J|A|C|S
COMMUNICATIONS

Published on Web 08/30/2003

A Stereoselective Synthesis of (−)-Tetrodotoxin

Andrew Hinman and J. Du Bois*

Department of Chemistry, Stanford University, Stanford, California 94305-5080

Received June 22, 2003; E-mail: jdubois@stanford.edu

Tetrodotoxin (TTX), the guanidium poison synonymous with the Japanese *fugu*, is one of Nature's great marvels.[1,2] Its elaborate chemical architecture, crafted from a densely oxygenated cyclohexane framework and having affixed unique ortho-acid and guanidine aminal functionalities, is matched only by its awesome potency as a selective blocker of voltage-gated Na^+ ion channels.[3] Thirty years following the first synthesis by Kishi and co-workers[4] and despite numerous attempts,[5] only one additional route to the poison stands, having been disclosed recently by Isobe and his lab.[6,7] Herein, we describe our finished path to an asymmetric synthesis of (−)-TTX. The successful realization of this program offers testament to the power of modern catalytic methods for C−H bond functionalization and portends the coming of age of such tools for the preparation of highly complex natural products.

Guided by previous efforts to synthesize (−)-TTX, we devised an approach that would delay installation of the polar ortho-acid and guanidine functional groups until the final step of the plan.[4,7] Added knowledge of the base-lability of the target prompted the selection of blocking groups that could be cleaved under acidic conditions to reveal the assembled toxin.[2] Such a strategy would make inevitable, however, formation of both TTX and 4,9-anhydro-TTX, which exist as a mixture in aqueous acid (Figure 1).[2,6] Pursuant to this analysis, a masked form of the conjured structure "tetrodamine" became the focal point of our route.[4a] Construction of this highly oxygenated cyclohexylamine derivative affords numerous challenges, most notably in the form of two tetrasubstituted stereocenters at C6 and C8a (Figure 2). Of these, the requisite C8a carbinolamine presented an irresistible test for our recently developed intramolecular C−H amination method.[8] A decisive strategy thus evolved that would install the amine unit in the late stages of the synthesis through a stereospecific oxidation reaction. As such, retrosynthetic planning was further simplified to cyclohexane **1**. A solution to this problem in cyclic stereocontrolled synthesis was envisioned from diazoketone **2**. Selective decomposition of **2** could fashion both the cyclohexane framework and the tetrasubstituted C6 stereocenter in a single event.[9] Accordingly, stereospecific metal-mediated nitrene and carbene C−H insertion reactions would hallmark the defining bond constructions in our approach to TTX.

Synthesis of (−)-TTX commenced from amide **3**, readily available in four steps from D-isoascorbic acid (Scheme 1).[10] Reduction of this intermediate using a combination of *i*-Bu_2AlH and *n*-BuLi yielded aldehyde **4**.[11] A convenient and easily scalable aldol reaction with dibenzyloxalacetate **5** facilitated conversion of **4** to the desired lactone **6**.[12] For this step NaOAc served as an optimal base, generating the product with >10:1 C7,C8-anti diastereocontrol (TTX numbering). Following treatment with *t*-BuCOCl, pivaloate **7** was isolated as a single stereoisomer in 85% overall yield (three steps). Hydrogenolysis of **7** afforded a carboxylic acid, which could be processed to diazoketone **8** without event.

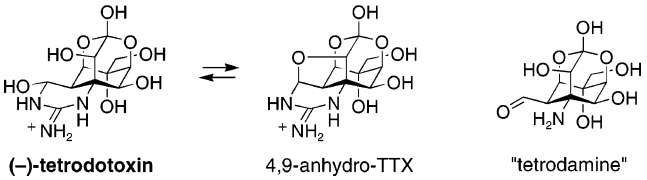


Figure 1. (−)-TTX, the active poison of the Japanese *fugu*.

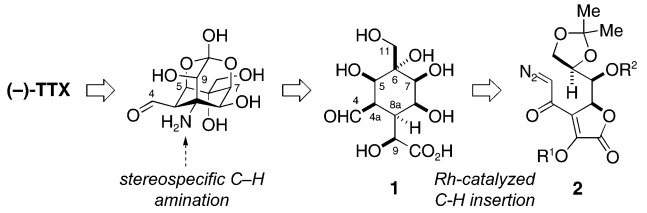


Figure 2. Retrosynthetic analysis of (−)-TTX.

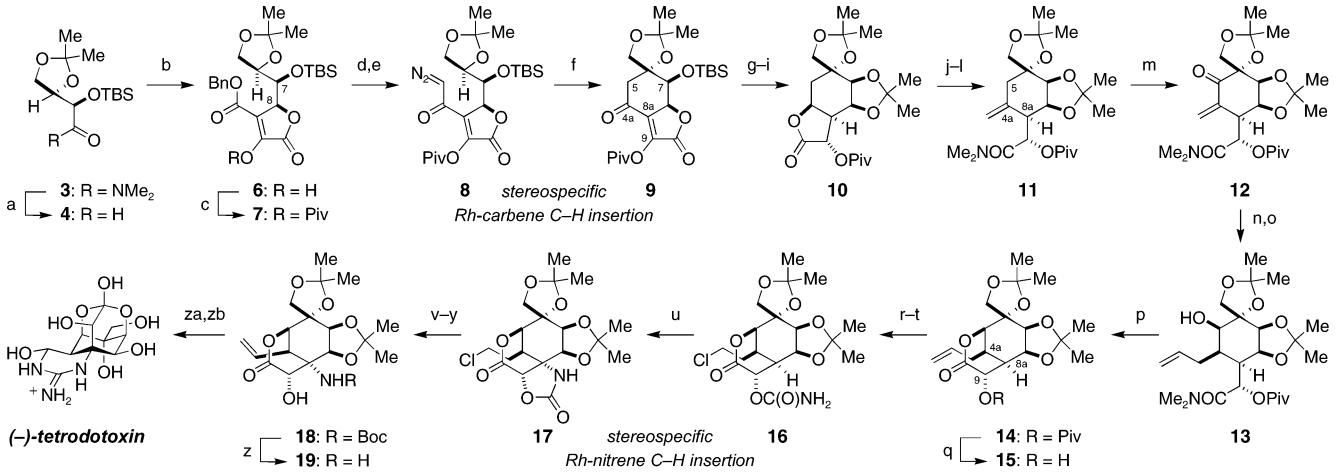
Selective formation of cyclohexanone **9** from diazoketone **8** was anticipated to occur under Rh-catalysis. Our first attempts to effect this transformation using $Rh_2(OAc)_4$, however, yielded complex product mixtures. Cognizant of the exceptional performance of Rh-acetamide catalysts for C−H insertion reactions, we screened several of these agents.[13] Much to our satisfaction, a reaction performed with 1.5 mol % $Rh_2(HNCOCPh_3)_4$ resulted in the exclusive production of cyclic ketone **9**. The outstanding efficiency of this process made possible subsequent use of **9** without purification.

Having established a 10-step route to the functionalized cyclohexanone **9**, our attention turned to installation of the C8a and C9 stereocenters. The capricious nature of the vinylogous anhydride in **9** necessitated initial reduction of the C4a carbonyl. For this unusual transformation, $BH_3{\cdot}NH_3$ offered superior regio- and stereocontrol, yielding the C4a alcohol as a single epimer.[14] The strong bias for reactions to proceed from the convex face of the bicyclic framework in structures such as **9** was again exploited in the ensuing alkene hydrogenation step.[15] Acetonide protection of the resulting tetraol was followed by a straightforward three-step sequence to complete the TTX carbon skeleton **11**.

Our decision to install the C5 oxygen at this stage of the synthesis was part of a larger strategic plan to access the bridged C5 lactone (**14**). Modification of a little-known procedure for allylic oxidation provided enone **12** (70%).[16] This versatile intermediate was well crafted for establishing both the C4 functionality and the C4a and C5 stereocenters. Accordingly, 1,4-addition of vinyl cuprate to **12** occurred with selective protonation of the intermediate Cu-enolate to yield a single product.[17] Subsequent reduction of the C5 ketone also proceeded from the convex face of the fused bicycle to give **13**. The axial C5 alcohol could now be used to cleave the robust 3° amide and to assemble δ-lactone **14**. Selective removal of the pivaloate ester afforded **15**, an intermediate suitably configured for the impending C−H insertion reaction.

10.1021/ja0368305 CCC: $25.00 **© 2003 American Chemical Society**

Scheme 1 [a]



[a] Conditions: (a) *i*-Bu_2AlH, *n*-BuLi, THF/hexanes; (b) $BnO_2CCH_2C(O)CO_2Bn$ **5**, NaOAc, THF; (c) *t*-BuCOCl, C_5H_5N, THF, 85% (three steps); (d) H_2, Pd−C, THF, 88%; (e) $(COCl)_2$, cat. DMF, THF; then CH_2N_2, CH_2Cl_2, 63−70%; (f) 1.5 mol % $Rh_2(HNCOCPh_3)_4$, CCl_4; (g) $NH_3{\cdot}BH_3$, CH_2Cl_2/MeOH, 75% (two steps); (h) H_2 (1200 psi), 5 mol % Rh−C, 2:1 CF_3CO_2H/MeOH; (i) *p*-TsOH, 2,2-DMP, THF, 77% (two steps); (j) Me_2NH, THF, 83%; (k) cat. (*n*-Pr_4N)RuO_4, NMO, 4 Å MS, CH_2Cl_2, 94%; (l) Zn, $TiCl_4$, CH_2I_2, cat. $PbCl_2$, THF, 72%; (m) Ph_2Se_2, $PhIO_2$, C_5H_5N, C_6H_5Cl, 100 °C, 70%; (n) $H_2C{=}CHMgBr$, CuI, THF; (o) *t*-$BuNH_2{\cdot}BH_3$, DCE, 77% (two steps); (p) *t*-$BuCO_2H$, C_6H_5Cl, 200 °C; (q) NaOMe, THF/MeOH 78% (two steps), (r) $Cl_3CC(O)NCO$, CH_2Cl_2; Zn, MeOH, 93%; (s) O_3; then $NaBH_4$, CH_2Cl_2/MeOH, 83%; (t) $MeSO_2Cl$, C_5H_5N, DCE, 86%; (u) 10 mol % $Rh_2(HNCOCF_3)_4$, $PhI(OAc)_2$, MgO, C_6H_6, 65 °C, 77%; (v) NaSePh, THF/DMF, 77%; (w) *m*-CPBA; C_5H_5N, DCE, 55 °C, 92%; (x) Boc_2O, Et_3N, DMAP, THF; (y) K_2CO_3, THF/MeOH, 84% (two steps); (z) H_2O, 110 °C, 95%; (za) $BocN{=}C(SMe)NHBoc$, $HgCl_2$, Et_3N, $MeCN/CH_2Cl_2$, 80%; (zb) O_3, CH_2Cl_2/MeOH; Me_2S; then aq CF_3CO_2H, 65%.

Installation of the tetrasubstituted carbinolamine at C8a would be accomplished through stereospecific C−H amination.[8] To this end, the requisite 1° carbamate was affixed at C9. An efficient two-step sequence made available chloride **16**, properly outfitted for the critical insertion step. Surprisingly, the conditions we originally reported for C−H amination gave only trace amounts of oxazolidinone **17**. After further testing, however, a reaction performed with 10 mol % $Rh_2(HNCOCF_3)_4$ was found to yield 77% of the desired adduct **17**. This result was particularly satisfying, given the structural complexity of the substrate.

The C8a nitrogen center in place, straightforward assembly of **19**, a masked form of "tetrodamine", was now possible. Guanidinylation of **19** provided the penultimate intermediate, which was converted to (−)-TTX following ozonolysis and treatment with aqueous CF_3CO_2H. In the final event, all protecting groups were removed, and the assembled poison was isolated as a 1:1 mixture with its anhydro form. Standing in aqueous acid for 5 days, the ratio of TTX could be enriched to ∼4:1, from which purified toxin was shown to match a commercial sample in all respects (NMR, HRMS, HPLC).[2,18] The finished work underscores C−H functionalization as a unique strategy for assembling complex targets and further validates our Rh-catalyzed nitrene insertion as a powerful new tool in synthesis.

Acknowledgment. To our friend and colleague Harry S. Mosher (1915−2001) for his inspiration and in tribute to his work on tetrodotoxin (tarichatoxin). A.H. is grateful to the NSF and to Eli Lilly for fellowship awards. This work has been supported by the NIH and by generous gifts from Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, GlaxoSmithKline, Johnson Matthey, Merck, and Pfizer.

Supporting Information Available: Experimental details and analytical data for select compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel*; Kao, C. Y., Levinson, S. R., Eds.; New York Academy of Sciences: New York, 1986; Vol. 479.

(2) (a) Goto, T.; Kishi, Y.; Takahashi, S.; Hirata, Y. *Tetrahedron* **1965**, *21*, 2059−2088. (b) Mosher, H. S.; Fuhrman, F. A.; Buchwald, H. D.; Fischer, H. G. *Science* **1964**, *144*, 1100−1110. (c) Tsuda, K.; Ikuma, S.; Kawamura, M.; Tachikawa, R.; Sakai, K.; Tamura, C.; Amakasu, O. *Chem. Pharm. Bull.* **1964**, *12*, 1357−1374. (d) Woodward, R. B. *Pure Appl. Chem.* **1964**, *9*, 49−74.

(3) For recent references, see: (a) Choudhary, G.; Yotsu-Yamashita, M.; Shang, L.; Yasumoto, T.; Dudley, S. C., Jr. *Biophys. J.* **2003**, *84*, 287−294. (b) Narahashi, T. *J. Toxicol., Toxin Rev.* **2001**, *20*, 67−84.

(4) (a) Kishi, Y.; Aratani, M.; Fukuyama, T.; Nakatsubo, F.; Goto, T.; Inoue, S.; Tanino, H.; Sugiura, S.; Kakoi, H. *J. Am. Chem. Soc.* **1972**, *94*, 9217−9219. (b) Kishi, Y.; Fukuyama, T.; Aratani, M.; Nakatsubo, F.; Goto, T.; Inoue, S.; Tanino, H.; Sugiura, S.; Kakoi, H. *J. Am. Chem. Soc.* **1972**, *94*, 9219−9221.

(5) For recent progress, see: (a) Ohtani, Y.; Shinada, T.; Ohfune, Y. *Synlett* **2003**, 619−622. (b) Itoh, T.; Watanabe, M.; Fukuyama, T. *Synlett* **2002**, 1323−1325 and references therein.

(6) Ohyabu, N.; Nishikawa, T.; Isobe, M. *J. Am. Chem. Soc.* **2003**, *125*, 8798−8805.

(7) Isobe has described syntheses of several deoxy-TTX forms, see: Nishikawa, T.; Urabe, D.; Yoshida, K.; Iwabuchi, T.; Asai, M.; Isobe, M. *Pure Appl. Chem.* **2003**, *75*, 251−257 and references therein.

(8) Espino, C. G.; Du Bois, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 598−600.

(9) For recent examples of intramolecular C−H insertion in synthesis, see: (a) Kurosawa, W.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 8112−8113. (b) White, J. D.; Hrnciar, P.; Stappenbeck, F. *J. Org. Chem.* **1999**, *64*, 7871−7884. (c) Taber, D. F.; Stiriba, S.-E. *Chem. Eur. J.* **1998**, *4*, 990−992 and references therein.

(10) Carreira, E. M.; Du Bois, J. *J. Am. Chem. Soc.* **1994**, *117*, 8106−8125.

(11) (a) Trost, B. M.; Nishimura, Y.; Yamamoto, K.; McElvain, S. S. *J. Am. Chem. Soc.* **1979**, *101*, 1328−1330. (b) Kovács, G.; Galambos, G.; Juvancz, Z. *Synthesis* **1977**, 171−172.

(12) Barrett, A. G. M.; Sheth, H. G. *J. Org. Chem.* **1983**, *48*, 5017−5022.

(13) Doyle, M. P.; McKervey, M. A.; Ye, T. *Modern Catalytic Methods for Organic Synthesis with Diazo Compounds*; Wiley-Interscience: New York, 1998.

(14) Andrews, G. C.; Crawford, T. C. *Tetrahedron Lett.* **1980**, *21*, 693−696.

(15) Under the acidic hydrogenation conditions, cleavage of the protecting groups and isomerization of the reduced lactone occurred.

(16) Barton, D. H. R.; Crich, D. *Tetrahedron* **1985**, *41*, 4359−4364.

(17) Zimmerman, H. E. *Acc. Chem. Res.* **1987**, *20*, 263−268.

(18) Yasumoto, T.; Yotsu, M.; Murata, M.; Naoki, H. *J. Am. Chem. Soc.* **1988**, *110*, 2344−2345.

JA0368305