the authentic *N*-guanidine derivative of ^{12}C-**13**.[12]



Scheme 2.



Scheme 3. Reagent and conditions: a) $Na^{13}CN$, $MgCl_2$, MeOH, rt; b) TMSOTf, 2,6-lutidine, CH_2Cl_2, rt; c) **14**, AgOTf, Et_3N, DMF, rt; d) O_3, Me_2S, MeOH, −78 °C to rt (4 steps, 31%); e) 20% TFA, rt, quant.

attempted the same conditions for the aldehyde **6** to provide (9*R*)-**12** in a highly stereoselective manner (Scheme 2, eq 2).[17] These results indicated that the magnesium cation plays an important role in the stereocontrolled addition reaction of the acetylide anion. Inspired by these results, we attempted the addition of NaCN to **6** in the presence of a magnesium salt (Table 1, Entry 6). To our delight, the stereoselectivity was improved to provide (9*S*)-**7** as the major product (9*S*:9*R* = 85:15). Thus, Na-^{13}CN was successfully incorporated to give ^{13}C-**13** (Scheme 3).

The cyanohydrin ^{13}C-**13** was transformed into the target ^{13}C-**1** and -**2** in a manner similar to our previous total synthesis of **1** and **2** (Scheme 3). The Boc group of ^{13}C-**13** was removed by treatment with TMSOTf in the presence of 2,6-lutidine to give an amine. The amidination reaction with **14** in the presence of AgOTf as a sulfide scavenger produced the guanidine ^{13}C-**15**. The ^{13}C NMR analysis of ^{13}C-**15** showed enhancement of the peak intensity of the nitrile group (118 ppm) compared with the nonlabeled specimen, confirming the ^{13}C atom incorporation by this route (Supporting Information).[18] Ozonolysis of ^{13}C-**15** gave the bicyclic acetal ^{13}C-**16**, which was transformed into a mixture of the ^{13}C-trideoxyTTX (**1**), its 4-epimer **2**, and ^{13}C-**9** by treatment with 20% TFA in a quantitative manner.[19]

In summary, we have achieved the first synthesis of ^{13}C-**1** and ^{13}C-**2** via the $MgCl_2$-assisted stereoselective addition to the aldehyde **6**.[20,21] Biological and bioorganic studies including elucidation of the biosynthetic and metabolic pathways of TTX using ^{13}C-**1** and ^{13}C-**2** are in progress.

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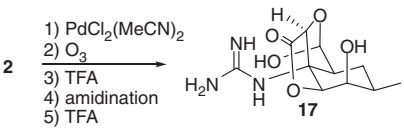
References and Notes

1 M. Yotsu-Yamashita, Y. Yamagishi, T. Yasumoto, *Tetrahedron Lett.* **1995**, *36*, 9329.
2 M. Yotsu-Yamashita, *J. Toxicol., Toxin Rev.* **2001**, *20*, 51.
3 J. N. Wood, M. Baker, *Curr. Opin. Pharmacol.* **2001**, *1*, 17.
4 S. Cestèle, W. A. Catterall, *Biochimie* **2000**, *82*, 883.
5 J. Kobayashi, M. Ishibashi, *Comprehensive Natural Products Chemistry: Miscellaneous Natural Products Including Marine Natural Products, Pheromones, Plant Hormones, and Aspects of Ecology*, ed. by D. Barton, K. Nakanishi, O. Meth-Cohn, Pergamon Press, **1999**, Vol. 8, p. 480.
6 M. Yotsu-Yamashita, A. Sugimoto, A. Takai, T. Yasumoto, *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1688.
7 C. Y. Kao, *Ann. N.Y. Acad. Sci.* **1986**, *479*, 52.
8 P. Rodriguez, A. Alfonso, C. Vale, C. Alfonso, P. Vale, A. Tellez, L. M. Botana, *Anal. Chem.* **2008**, *80*, 5622.
9 M. Diener, B. Christian, M. S. Ahmed, B. Luckas, *Anal. Bioanal. Chem.* **2007**, *389*, 1997.
10 J. Jang, M. Yotsu-Yamashita, *Toxicon* **2006**, *48*, 980.
11 T. Nakagawa, J. Jang, M. Yotsu-Yamashita, *Anal. Biochem.* **2006**, *352*, 142.
12 T. Umezawa, T. Hayashi, H. Sakai, H. Teramoto, T. Yoshikawa, M. Izumida, Y. Tamatani, T. Hirose, Y. Ohfune, T. Shinada, *Org. Lett.* **2006**, *8*, 4971.
13 A. Armstrong, Y. Bhonoah, S. E. Shanahan, *J. Org. Chem.* **2007**, *72*, 8019.
14 T. Dünnwald, M. Müller, *J. Org. Chem.* **2000**, *65*, 8608.
15 N. Komatsu, M. Uda, H. Suzuki, T. Takahashi, T. Domae, M. Wada, *Tetrahedron Lett.* **1997**, *38*, 7215.
16 T. Nishikawa, M. Asai, M. Isobe, *J. Am. Chem. Soc.* **2002**, *124*, 7847.
17 Stereochemistry of (9*R*)-**12** was determined by the conversion to tricyclic **17** in 5 steps.



18 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
19 The anhydro derivative was obtained as a major product (Supporting Information). We have reported that i) the product ratio of **1** and **2** was enhanced by an acid-catalyzed equilibration and ii) the purification conditions of the mixture are reported in ref. 12.
20 The use of a magnesium salt for the diastereoselecitive formation of cyanohydrins. D. E. Ward, M. J. Hrapchak, M. Sales, *Org. Lett.* **2000**, *2*, 57.
21 The role of $MgCl_2$ in the stereoselective formation of **13** has been unclear, because of the presence of the neighboring functional groups which could chelate to magnesium ion. Sales et al. discussed various possibilities for the diastereoselective formation of cyanohydrins from α-alkoxy aldehydes using $Et_4NAg(CN)_2$ or Me_3SiCN in the presence of $MgBr_2$.

