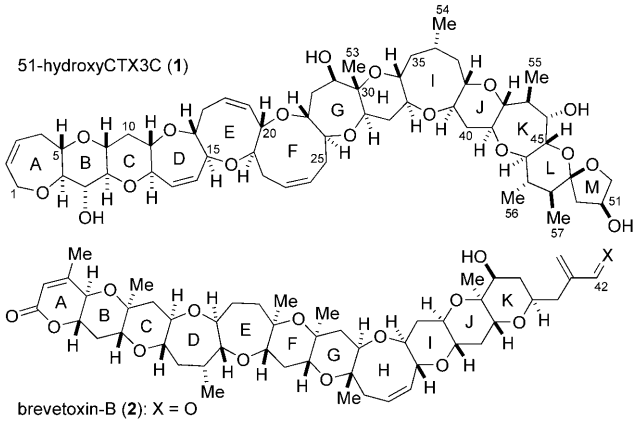
VIP Structure–Activity Relationships

DOI: 10.1002/anie.200803921

Critical Importance of the Nine-Membered F Ring of Ciguatoxin for Potent Bioactivity: Total Synthesis and Biological Evaluation of F-Ring-Modified Analogues**

Masayuki Inoue, Nayoung Lee, Keisuke Miyazaki, Toyonobu Usuki, Shigeru Matsuoka, and Masahiro Hirama**

Trans-fused polycyclic ethers such as ciguatoxins and brevetoxins are among the most spectacular classes of compounds isolated from marine sources (Scheme 1).[1] These molecules



Scheme 1. Structures of ciguatoxin 51-hydroxyCTX3C and brevetoxins.

are made up of a single carbon chain locked into a ladderlike structure with a length of 3 nm. The striking regularity with which the oxygen atoms bridge the polycyclic framework is a remarkable feature of these molecules. The cyclic ethers, with sizes ranging from five- to nine-membered rings, all fuse in a *trans*/*syn*/*trans* fashion.

Ciguatoxins[2] (**1**, Scheme 1) and brevetoxins[3] (**2** and **3**) have attracted intense interest from biologists and chemists alike because of their potent neurotoxicity and their association with the catastrophic effects of food poisoning and red-tide phenomena. Ciguatoxins were identified as the principle toxins in the widespread seafood poisoning known as ciguatera.[4] The toxins generated by the dinoflagellate *Gambierdiscus toxicus*[5] are transferred through the aquatic food chain, and ingestion of ciguateric fish by humans leads to neurological, gastrointestinal, and cardiovascular disorders. On the other hand, brevetoxins are potent ichthyotoxins, and have been isolated from the dinoflagellate *Karenia breve*.[6] Red tides, which are caused by blooms of *Karenia breve*, have killed great numbers of fish and caused intoxication in humans.

These toxins exert their toxicity by binding to the voltage-sensitive sodium channels (VSSC) of excitable membranes, causing them to open, and thereby allowing influx of sodium ions.[7,8] The specific binding site shared by ciguatoxins and brevetoxins was designated as site 5.[9–11] Interestingly, more structurally complex ciguatoxins are known to possess both a higher binding affinity to site 5 and more potent toxicities than brevetoxins. The extremely limited supply of ciguatoxins from the natural sources has prevented studies on structure–activity relationships (SARs), which would help in understanding the structural requirements necessary for this highly specific ligand–receptor interaction.[11–13] Thus, a synthetic supply of ciguatoxins is urgently needed.

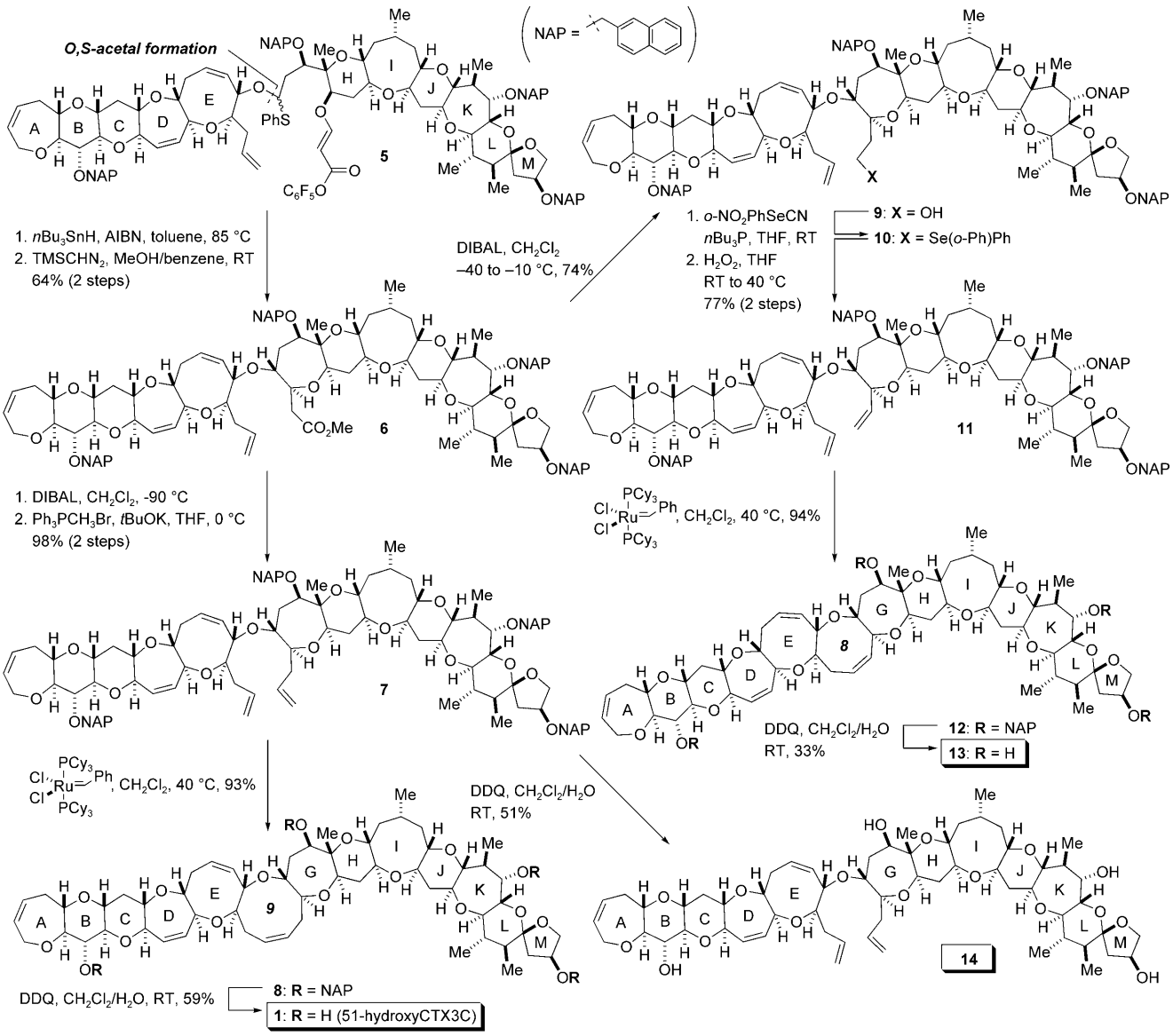
Recently, we achieved the first total synthesis of three members of the ciguatoxin family, namely CTX1B, CTX3C, and 51-hydroxyCTX3C (**1**), based on a unified synthetic strategy.[14] These routes allowed us to prepare new fully synthetic analogues of ciguatoxins for detailed SAR studies. Here we report the total synthesis and biological evaluation of F-ring-modified analogues of **1**.

Scheme 2 illustrates the last stage of our total synthesis of 51-hydroxyCTX3C (**1**),[14b] one of the most toxic congeners of the ciguatoxins. The comparably complex left ABCDE and right HIJKLM wings were coupled through O,S-acetal formation. The FG ring was then constructed in a stepwise manner: radical cyclization of **5** generated the seven-membered G ring of **6**, and ring-closing olefin metathesis (RCM)[15] built the nine-membered F ring (**7**→**8**). Final removal of the 2-naphthylmethyl (NAP) groups[16] from **8** afforded **1**. This synthetic scheme offered a unique oppor-

[*] Prof. Dr. M. Inoue, Dr. S. Matsuoka
Graduate School of Pharmaceutical Sciences
The University of Tokyo, Hongo, Bunkyo-ku
Tokyo 113-0033 (Japan)
Fax: (+81) 3-3395-5214
E-mail: inoue@mol.f.u-tokyo.ac.jp
Dr. N. Lee, Dr. K. Miyazaki, Dr. T. Usuki, Prof. Dr. M. Hirama
Department of Chemistry, Graduate School of Science
Tohoku University, Sendai 980-8578 (Japan)
Fax: (+81)-22-795-6566
E-mail: hirama@mail.tains.tohoku.ac.jp

[**] This work was supported financially by SORST (Japan) Science and Technology Agency (JST) and a Grant-in-Aid for Scientific Research (S) from the Japan Society for the Promotion of Science (JSPS). A fellowship for K.M. and T.U. from the Japan Society for the Promotion of Science is gratefully acknowledged. We thank Prof. M. Satake (The University of Tokyo) for valuable suggestions. [3H]PbTx-3 was kindly provided by Prof. K. Tachibana (The University of Tokyo).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200803921.



Scheme 2. Total synthesis of the F-ring-modified analogues of 51-hydroxyCTX3C. AIBN = 2,2′-azobisisobutyronitrile, Cy = cyclohexyl, DIBAL = diisobutylaluminum hydride, TMS = trimethylsilyl.

tunity for us to access a variety of F-ring-modified analogues by altering a minimum sequence of reactions.

The F ring of ciguatoxins is conceivably the most important structural element for governing the 3 nm long molecular shape, as slight structural changes in the central portion drastically affect the relative orientation of the two molecular ends. To understand the function of the central fused-ring system at an atomic level, we designed two F-ring-modified analogues, the eight-membered-ring-containing **13** and the open-chain O-linked **14**. Specifically, the ABCDE ring and the GHIJKLM ring systems in **13** and **14** are connected by spacers that are conformationally distinct from the nine-membered ring of **1**.

These analogues were synthesized from the previously reported intermediates generated in the total synthesis of 51-hydroxyCTX3C (Scheme 2). Oxidative removal of the NAP groups of protected tetraol **7** was realized using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to generate the open chain **14**. The eight-membered compound **13** was synthesized from the intermediate **6** through a four-step procedure. First, reduction of ester **6** with DIBAL generated primary alcohol **9**. Treatment of **9** with 2-nitrophenyl selenocyanate and tributylphosphine in THF produced selenide **10**,[17] which was oxidized with hydrogen peroxide to afford olefin **11**. Finally, an RCM reaction of **11** resulted in the successful formation of **12**, DDQ-mediated deprotection of which provided the target **13**.

These synthetic analogues were subjected to three levels of biological assays: ligand–receptor interaction, in vitro cell-based activity, and in vivo activity. Interaction of the analogues with the receptor was assessed by the competitive displacement of radiolabeled brevetoxin-3 ([^{3}H]PbTx-3, **4**, Scheme 1) using rat brain synaptosome, according to the protocol developed by Baden and co-workers.[9] The synthetic

polyethers **1**, **13**, and **14** were able to displace [^{3}H]PbTx-3 from site 5 in a concentration-dependent manner (Figure 1). As shown in Table 1, the K_i value of 51-hydroxyCTX3C (**1**; 64.6 pM) showed a greater affinity (16-fold) to site-5 than that of brevetoxin-3 (**3**; 1.05 nM). To our surprise, the affinity of **13** and **14** were significantly lower (173- and 1930-fold, respectively) in comparison to the natural product **1**.

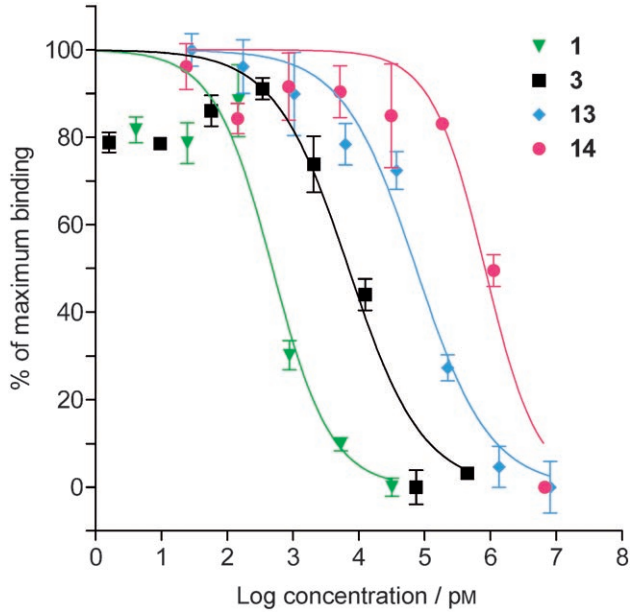


Figure 1. Inhibition of binding of the [^{3}H]-PbTx-3 to site 5 of the voltage-sensitive sodium channel by 51-hydroxyCTX3C (**1**), PbTx-3 (**3**), as well as F-ring-modified analogues **13** and **14**.

Table 1: Biological assays of 51-hydroxyCTX3C (**1**), brevetoxin-3 (**3**), and F-ring-modified analogues **13** and **14**.

Polyethers	Receptor interaction[a] K_i [nM]	Cytotoxicity[b] EC_{50} [nM]	Acute toxicity[c] LD_{50} [μg kg^{-1}]
1	0.0646	0.00326	0.31
3	1.05	18.6	>200[d]
13	11.2	103	>667
14	125	170	>667

[a] Dissociation constants were measured against 5.0 nM [^{3}H]-PbTx-3. [b] Cytotoxicity was determined as the EC_{50} value on mouse neuroblastoma cells Neuro-2A. [c] Acute toxicity was determined as the LD_{50} value on intraperitoneal injected mice. [d] Ref. [11a].

In agreement with the diminished affinities to the target protein, the in vitro and in vivo toxicities of the two new analogues were found to be markedly weaker. EC_{50} values, which were evaluated using mouse neuroblastoma Neuro-2A cells,[18] indicated that **13** and **14** were 1/31 600 and 1/52 100 less cytotoxic than **1**, respectively. Furthermore, in contrast to the high in vivo toxicity of 51-hydroxyCTX3C (0.31 μg kg^{-1}), as determined by intraperitoneal injection into mice, both **13** and **14** were not toxic to mice up to 667 μM kg^{-1}. Clearly, the perturbation of the F-ring structure had a detrimental effect on all three levels of bioactivities.

It was an attractive possibility that the assay data could be related to the overall shapes of the molecules. Accordingly, a preliminary molecular modeling study of **1**, **13**, and **14** was performed to visualize their three-dimensional structures. Many conformers for each polyether were generated by the Monte Carlo method, in which energy minimization was performed with the MM2* force field (MacroModel Ver. 8.1).[19] Next, the most stable conformers that generally satisfied the reported spectroscopic data were chosen from the pool of energy-minimized structures. As shown in Figure 2 A, 51-hydroxyCTX3C (**1**) adopts a relatively straight conformation with a length of 3 nm and only a slight curvature. Intriguingly, the three-dimensional orientations of the two halves of **13** and **14** significantly deviated from that of ciguatoxin **1** (Figure 2 B). These data confirmed the profound influence of slight modifications of the central ring on the overall shape of the molecule.

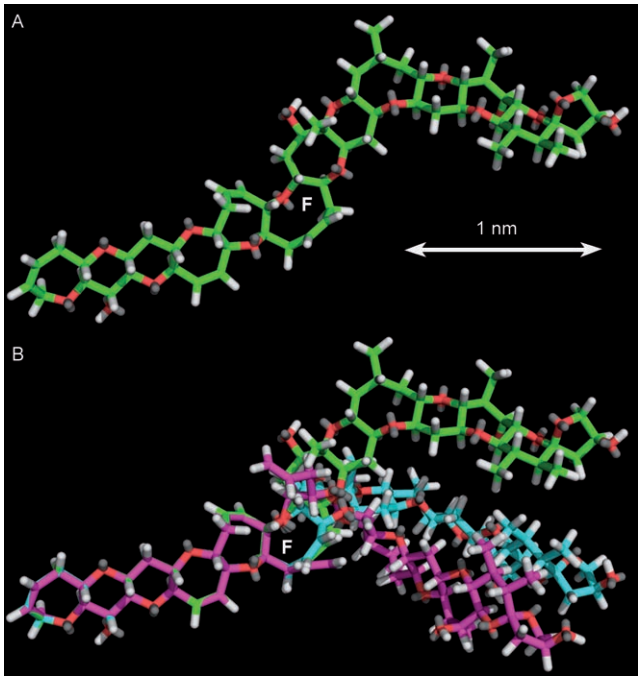


Figure 2. A) Energy-minimized structure of 51-hydroxyCTX3C (**1**). B) Energy-minimized structures of **1** (green), as well as F-ring-modified analogues **13** (cyan) and **14** (magenta) with the ABCDE rings superimposed (MM2*, MacroModel Ver. 8.1).

In conclusion, we have shown that the nine-membered F ring plays a major role in organizing the ciguatoxin molecule into a shape suitable for potent bioactivity. The present synthetic scheme and the bioassay data should help launch design strategies for new fully synthetic ciguatoxin analogues that can modulate the VSSC function in a desired fashion. Further studies toward understanding the SARs of ciguatoxins as well as synthesizing new ligands for site 5 are currently underway.

Received: August 8, 2008
Published online: October 9, 2008

Keywords: biological activity · medium-ring compounds · natural products · structure–activity relationships · total synthesis

[1] For reviews, see: a) T. Yasumoto, M. Murata, *Chem. Rev.* **1993**, *93*, 1897; b) T. Yasumoto, *Chem. Rec.* **2001**, *1*, 228; c) G. M. Nicholson, R. J. Lewis, *Mar. Drugs* **2006**, *4*, 82.

[2] a) M. Murata, A.-M. Legrand, Y. Ishibashi, M. Fukui, T. Yasumoto, *J. Am. Chem. Soc.* **1990**, *112*, 4380; b) M. Satake, M. Fukui, A.-M. Legrand, P. Cruchet, T. Yasumoto, *Tetrahedron Lett.* **1998**, *39*, 1197; c) T. Yasumoto, T. Igarashi, A.-M. Legrand, P. Cruchet, M. Chinain, T. Fujita, H. Naoki, *J. Am. Chem. Soc.* **2000**, *122*, 4988.

[3] a) Y.-Y. Lin, M. Risk, S. M. Ray, D. Van Engen, J. Clardy, J. Golik, J. C. James, K. Nakanishi, *J. Am. Chem. Soc.* **1981**, *103*, 6773; b) Y. Shimizu, H.-N. Chou, H. Bando, G. Van Duyne, J. C. Clardy, *J. Am. Chem. Soc.* **1986**, *108*, 514.

[4] For reviews, see: a) P. J. Scheuer, *Tetrahedron* **1994**, *50*, 3–18; b) R. J. Lewis, *Toxicon* **2001**, *39*, 97–106.

[5] T. Yasumoto, I. Nakajima, R. Bagnis, R. Adachi, *Bull. Jpn. Soc. Sci. Fish.* **1977**, *43*, 1021.

[6] D. G. Baden, *FASEB J.* **1989**, *3*, 1807.

[7] a) G. Jeglitsch, K. Rein, D. G. Baden, D. J. Adams, *J. Pharmacol. Exp. Ther.* **1998**, *284*, 516; b) S. L. Purkerson, D. G. Baden, L. A. Fieber, *Neurotoxicology* **1999**, *20*, 909.

[8] a) L. C. Strachan, R. J. Lewis, G. M. Nicholson, *J. Pharmacol. Exp. Ther.* **1999**, *288*, 379; b) K. Yamaoka, M. Inoue, H. Miyahara, K. Miyazaki, M. Hirama, *Br. J. Pharmacol.* **2004**, *142*, 879.

[9] M. A. Poli, T. J. Mende, D. G. Baden, *Mol. Pharmacol.* **1986**, *30*, 129.

[10] a) J.-N. Bidard, H. P. M. Vijverberg, C. Frelin, E. Chungue, A.-M. Legrand, R. Bagnis, M. Lazdenski, *J. Biol. Chem.* **1984**, *259*, 8353; b) A. Lombet, J.-N. Bidard, M. Lazdunski, *FEBS Lett.* **1987**, *219*, 355.

[11] a) M.-Y. Dechraoui, J. Naar, S. Pauillac, A.-M. Legrand, *Toxicon* **1999**, *37*, 125; b) M. Inoue, M. Hirama, M. Satake, K. Sugiyama, T. Yasumoto, *Toxicon* **2003**, *41*, 469.

[12] Y. Hokama, K. E. Chun, C. E. Campora, N. Higa, C. Suma, A. Hamajima, M. Isobe, *J. Clin. Lab. Anal.* **2006**, *20*, 126.

[13] For extensive SAR studies of brevetoxins, see: a) K. S. Rein, B. Lynn, R. E. Gawley, D. G. Baden, *J. Org. Chem.* **1994**, *59*, 2107; b) K. S. Rein, D. G. Baden, R. E. Gawley, *J. Org. Chem.* **1994**, *59*, 2101; c) R. E. Gawley, K. S. Rein, G. Jeglitsch, D. J. Adams, E. A. Theodorakis, J. Tiebes, K. C. Nicolaou, D. G. Baden, *Chem. Biol.* **1995**, *2*, 533; d) S. L. Purkerson-Parker, L. A. Fieber, K. S. Rein, T. Podona, D. G. Baden, *Chem. Biol.* **2000**, *7*, 385; e) S. Michelliza, W. M. Abraham, H. M. Jacocks, T. Schuster, D. G. Baden, *ChemBioChem* **2007**, *8*, 2233.

[14] CTX3C: a) M. Inoue, K. Miyazaki, H. Uehara, M. Maruyama, M. Hirama, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12013; 51-hydroxyCTX3C and CTX1B: b) M. Inoue, K. Miyazaki, Y. Ishihara, A. Tatami, Y. Ohnuma, Y. Kawada, K. Komano, S. Yamashita, M. Hirama, *J. Am. Chem. Soc.* **2006**, *128*, 9352; for reviews, see: c) M. Inoue, M. Hirama, *Acc. Chem. Res.* **2004**, *37*, 961; d) M. Hirama, *Chem. Rec.* **2005**, *5*, 240.

[15] For reviews, see: a) T. M. Trnka, R. H. Grubbs, *Acc. Chem. Res.* **2001**, *34*, 18; b) A. Fürstner, *Angew. Chem.* **2000**, *112*, 3140; *Angew. Chem. Int. Ed.* **2000**, *39*, 3012; c) A. Deiters, S. F. Martin, *Chem. Rev.* **2004**, *104*, 2199; d) K. C. Nicolaou, P. Bulger, D. Sarlah, *Angew. Chem.* **2005**, *117*, 4564; *Angew. Chem. Int. Ed.* **2005**, *44*, 4490.

[16] a) M. Inoue, H. Uehara, M. Maruyama, M. Hirama, *Org. Lett.* **2002**, *4*, 4551; b) M. J. Gaunt, J. Yu, J. B. Spencer, *J. Org. Chem.* **1998**, *63*, 4172; c) J. A. Wright, J. Yu, J. B. Spencer, *Tetrahedron Lett.* **2001**, *42*, 4033; d) J. Xia, J. L. Alderfer, C. F. Piskorz, K. L. Matta, *Chem. Eur. J.* **2001**, *7*, 356.

[17] P. A. Grieco, S. Gilman, M. Nishizawa, *J. Org. Chem.* **1976**, *41*, 1485.

[18] a) R. L. Manger, L. S. Leja, S. Y. Lee, J. M. Hungerford, M. M. Wekell, *Anal. Biochem.* **1993**, *214*, 190; b) T. Yasumoto, M. Fukui, K. Sasaki, K. Sugiyama, *J. AOAC Int.* **1995**, *78*, 574; c) R. L. Manger, L. S. Leja, S. Y. Lee, J. M. Hungerford, Y. Hokama, R. W. Dickey, H. R. Granade, R. Lewis, T. Yasumoto, M. M. Wekell, *J. AOAC Int.* **1995**, *78*, 521.

[19] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, . M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440.

