Tetrahedron Letters,Vol.28,No.51,pp 6485-6488,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

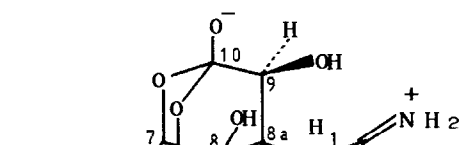
# SYNTHETIC STUDIES ON TETRODOTOXIN (1) STEREOCONTROLLED SYNTHESIS OF THE CYCLOHEXANE MOIETY

Minoru Isobe,* Toshio Nishikawa, Stanislaw Pikul* and Toshio Goto
Laboratory of Organic Chemistry, Faculty of Agriculture,
Nagoya University, Chikusa, Nagoya 464, Japan

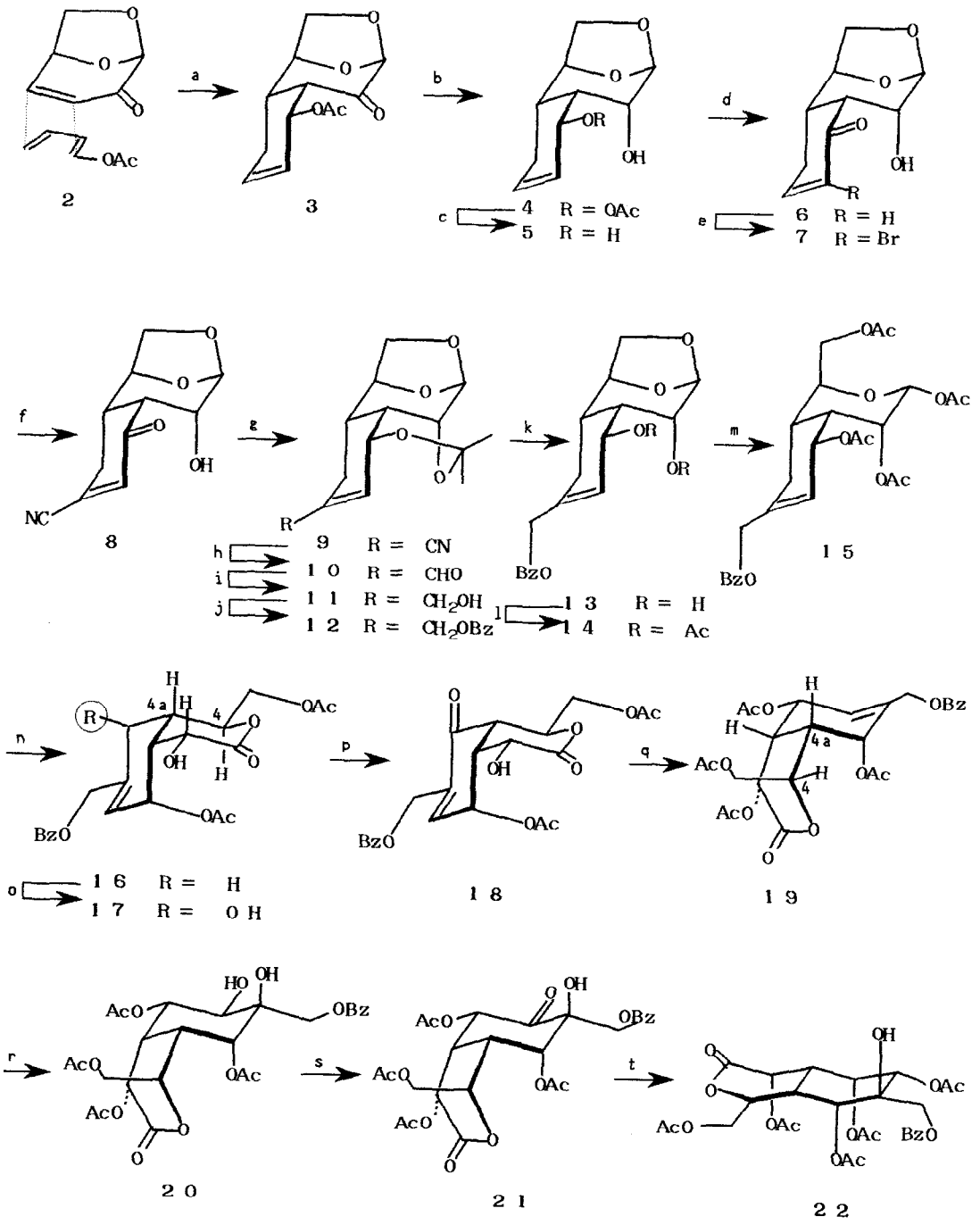
***Abstract*** *Tetrodotoxin is a potent neurotoxin, for which we planned a chemical synthesis in the optically active form. The key steps are i) Diels-Alder cycloaddition to form the optically active system with the cyclohexane moiety, ii) introduction of one-carbon fragment in the form of nitrile and iii) stereoselective hydroxyl group introductions. Stereocontroled synthesis of the cyclohexane part was accomplished.*

Tetrodotoxin ***(TTX, 1)*** has long been known as one of the most famous food toxins caused by puffer fish, *Spheroides rubripes*.[1] TTX is responsible for blocking the sodium ion current in the nervous system of mammals,[2] and the extensive biochemical studies on the sodium channel protein have recently culminated in the elucidation of the 1820 amino acid sequence of this protein.[3] Recently, the origin of this toxin was identified to be a gram negative bacteria.[4] The chemical approach to uncover biologically significant problems will be best achieved chemically by preparing labeled tetrodotoxin, which should play central roles in understanding nerve cell mechanism in molecular level. Therefore, we became interested in the chemical synthesis of TTX which will allow incorporation of $^{13}C$'s or $^{14}C$'s into specific positions of the molecule. In this paper we deal with a new design for the total synthesis of optically active tetrodotoxin.

The key feature in the designing of this chemical synthesis lies in the elaboration of the chiral cyclohexane, for which we have developed a strategy involving Diels-Alder cycloaddition to a chiral dienophile, *levoglucosenone (2)*.[5] The adduct ***3*** was converted into the enone ***6*** *via* ***4*** and ***5*** by the same route as in the case that has been developed for the indole alkaloid synthesis.[5] $C_1$-Fragment was introduced at the C-6 position instead of the C-8 position for the alkaloid.[5b]




Tetrodotoxin (1)

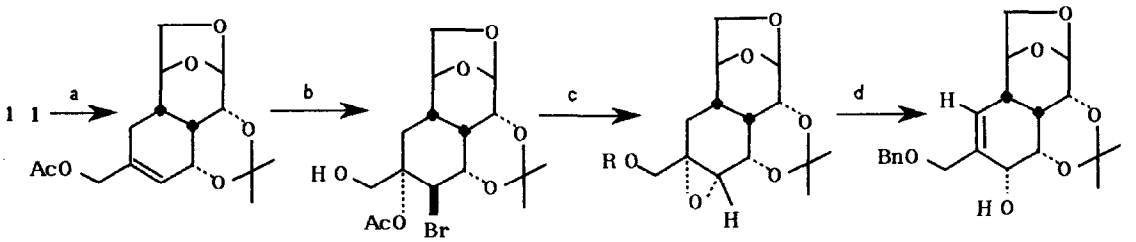


**Scheme 1**

a) heat at 80°C[5b]. b) $NaBH_4$. c) NaOMe. d) $MnO_2$. e) $Br_2$; $Et_3N$. f) KCN/$NH_4Cl$. g) $NaBH_4$; $Me_2C(OMe)_2$/CSA. h) DIBAL-H. i) $LiAlH_4$. j) BzCl/Py. k) AcOH-THF-$H_2O$. l) $Ac_2O$/Py. m) $Ac_2O$/TFA. n) $NH_2NH_2$/DMF; Jones. o) $SeO_2$; p) $CrO_3$-2Py. q) $NaBH_4$, $CeCl_3$; $Ac_2O$/Py. r) $OsO_4$/NMO; $Ac_2O$/Py. s) Swern. t) $NaBH_4$; $Ac_2O$/Py.

The enone bromide *7* was obtained from the enone *6* by addition of 1 equiv. bromine in dichloromethane solvent at 0°C and then by treatment with triethylamine (96%).[6] The addition with 2 equiv. of KCN was run in EtOH in the presence of ammonium chloride at room temperature to give crystalline *8* in 96% yield. Reduction of the carbonyl group in *8* with sodium borohydride (86%) was followed by protection of the diol with 2,2-dimethoxypropane and camphorsulfonic acid as catalyst to yield *9*. Two step reduction of the nitrile group into hydroxy methyl was effected by diisobutylaluminum hydride (1.5*M* toluene) in dichloromethane (to 10) and subsequently by lithiumaluminum hydride (1.2*M* in THF) in THF. The product alcohol *11* was obtained in 67% overall yield as crystals: mp 110°C, $[\alpha]_D$= +21.18° (c=0.97, $CHCl_3$). After the alcohol was protected as its benzoate, *12* (mp 119°C) and the acetonide was hydrolized in a mixture of acetic acid, water and THF (8:1:4), the diol *13* was finally protected as the acetate *14*, $[\alpha]_D$= +21.78° (c=1.01, $CHCl_3$).

Introduction of a hydroxyl group at the C-5 position of TTX may be possible from a precursor olefin with C-5/C-6 double bond. In Scheme 2, is illustrated a possible route directed to *26* from *11*. Treatment of the acetate *23* with N-bromosuccinimide in aq. acetonitrile gave the bromide *24*, which was heated with a base (1,8-diazabicycloundecene) to afford the epoxide *25* (epoxidic H at $\delta$3.13(d, J= 6Hz)).[7] After benzylation of this alcohol, epoxide ring in *25* was opened with lithium diethylamide to give the desired olefin *26* ($^1$H nmr $\delta$ 5.98(m)ppm), but in a low yield. The unexpected low reactivity of the epoxide *25* suggests remarkable strain caused by the tetracyclic ring system.



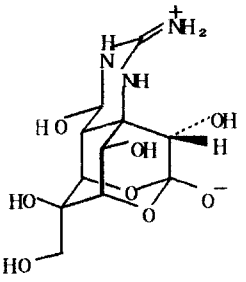

**Scheme 2**

a) $Ac_2O$. b) NBS. c) DBU; BnBr. d)$Et_2NLi$

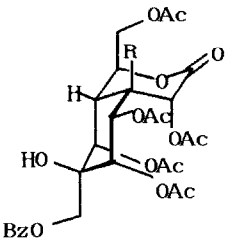
Attempted allylic oxidation at the C-5 position of *12* and *14* in Scheme 1 was difficult again due to the rigid conformation of the ring system.[8] We determined to cleave the 1,6-anhydro bridge to change the fixed conformation. Tri-cyclic *14* was hydrolyzed with trifluoroacetic acid and acetic anhydride to afford the bicyclic *15* in 96% yield.[9] Acidic hydrolysis of the anomeric acetate yielded the corresponding hemiacetal (in a maximum 70% yield) but with a side product.[10] The selective hydrolysis was better achieved with hydrazine (1.2 equiv. in DMF at 60°C).[11] Jones oxidation of this hemiacetal afforded the lactone *16* in 55% overall yield. The crucial allylic oxidation of *16* with $SeO_2$ was successful from the inverted chair-chair conformation ($J_{4,4a}$=12 Hz)[12] in refluxing xylene to yield *17* (48% yield). The hydroxy group in *17* was inverted in the following two steps involving oxidation of the alcohol with $CrO_3$-2Py to give the ketone *18* and reduction with $NaBH_4$-$CeCl_3$ to the alcohol which was isolated as the corresponding acetate *19* in 79% overall yield. At this moment, the conformation of the ring system altered into boat-chair judging from the coupling constants in $^1$H nmr $J_{4,4a}$ into 5Hz.

The last oxidation of the double bond in *19* was catalyzed with $OsO_4$ in a mixture of $CH_3CN$ and water in the presence of N-methylmorpholinium oxide. The configuration of the

secondary hydroxy group in the diol *20* was simply inverted into the tetrodotoxin type *via* Swern oxidation (*21*) and then sodium borohydride reduction and acetylation into *22*[13] in 39% overall yield. The final product *22* in this paper is drawn as *22a* for comparison of the stereochemistry with tetrodotoxin *1a*. Stereochemical problems on the cyclohexane ring have been solved in these studies. Introduction of the nitrogen functionality (*22a* R= $NH_2$) is currently studied.



1 a *Tetrodotoxin*



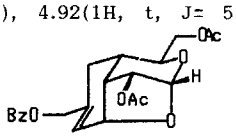
2 2 a *R= H, $NH_2$*

*Acknowledgements* Authors are indebted to Shourai Foundation and Grant-in-aid for Scientific Research for financial supports.

*References and Notes*


* To whom all correspondences should be addressed. * JSPS Postdoctral research associate fellow (Japan Society for the Promotion of Science) on leave from Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland.

1. For structure: a) K. Tsuda, C. Tamura, R. Tachikawa, K. Sakai, O. Amakasu, M. Kawamura, S. Ikuma, *Chem. Pharm. Bull.*, 11, 1473 (1963); K. Tsuda, R. Tachikawa, C. Tamura, O. Amakasu, M. Kawamura, S. Ikuma, *ibid*, 12, 634, 642, 1357 (1964). b) T. Goto, Y. Kishi, S. Takahashi, Y. Hirata, *Tetrahedron Lett.*, 2105, 2115 (1963); *ibid*, 779, 1831 (1964); *Tetrahedron*, 21, 2059 (1965). c) R.B. Woodward et al., *Pure and Appl. Chem.*, 9, 49 (1964); R.B. Woodward, J.Z. Gougoutas, *J. Am. Chem. Soc.*, 86, 5030 (1964). For synthetic studies to racemic TTX: a) Kishi *et al.*, *Tetrahedron Lett.*, 5127, 5129 (1970); 335 (1974); *J. Am. Chem. soc.*, 94, 9217, 9219 (1972).
2. a) T. Narahashi, J.W. Moore, R.N. Poson, *Science*, 156, 976 (1967). b) W.A. Catterall, *Ann. Rev. Pharmacol. Toxicol.*, 20, 15 (1980).
3. M. Noda, S. Shimizu, T. Tanabe, T. Takai, T. Kayano, T. Ikeda, H. Takahashi, H. Nakayama, Y. Kanaoka, N. Minamino, K. Kangawa, H. Matsuo, M.A. Raftery, T. Hirose, S. Inayama, H. Hayashida, T. Miyata, S. Numa, *Nature*, 312, 121 (1984).
4. a) T. Yasumoto, D. Yasumura, M. Yotsu, T. Michishita, A. Endo and Y. Kotaki, *Agric. Biol. Chem.*, 50, 793 (1986). b) Y. Shimizu, *Ann. New York. Acad. Sci.*, 479, 24 (1986).
5. a) M. Isobe, N. Fukami and T. Goto, *Chemistry Lett.*, 71 (1985). b) M. Isobe, N. Fukami, T. Nishikawa and T. Goto, *Heterocycles*, 25, 521 (1987).
6. This bromoenone *7* has an advantage for the future introduction of $^{13}C$ labeling because it readily receives the attack by KCN as C-1 source.
7. This epoxide was clearly different from the other $\beta$-epoxide (epoxidic H at $^1H$ nmr $\delta$ 3.24(d, J= 1Hz) obtained from *11* by treatment with m-chloroperbenzoic acid.
8. A selenium dioxide oxidation is known to start in an ene reaction to the enophile, the allylic system; thus, the reaction occurs only on the convex face and only when it has axial allylic proton.
9. Although only one product *15* was obtained, its conformation could not be determined as shown in Scheme 1. 15 $^1H$ nmr $\delta$ 1.99(3H, s), 2.09(3H, s), 2.11(3H, s), 2.13(3H, s), 2.93(1H, dt, J= 7, 4), 4.08-4.34(3H, m), 4.76(2H, brs), 4.92(1H, t, J= 5), 5.60(1H, m), 5.74(1H, brs), 6.14(1H, d, J= 5).
10. A tricyclic acetal with tetrahydrofuran ring such as the following was formed.



11. G. Excoffeier, D. Gagnaire and J.P. Utille, *Carbohydrate Res.*, 39, 368 (1975).
12. The nmr analysis of *16* as its acetate *17*: $^1H$ nmr $\delta$ 2.02(3H, s), 2.12(3H, s), 2.16(3H, s), 4.17(1H, dt, J= 12, 3), 5.52(1H, d, J= 6), 5.57(1H, t, J= 5), 6.00(1H, brd, J=5).
13. *22* $^1H$ nmr $\delta$ 1.87(3H, s), 1.94(3H, s), 2.14(3H, s), 2.16(3H, s), 2.19(3H, s), 2.87(1H, td, J= 6, 4), 3.12(1H, dt, J= 9, 6), 4.16(1H, dd, J= 12, 4), 4.40(1H, dd, 12, 2), 5.00(2H, AB), 5.03(1H, d, J= 4), 5.10-5.24(1H, overlap), 5.22(1H, d, J= 6), 5.53(1H, d, J= 6), 5.65(1H, t, J= 4), 7.42-7.69(3H, m), 8.01-8.10(2H, m).
14. All crystalline new compounds have shown proper combustion analyses.


(Received in Japan 24 September 1987)