Japan Academy Prize to:

Masahiro HIRAMA
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for "Total Synthesis of Ciguatoxins and Structurally Complex Bioactive Natural Products"

Outline of the work:

Nature has the incredible ability to create molecules with interesting structures and remarkable biological functions, which often present new challenges to researchers worldwide. A recurrent question regarding natural products is if and how we can synthesize them from easily available starting materials. The total synthesis of natural products with complex architectures and potent bioactivities is a most rewarding and challenging endeavor in the chemical sciences. Such work stimulates the development of powerful synthetic strategies, tactics and methodologies, as well as the design of new functional molecules. This constitutes the basis for bioscience and material sciences, and can help address public health problems. Dr. Masahiro Hirama believes that success in total synthesis is not the end of research but rather the beginning of new scientific endeavors enabled by the power and versatility of chemical synthesis.

1. Total synthesis of ciguatoxins

The landmark of Dr. Hirama's scientific accomplishments is the total synthesis of ciguatoxins (CTXs), the neurotoxins that cause ciguatera fish poisoning. This is produced by the ingestion of a variety of reef fish that have accumulated trace amounts of CTXs, which are large 3 nm-long ladder-like polycyclic ether systems and more toxic than the famous Puffer fish tetrodotoxin. The extremely low availability of ciguatoxins in fish hampered the detection, prevention, and treatment of ciguatera. To investigate how to combat ciguatera poisoning, it was necessary to find a reliable source of the chemical. However, the huge and complex molecular structure of CTXs was an issue for their chemical synthesis, which was the only realistic solution for obtaining enough CTXs for research procedures. Dr. Hirama's 12-year formidable efforts culminated in the first total synthesis of CTX3C in 2001, using a new unified convergent strategy, which was acknowledged in the publication "The Art of Total Synthesis" in *Science*. This efficient strategy enabled the total syntheses of four important Pacific related compounds as well as F-ring modified analogs, which demonstrated that the 9-membered F ring plays a critical role in the binding of CTX to the sodium channel and the subsequent toxicity. Since natural CTXs are not readily available, synthetic CTXs accelerated biological and neurological studies, and were used as standards for toxins analyses. The total synthesis also successfully allowed to develop antibodies by a synthesis-based approach using rationally designed synthetic haptens. Specific anti-CTX monoclonal antibodies were prepared, and a highly-sensitive sandwich enzyme-linked immunosorbent assay (ELISA) method for the reliable detection of CTXs in fish was established. The ELISA detection kit is now commercially available worldwide. The successful total synthesis of CTXs has significantly impacted biological and pharmacological studies, and would facilitate both the prevention and treatment of ciguatera poisoning.

2. Total syntheses of chromoprotein enediyne antitumor antibiotics

Macromolecular chromoprotein antitumor antibiotics, such as neocarzinostatin, maduropeptin, C-1027, and kedarcidin, are composed of a highly-strained and reactive 9-membered enediyne chromophore and an apoprotein. After considerable efforts, Dr. Hirama developed a strategy to synthesize such complex enediyne system, achieving the total syntheses of the unstable chromophores. Through the synthetic challenges of natural chromophores and model compounds, he discovered that the 9-membered enediynes equilibrate with the p-benzyne biradical intermediates. Furthermore, this finding clarified how the chromophores cut the DNA double strand. He also observed that the natural chromophore-apoprotein complex of C-1027 and the synthetic bicyclic 9-membered enediyne are paramagnetic in the solid form and in solution, respectively. They exhibit steady ESR signals, albeit the observed paramagnetic species is not directly attributed to the equilibrated p-benzyne biradical, but rather to the more stable secondary radical species. Based on the NMR structural analysis of the chromophore-apoprotein complex of C-1027, he designed and prepared a recombinant deuterated (D-Gly) apoprotein to improve the chromophore-stabilizing activity due to the kinetic deuterium-isotope effect. The results of this work demonstrated that the kinetic stabilization of the reactive chromophore enhances the overall stability of the small molecule-protein complex, thus achieving more effective antitumor activities compared to the natural C-1027.

3. Total syntheses of other natural products

Dr. Hirama has accomplished the total syntheses of more than 35 architecturally interesting bioactive natural products, such as compactin, lovastatin, and avermectin.

In summary, Dr. Masahiro Hirama has successfully worked on the total syntheses of biologically active natural products, that have led to remarkable progress in bioscience and materials science, and, most importantly, have helped resolve public health problems. His seminal work has already been recognized by the various awards and honors he has received, including the Inoue Prize for Science (1998), Synthetic Organic Chemistry Award, Japan (2000), the Chemical Society of Japan Award (2004), the Fujihara Award (2010), and Medal with Purple Ribbon (2011).

List of Main Publications

(1) **M. Hirama** and M. Uei, A Chiral Total Synthesis of Compactin, *J. Am. Chem. Soc.*, **104**, 4251–4253 (1982).

(2) **M. Hirama**, K. Fujiwara, K. Shigematsu, and Y. Fukazawa, The 10-Membered Ring Analogs of Neocarzinostatin Chromophore: Design, Synthesis and Mode of Decomposition, *J. Am. Chem. Soc.*, **111**, 4120–4122 (1989).

(3) **M. Hirama**, T. Noda, S. Yasuda, and S. Itô, Simple Strategy for the Synthesis of the Avermectin-Milbemycin Family. Total Synthesis of Milbemycin α1, *J. Am. Chem. Soc.*, **113**, 1830–1832 (1991).

(4) **M. Hirama**, T. Gomibuchi, K. Fujiwara, Y. Sugiura, and M. Uesugi, Synthesis and DNA Cleaving Abilities of Functional Neocarzinostatin Chromophore Analogs. Base Discrimination by a Simple Alcohol, *J. Am. Chem. Soc.*, **113**, 9851–9853 (1991).

(5) K. Iida and **M. Hirama**, Efficient Route to the Nine-Membered Cyclic Diyne System: Tuning of the Extremely Facile Cope Rearrangement of 1,5-Diyne, *J. Am. Chem. Soc.*, **116**, 10310–10311 (1994).

(6) K. Iida and **M. Hirama**, Synthesis and Characterization of Nine-Membered Cyclic Enediynes, Models of the C-1027 and Kedarcidin Chromophores: Equilibration with a p-Benzyne Biradical and Kinetic

Stabilization, *J. Am. Chem. Soc.*, **117**, 8875–8876 (1995).
(7) M. Satake, A. Morohashi, H. Oguri, T. Oishi, **M. Hirama**, N. Harada, and T. Yasumoto, The Absolute Configuration of Ciguatoxin, *J. Am. Chem. Soc.*, **119**, 11325–11326 (1997).
(8) S. Kawata, S. Ashizawa, and **M. Hirama**, Synthetic Study of Kedarcidin Chromophore: Revised Structure, *J. Am. Chem. Soc.*, **119**, 12012–12013 (1997).
(9) **M. Hirama**, K. Akiyama, T. Tanaka, T. Noda, K. Iida, I. Sato, R. Hanaishi, S. Fukuda-Ishisaka, M. Ishiguro, T. Otani, and J. E. Leet, Paramagnetic Enediyne Antibiotic C-1027: Spin Identification and Characterization of Radical Species, *J. Am. Chem. Soc.*, **122**, 720–721 (2000).
(10) S. Kobayashi, S. Ashizawa, Y. Takahashi, Y. Sugiura, M. Nagaoka, M. J. Lear, and **M. Hirama**, The First Total Synthesis of N1999-A2: Absolute Stereochemistry and Stereochemical Implications into DNA Cleavage, *J. Am. Chem. Soc*., **123**, 11294–11295 (2001).
(11) **M. Hirama**, T. Oishi, H. Uehara, M. Inoue, M. Maruyama, H. Oguri, and M. Satake, Total Synthesis of Ciguatoxin CTX3C, *Science*, **294**, 1904–1907 (2001).
(12) H. Oguri, **M. Hirama**, T. Tsumuraya, I. Fujii, M. Maruyama, H. Uehara, and Y. Nagumo, Synthesis-Based Approach toward Direct Sandwich Immunoassay for Ciguatoxin CTX3C, *J. Am. Chem. Soc.*, **125**, 7608–7612 (2003).
(13) T. Usuki, M. Inoue, **M. Hirama**, and T. Tanaka, Rational Design of a Supra C-1027: Kinetically Stabilized Analogue of the Antitumor Enediyne Chromoprotein, *J. Am. Chem. Soc.*, **126**, 3022–3023 (2004).
(14) M. Inoue and **M. Hirama**, Evolution of a Practical Total Synthesis of Ciguatoxin CTX3C, *Acc. Chem. Res*., **37**, 961–968 (2004).
(15) T. Usuki, T. Mita, M. J. Lear, P. Das, F. Yoshimura, M. Inoue, **M. Hirama**, K. Akiyama, and S. Tero-Kubota, Spin-Trapping of ^{13}C-Labeled *p*-Benzynes Generated by Masamune–Bergman Cyclization of Bicyclic Nine-Membered Enediynes, *Angew. Chem. Int. Ed.*, **43**, 5249–5253 (2004).
(16) M. Inoue, K. Miyazaki, H. Uehara, M. Maruyama, and **M. Hirama**, First- and Second-Generation Total Synthesis of Ciguatoxin CTX3C, *Proc. Natl. Acad. Sci. USA*, **101**, 12013–12018 (2004).
(17) **M. Hirama**, Total Synthesis of Ciguatoxin CTX3C: A Venture into the Problems of Ciguatera Seafood Poisoning, *Chem. Rec.*, **5**, 240–250 (2005).
(18) M. Inoue, T. Usuki, N. Lee, **M. Hirama**, T. Tanaka, F. Hosoi, S. Ohie, and T. Otani, Antitumor Enediyne Chromoprotein C-1027: Mechanistic Investigation of the Chromophore-Mediated Self-Decomposition Pathway, *J. Am. Chem. Soc*., **128**, 7896–7903 (2006).
(19) M. Inoue, K. Miyazaki, Y. Ishihara, A. Tatami, Y. Ohnuma, Y. Kawada, K. Komano, S. Yamashita, N. Lee, and **M. Hirama**, Total Synthesis of Ciguatoxin and 51-HydroxyCTX3C, *J. Am. Chem. Soc*., **128**, 9352–9354 (2006).
(20) K. Komano, S. Shimamura, M. Inoue, and **M. Hirama**, Total Synthesis of the Maduropeptin Chromophore Aglycon, *J. Am. Chem. Soc*., **129**, 14184–14186 (2007).
(21) M. Inoue, I. Ohashi, T. Kawaguchi, and **M. Hirama**, Total Synthesis of the C-1027 Chromophore Core: Extremely Facile Enediyne Formation through SmI_2-Mediated 1,2-Elimination, *Angew. Chem. Int. Ed.*, **47**, 1777–1779 (2008).
(22) M. Inoue, N. Lee, K. Miyazaki, T. Usuki, S. Matsuoka, and **M. Hirama**, Critical Importance of the Nine-Membered F Ring of Ciguatoxin for Potent Bioactivity: Total Synthesis and Biological Evaluation of F-Ring-Modified Analogues, *Angew. Chem. Int. Ed*. (VIP), **47**, 8611–8614 (2008).
(23) K. Ogawa, Y. Koyama, I. Ohashi, I. Sato, and **M. Hirama**, Total Synthesis of a Protected Aglycon of the Kedarcidin Chromophore, *Angew. Chem. Int. Ed*., **48**, 1110–1113 (2009).
(24) K. Komano, S. Shimamura, Y. Norizuki, D. Zhao, C. Kabuto, I. Sato, and **M. Hirama**, Total Synthesis

and Structure Revision of the (−)-Maduropeptin Chromophore, *J. Am. Chem. Soc*., **131**, 12072–12073 (2009).

(25) T. Tsumuraya, I. Fujii, and **M. Hirama**, Production of Monoclonal Antibodies for Sandwich Immunoassay Detection of Pacific Ciguatoxins, *Toxicon*, **56**, 797–803 (2010).

(26) S. Yamashita, Y. Ishihara, H. Morita, J. Uchiyama, K. Takeuchi, M. Inoue, and **M. Hirama**, Stereoselective 6-Exo Radical Cyclization Using *cis*-Vinyl Sulfoxide: Practical Total Synthesis of CTX3C, *J. Nat. Prod*., **74**, 357–364 (2011).

(27) T. Tsumuraya, K. Takeuchi, S. Yamashita, I. Fujii, and **M. Hirama**, Development of a Monoclonal Antibody against the Left Wing of Ciguatoxin CTX1B: Thiol Strategy and Detection Using a Sandwich ELISA, *Toxicon*, **60**, 348–357 (2012).

(28) S. Yamashita, K. Takeuchi, T. Koyama, M. Inoue, Y. Hayashi, and **M. Hirama**, Practical Route to the Left Wing of CTX1B and Total Syntheses of CTX1B and 54-deoxyCTX1B, *Chem. Eur. J.*, **21**, 2621–2628 (2015).

(29) S. Yamashita, D. Hayashi, A. Nakano, Y. Hayashi, and **M. Hirama**, Total Synthesis of Avermectin B_{1a} Revisited, *J. Antibiot*., **69**, 31–50 (2016).

(30) **M. Hirama**, Total Synthesis and Related Studies of Large, Strained, and Bioactive Natural Products, *Proc. Jpn. Acad., Ser. B*, **92**, 290–329 (2016).

(31) T. Tsumuraya, T. Sato, **M. Hirama**, and I. Fujii, Highly Sensitive and Practical Fluorescent Sandwich ELISA for Ciguatoxins, *Anal. Chem*., **90**, 7318–7324 (2018).