Remarkable Selectivity in Derivatization and Protection of Hydroxy Groups of 51-hydroxyCTX3C: Chemoselective Synthesis of Biotin-conjugated Ciguatoxin Derivatives

Yuuki Ishihara,[1] Keisuke Miyazaki,[1] Shuji Yamashita,*[1] and Masahiro Hirama*[1,2]
[1]*Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578*
[2]*Research and Analytical Centar for Giant Molecules, Graduate School of Science, Tohoku University, Sendai 980-8578*

(Received June 24, 2009; CL-090596; E-mail: s-yamashita@mail.tains.tohoku.ac.jp, hirama@mail.tains.tohoku.ac.jp)

Ciguatoxins, principal causative toxins of ciguatera seafood poisoning, are large ladder-like polycyclic ethers that exert their toxicities by binding to the voltage-sensitive sodium channels. We report C7/C51 chemoselective derivatization of 51-hydroxyCTX3C applicable for synthesis of biological probes.

Ciguatoxins are large 3-nm long ladder-like marine polycyclic ethers that possess several hydroxy groups at the specific positions of the 13 rings among the congeners. They display their toxicities by binding to the voltage-sensitive sodium channels (VSSC),[1] leading to the widespread seafood poisoning known as ciguatera.[2] Chemical and biological studies of ciguatoxins have been hampered by the extreme difficulty to isolate them from toxic fishes. Thus, we have been attempting total synthesis of ciguatoxins and have successfully synthesized three members of the ciguatoxin family, CTX3C (**1**),[3] 51-hydroxyCTX3C (**2**),[4] and CTX1B (**3**),[5] based on a unified synthetic strategy (Figure 1).[6] The pharmacological behavior against VSSC of synthetic CTX3C (**1**), the first congener to be synthesized, has been documented in detail: **1** exerts multimodal effects on VSSC with simultaneous stimulatory and inhibitory aspects.[7] We therefore developed derivatives of **1** containing biotin linkers at either of its terminals (B- and M-rings) to further investigate the pharmacology and molecular mechanism for the interaction with VSSC. An invaluable congener 51-hydroxyCTX3C (**2**) possesses a hydroxy group on the M-ring which is lacking in **1**. We describe herein the chemoselective derivatization and protection of hydroxy groups of **2** and a novel method for selective synthesis of C7 and C51 biotin-linked conjugates of **2**.

To evaluate the global relative reactivity of the hydroxy groups of **2** toward protection reactions, we first examined triethylsilylation. Treatment of **2** with a large excess chlorotriethylsilane (TESCl) and Et_3N in CH_2Cl_2 at $-30\,^{\circ}C$ gave almost exclusively the C51-TES ether in 58% yield with a trace amount of the C7, C51-diTES ether; the C29 (G-ring) and C44 (K-ring) hydroxy groups of **2** were much less reactive than those at C7 and C51. Additional studies were performed using a 1:1 mixture of the ABCDE-ring **4**[8] and HIJKLM-ring fragments **5**[9] instead of invaluable **2** (Table 1). The M-ring hydroxy of **5** selectively reacted with TESCl/Et_3N at $-30\,^{\circ}C$ (Entry 1) and di-*tert*-butyl dicarbonate [$(Boc)_2O$]/*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl)/4-pyrrolidinopyridine[10] in CH_2Cl_2 at room temperature (Entry 2). On the other hand, the C7 hydroxy of **4** was selectively esterified by condensation with mono-Boc–glycine/EDC·HCl/4-(dimethylamino)pyridine (DMAP) in CH_2Cl_2 at room temperature (Entry 3). After considerable experiments we found that di-Boc-protected glycine using EDC·HCl/4-pyrrolidinopyridine gave rise to the C51 protection derivative **7** as a major product (Entry 4). The similar chemoselectivities were also observed for **2** using these conditions: The C7-protected **8** was obtained in 68% yield, and the C51-protected derivative **10** in 78% yield together with the minor C7 derivative **9** (18% yield) from **2**, respectively (Scheme 1).[11] This chemoselectivity is likely due to a lack of the hydrogen on the glycine nitrogen, which is needed for hydrogen bonding with the oxygen of the A- and/or C-ring ether.

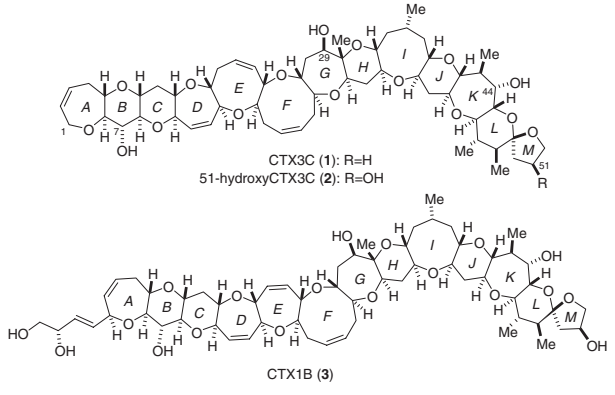
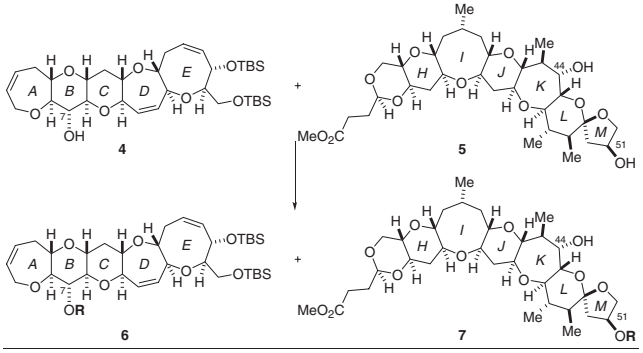
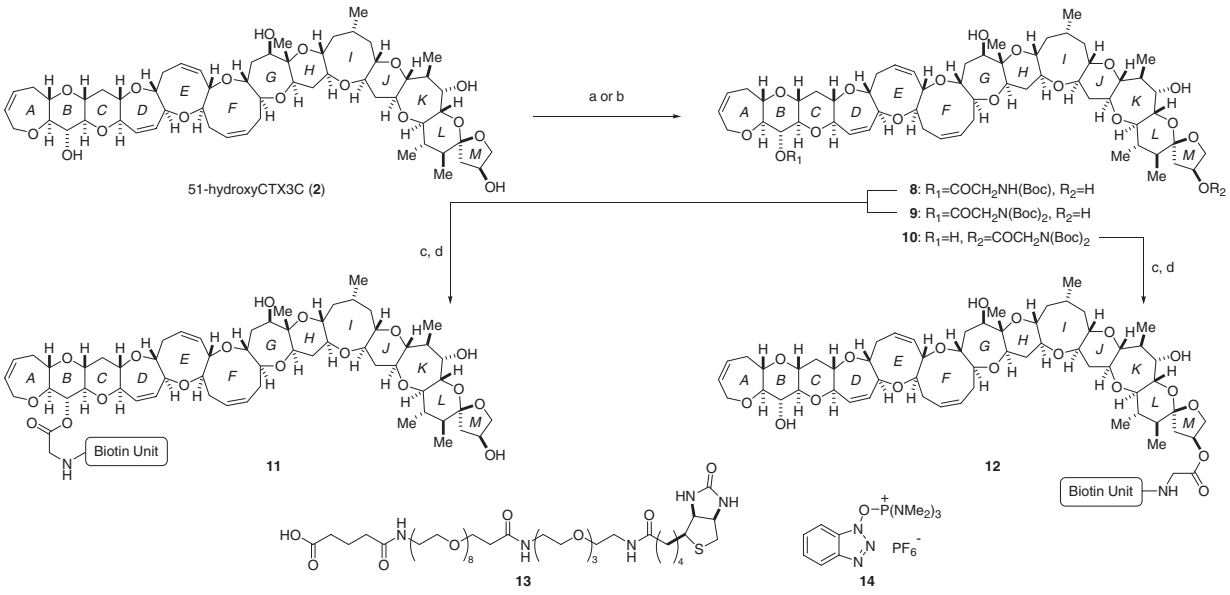


Figure 1. Structures of ciguatoxins.

Table 1. Chemoselective functionalization of a 1:1 mixture of **4** and **5**



Entry	R	Conditions	Major product
1		TESCl, Et_3N CH_2Cl_2, -30 °C.	**7**
2		$(Boc)_2O$, EDC·HCl 4-pyrrolidinopyridine CH_2Cl_2, rt	**7**
3		mono-Boc-Gly, EDC·HCl DMAP, CH_2Cl_2, rt	**6**
4		di-Boc-Gly, EDC·HCl 4-pyrrolidinopyridine CH_2Cl_2, rt	**7**



Scheme 1. a) mono-Boc–Gly, EDC·HCl, DMAP, CH_2Cl_2, rt, **8** (68%); b) di-Boc–Gly, EDC·HCl, 4-pyrrolidinopyridine, CH_2Cl_2, rt, **9** (18%), **10** (78%); c) TFA/CH_2Cl_2, rt; d) **13**, Castro's reagent (**14**), *i*-Pr_2NEt, DMF/DMSO, rt, **11** (100%), **12** (100%).

Selective esterification of C7- and C51-hydroxy groups with glycine derivatives is now attainable. The chemoselectivity is interchangeable by using mono-Boc–glycine or di-Boc–glycine as a glycine derivative. Further functionalization of **8** and **10** were exemplified by formation of their corresponding biotin derivatives. Removal of the Boc-protecting groups of **8** and **10** with trifluoroacetic acid (TFA) furnished the corresponding amines, which were directly reacted with **13**, Castro's reagent **14**,[12] and *i*-Pr_2NEt to give the biotin-linked **11** and **12** in quantitative yields, respectively. We believe these derivatives will play a key role in electrophysiological and electron microscopy studies of ciguatoxin binding to VSSC.[7,13]

In summary, we established a C7/C51 chemoselective synthetic method for ciguatoxin–biotin conjugates **11** and **12**. Our method is applicable for synthesis of biotin conjugates containing various linkers, as well as other C7 and C51 derivatives such as photoaffinity probes and fluorescent probes, to facilitate studies on the interaction between ciguatoxins and VSSC.

This work was supported financially by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan. Fellowships for Young Scientists to Y. I. and K. M. from Japan Society for the Promotion of Science (JSPS) are gratefully acknowledged.

References and Notes

1 L. C. Strachan, R. J. Lewis, G. M. Nicholson, *J. Pharmacol. Exp. Ther.* **1999**, *288*, 379.
2 a) P. J. Scheuer, *Tetrahedron* **1994**, *50*, 3. b) R. J. Lewis, *Toxicon* **2001**, *39*, 97. c) T. Yasumoto, M. Murata, *Chem. Rev.* **1993**, *93*, 1897. d) T. Yasumoto, *Chem. Rec.* **2001**, *1*, 228.
3 M. Satake, M. Murata, T. Yasumoto, *Tetrahedron Lett.* **1993**, *34*, 1975.
4 M. Satake, M. Fukui, A.-M. Legrand, P. Cruchet, T. Yasumoto, *Tetrahedron Lett.* **1998**, *39*, 1197.
5 a) M. Murata, A.-M. Legrand, Y. Ishibashi, T. Yasumoto, *J. Am. Chem. Soc.* **1989**, *111*, 8929. b) M. Murata, A.-M. Legrand, Y. Ishibashi, M. Fukui, T. Yasumoto, *J. Am. Chem. Soc.* **1990**, *112*, 4380. c) M. Murata, A.-M. Legrand, P. J. Scheuer, T. Yasumoto, *Tetrahedron Lett.* **1992**, *33*, 525. d) M. Satake, A. Morohashi, H. Oguri, T. Oishi, M. Hirama, N. Harada, T. Yasumoto, *J. Am. Chem. Soc.* **1997**, *119*, 11325.
6 a) M. Hirama, T. Oishi, H. Uehara, M. Inoue, M. Maruyama, H. Oguri, M. Satake, *Science* **2001**, *294*, 1904. b) M. Inoue, H. Uehara, M. Maruyama, M. Hirama, *Org. Lett.* **2002**, *4*, 4551. c) M. Inoue, K. Miyazaki, H. Uehara, M. Maruyama, M. Hirama, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12013. d) M. Inoue, M. Hirama, *Acc. Chem. Res.* **2004**, *37*, 961. e) M. Hirama, *Chem. Rec.* **2005**, *5*, 240. f) M. Inoue, K. Miyazaki, Y. Ishihara, A. Tatami, Y. Ohnuma, Y. Kawada, K. Komano, S. Yamashita, N. Lee, M. Hirama, *J. Am. Chem. Soc.* **2006**, *128*, 9352.
7 a) K. Yamaoka, M. Inoue, H. Miyahara, K. Miyazaki, M. Hirama, *Br. J. Pharmacol.* **2004**, *142*, 879. b) V. Ghiaroni, H. Fuwa, M. Inoue, M. Sasaki, K. Miyazaki, M. Hirama, T. Yasumoto, G. P. Rossini, G. Scalera, A. Bogoani, *Chem. Senses* **2006**, *31*, 673. c) K. Yamaoka, M. Inoue, K. Miyazaki, M. Hirama, C. Kondo, E. Kinoshita, H. Miyoshi, I. Seyama, *J. Biol. Chem.* **2009**, *284*, 7597.
8 a) M. Maruyama, M. Inoue, T. Oishi, H. Oguri, Y. Ogasawara, Y. Shindo, M. Hirama, *Tetrahedron* **2002**, *58*, 1835. b) S. Kobayashi, Y. Takahashi, K. Komano, B. H. Alizadeh, Y. Kawada, T. Oishi, S. Tanaka, Y. Ogasawara, S. Sasaki, M. Hirama, *Tetrahedron* **2004**, *60*, 8375. c) M. Inoue, S. Yamashita, Y. Ishihara, M. Hirama, *Org. Lett.* **2006**, *8*, 5805.
9 a) A. Tatami, M. Inoue, H. Uehara, M. Hirama, *Tetrahedron Lett.* **2003**, *44*, 5229. b) M. Inoue, S. Yamashita, A. Tatami, K. Miyazaki, M. Hirama, *J. Org. Chem.* **2004**, *69*, 2797.
10 It was reported that the bulkier acylating agent generated with 4-pyrrolidinopyridine would be more selective for less hindered alcohol. For reference, see: E. M. Carreira, J. Du Bois, *J. Am. Chem. Soc.* **1995**, *117*, 8106.
11 Derivatized hydroxy groups of **8**, **9**, and **10** were deteremined by comparison of ^{1}H NMR spectra (see Supporting Information). Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
12 B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron Lett.* **1975**, *16*, 1219.
13 C. Sato, Y. Ueno, K. Asai, K. Takahashi, M. Sato, A. Engel, Y. Fujiyoshi, *Nature* **2001**, *409*, 1047.

Published on the web (Advance View) August 1, 2009; doi:10.1246/cl.2009.886