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Concise Synthesis of Ciguatoxin ABC-Ring Fragments and Surface Plasmon Resonance Study of the Interaction of Their BSA Conjugates with Monoclonal Antibodies

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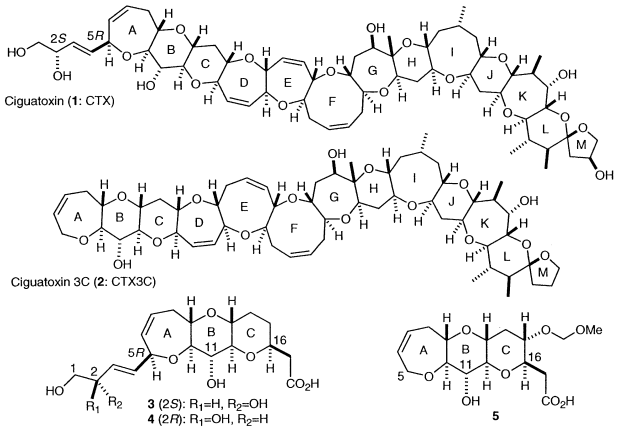
Abstract—Monoclonal antibodies (mAbs), 4H2 and 6H7, were prepared previously using a protein conjugate of a 1:1 epimeric mixture of the synthetic ABC-ring fragments of ciguatoxin (CTX), **3** and **4**. Here, the interactions of these mAbs with the fragments of CTX and CTX3C, **3** and **5**, were investigated by surface plasmon resonance (SPR) spectroscopy in an attempt to clarify an antigenic determinant. Compared with the previous synthesis, the fragment **3** possessing the 2*S* configuration was synthesized from tri-*O*-acetyl-D-glucal much more effectively. The mAb 4H2 was already known to show a dose-dependent binding to the bovine serum albumin (BSA) conjugate of **3**, but not to that of **5**. The present SPR study of 4H2 demonstrates that the A-ring side chain of **3** plays a decisive role as an epitope. Therefore, SPR can effectively replace the ELISA method for the analysis of mAbs.

Introduction

More than 20,000 people in the tropics and subtropics suffer annually from ciguatera, a disease that causes gastrointestinal, neurological and cardiovascular disorders.[1] Ciguatoxin (**1**: CTX)[2] and its congener (**2**: CTX3C)[3] are the principal causative toxins of ciguatera. Considerable effort has been directed toward the development of accurate detection methods for ciguatoxins, particularly to those involving the application of anti-ciguatoxin monoclonal antibodies.[4] We previously prepared three monoclonal antibodies (mAbs), 4H2, 6H7, and 6F12, using a synthetic ABC-ring fragment of **1** as a haptenic group, which was a 1:1 C2-epimeric mixture (**3** and **4**).[5,6] Preliminary ELISA analysis indicated that these mAbs differentiate the configuration of C2 carbinol between **3** and **4**: 4H2 binds to **3** which possesses the 2*S*-configuration and 6H7 binds to **4** but not to **3**.[5] To investigate the binding affinity more rapidly than afforded by the ELISA method, interactions between the mAbs and the bovine serum albumin (BSA) conjugates of synthetic fragments, **3** and **5**, were investigated using surface plasmon resonance (SPR) spectroscopy. In addition, concise syntheses of **3** and **5** were achieved.



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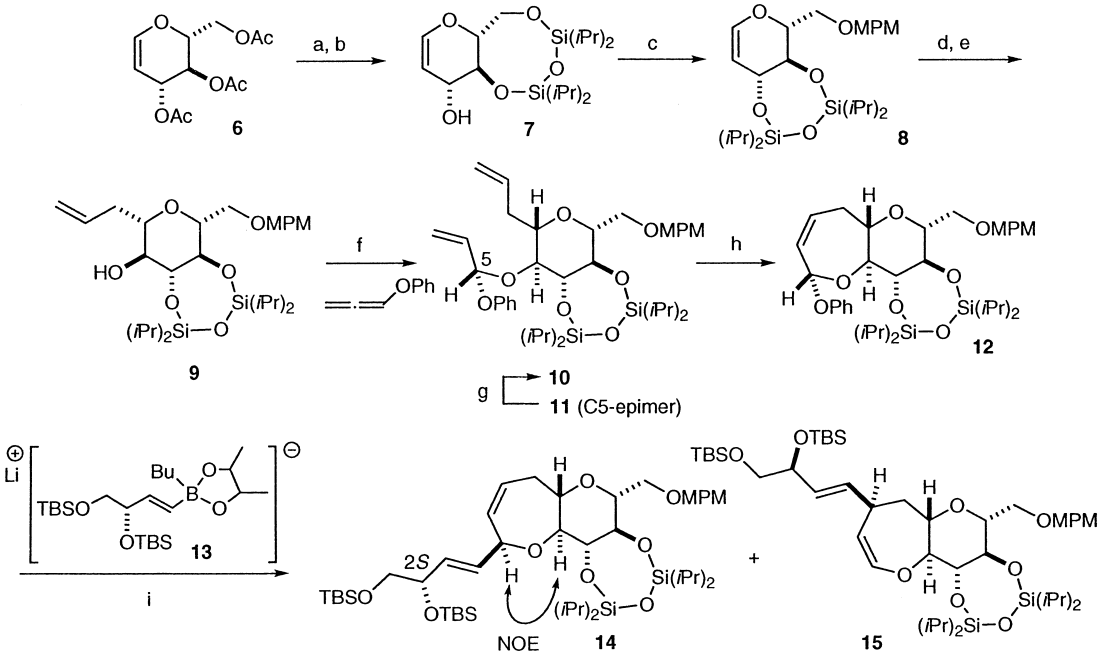
Synthesis

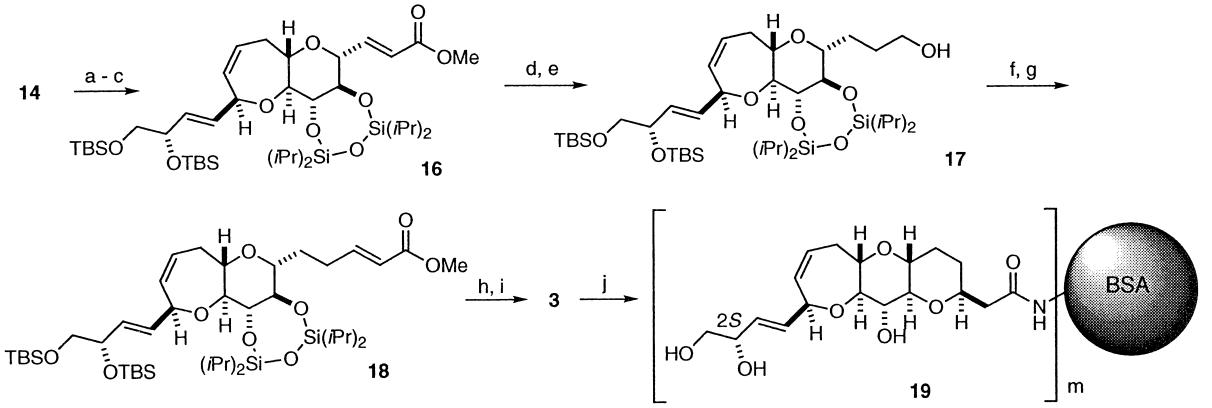
Since the previous synthesis of **3** was lengthy and laborious (47 steps from D-glucose),[5,7] we established a more concise and convergent route to the ABC-ring fragment.[8] The new synthesis of **3** began with commercially available tri-*O*-acetyl-D-glucal (**6**) (Scheme 1). Protecting group manipulation and stereoselective allylation using Spilling's protocol[9] gave the *C*-glycoside (**9**). The acid-sensitive key fragment (**14**), which has a 2*S* configuration, was synthesized from **9** via transition metal ([Pd], [Ru], and [Ni])-catalyzed sequential process only by altering the nature of the protecting groups from the previously reported synthesis.[10]

Construction of the C-ring and the synthesis of **3** were achieved through an intramolecular conjugate addition of **18** mediated by tetrabutylammonium fluoride (TBAF) according to our reported protocol[5] (Scheme 2). An established concise synthetic route to **3** from **6** includes only 17 steps and accelerated the preparation of the synthetic samples for the SPR study.

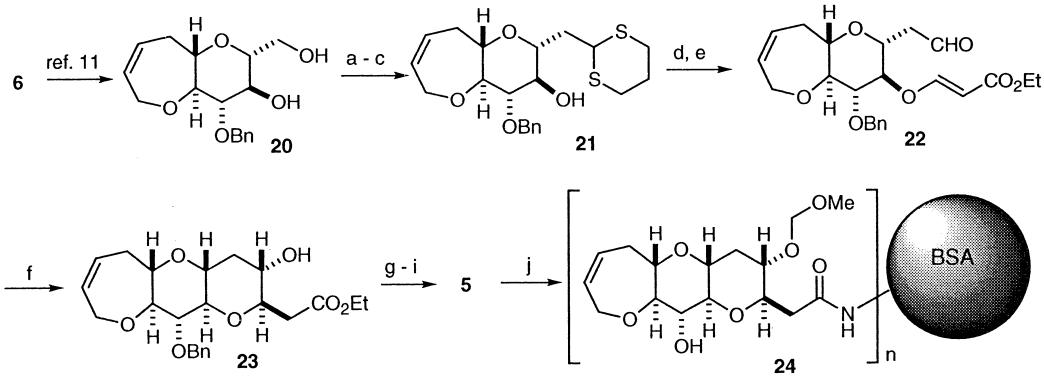
On the other hand, the ABC-ring fragment (**5**) of CTX3C (**2**) was synthesized from an intermediate **20**, which is readily available from **6** (Scheme 3).[11] One carbon homologation of **20** and conjugate addition to ethyl propiolate gave **22**. Cyclization of **22** with SmI_2[12] furnished steroselectively a *trans*-fused tricyclic ether



Scheme 1. Reagents and conditions: (a) K_2CO_3 (cat), MeOH; (b) $Cl(iPr)_2SiOSi(iPr)_2Cl$, pyridine, 73% (two steps); (c) NaH, THF/DMF = 2:1, 0 °C, 1 h, then MPMCl, 0 °C to rt, 42%; (d) *N*-bromosuccinimide, THF/H_2O = 10:1; (e) $K[N(SiMe_3)_2]$, 18-crown-6, toluene, −78 °C, 11 h, then CH_2=$CHCH_2MgBr$, −20 °C to rt, 33% (two steps); (f) $H_2CCCHOPh$ (8 equiv), $Pd(OAc)_2$ (10 mol%), $Ph_2P(CH_2)_3PPh_2$ (10 mol%), K_2CO_3 (1.5 equiv), CH_3CN, reflux, **10** (24%; 45% based on recovery of **9**), **11** (17%; 32%, based on recovery of **9**), **9** recovered (47%); (g) $Pd(OAc)_2$ (10 mol%), $Ph_2P(CH_2)_3PPh_2$ (10 mol%), PhOH (1 equiv), K_2CO_3 (1.5 equiv), CH_3CN, reflux, **10**:**11** = 2:1, 64% based on recovery of **11**: (h) $(PCy_3)_2Cl_2Ru$=CHPh (7 mol%), CH_2Cl_2, rt to 40 °C, 79%; (i) **13** (1.8 equiv), $(PPh_3)_2NiCl_2$ (10 mol%), NaI (1 equiv), *t*BuCN (2 equiv), THF, 0 °C to rt, **14** (39%), **15** (49%).



Scheme 2. Reagents and conditions: (**a**) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH_2Cl_2/H_2O = 10:1, 92%; (b) $(COCl)_2$, dimethyl sulfoxide (DMSO), Et_3N, CH_2Cl_2, −78 °C to rt; (c) Ph_3PCHCO_2Me, CH_2Cl_2, 99% (two steps); (d) L-Selectride (2 equiv), *t*BuOH (5 equiv), THF, −70 °C, then NaOH aq, H_2O_2 aq, 0 °C, 81%; (e) diisobutylaluminium hydride (DIBAL), Et_2O, −78 to −40 °C, 73%; (f) Dess–Martin periodinane, CH_2Cl_2, rt; (g) Ph_3PCHCO_2Me, CH_2Cl_2, rt; (g) Ph_3PCHCO_2Me, CH_2Cl_2, 84% (two steps); (h) TBAF, THF, then Ac_2O, pyridine, 87%; (i) LiOH·H_2O, *t*BuOH/H_2O = 4:1; (j) EDC·HCl, NHS, DMF, then BSA in phosphate buffer (pH 7.4).



Scheme 3. Reagents and conditions: (a) $(CF_3SO_2)_2O$ (1.1 equiv), 2,6-lutidine, CH_2Cl_2, −78 °C to rt; (b) NaI, acetone, rt, 87% (two steps); (c) *n*BuLi (4 equiv), 1,3-dithiane (5 equiv), THF/hexamethylphosphoramide (HMPA) = 9:1, −30 to 0 °C, 66%; (d) ethyl propiolate, *N*-methylmorpholine, CH_2Cl_2, 94%; (e) MeI (10 equiv), $NaHCO_3$ (5 equiv), CH_3CN/H_2O = 4:1, rt, 78%; (f) SmI_2 (2.2 equiv), MeOH (2.2 equiv), THF, rt, 94%; (g) CH_3OCH_2Cl, $(i\text{Pr})_2\text{NEt}$, Bu_4NI; (h) DDQ (4 equiv), CH_2Cl_2/H_2O = 10:1, rt, 80%; (i) LiOH·H_2O, *t*BuOH/H_2O = 4:1; (j) EDC·HCl, NHS, DMF, then BSA in phosphate buffer (pH 7.4).

(**23**) in good yield. The synthesis of **5** in 17 steps from **6** was completed by protecting the hydroxy group of **23** and removing the benzyl ether with DDQ before hydrolysis.

The fragments, **3** and **5**, were conjugated with BSA using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxysuccinimide (NHS) (Schemes 2 and 3, respectively). The resulting hapten–BSA conjugates, **19** and **24**, were purified by gel filtration (Amersham Pharmacia, PD-10 column), and MALDI-TOF-MS analysis determined the average number of the haptens attached to the BSA in the conjugates to be 14 and 15 for **19** and **24**, respectively.[13]

Analysis of Surface Plasmon Resonance

To develop a rapid screening system for antibodies using SPR spectroscopy in place of the ELISA protocol, the BSA conjugate (**19**) was immobilized through its amine group to the sensor chip [CM5 sensor chips (BIACORE)], and the interactions of **19** with the mAbs, 4H2 and 6H7, were analyzed using BIACORE 2000 instruments.[14] The mAbs diluted to a final concentration of 1.9 μg/mL in HBS buffer [10 mM Hepes (pH 7.4), 150 mM NaCl, 3 mM EDTA] were then passed over the coated sensor tip at a constant flow of 20 μL/min at 25 °C (Fig. 1). The 4H2 was bound to the immobilized **19** in a dose-dependent manner, although the 6H7 was not bound at all. These affinities of the mAbs with **19** are in agreement with the previous results using ELISA: 4H2 binds to **3** and 6H7 binds to **4** but not to **3**.[5] Thus, the SPR assay can effectively replace the ELISA method for the screening of antibodies that bind to the synthetic fragments.

Since epitope analyses of antibodies using SPR can be performed by passing various conjugates over the immobilized antibodies,[15] we next examined the interactions of immobilized 4H2 with the BSA conjugates, **19** and **24**. As shown in Figure 2, 4H2 was bound to **19**.

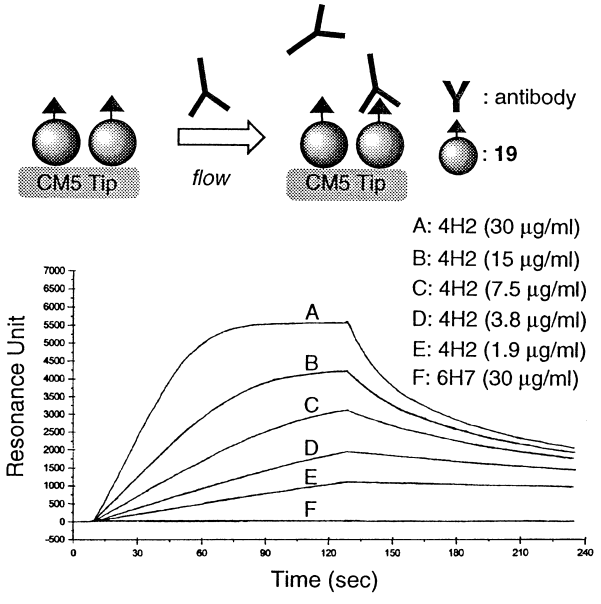


Figure 1. Sensorgrams obtained by passing mAbs [4H2 (A–E) and 6H7 (F)] over immobilized **19**; [HBS buffer, 25 °C, flow rate: 20 μL/min].

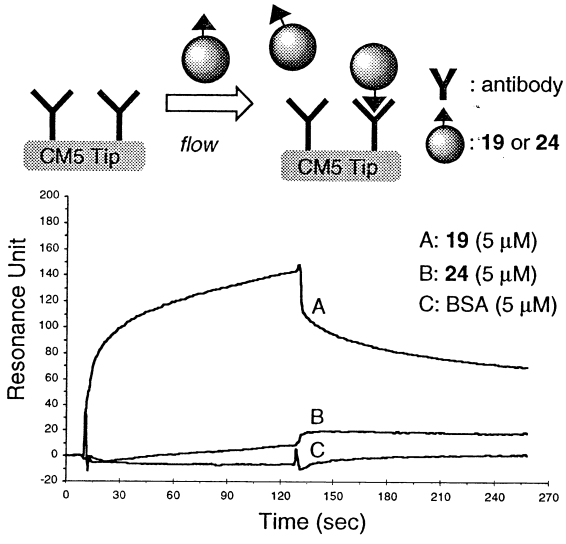


Figure 2. Sensorgrams obtained by passing **19**, **24** and BSA over immobilized 4H2; [HBS buffer, 25 °C, flow rate: 30 μL/min].

However, in the case of **24**, which lacks the A-ring side chain, 4H2 showed only negligible binding. Thus, the side chain of **3** is an essential antigenic determinant for 4H2.

Kinetic analysis for immobilized 4H2 by injection of **19** revealed the association rate constant (k_a) to be 5.8×10^3 M^{-1} s^{-1} and the dissociation rate constant (k_d) to be 3.5×10^{-3} s^{-1}.[16] Rapid association and slow dissociation take place in the present system and the equilibrium dissociation constant (K_d) is calculated to be 6.0×10^{-7} M.[17]

Conclusion

Concise synthetic routes to the ABC-ring fragments **3** and **5** were established, accelerating the SPR study of the interactions of mAbs, 4H2 and 6H7, with BSA conjugates, **19** and **24**. Binding analysis of 4H2 with **19** and **24** indicated that the A-ring side chain of **3** plays a critical role as an epitope. The present SPR method was shown to be convenient for not only epitope analysis but also the screening of antibodies, and thus can effectively replace the conventional ELISA protocol. Further detailed SPR analyses of mAbs are currently being performed at our laboratory.

Acknowledgements

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References and Notes

1. For recent reviews, see: (a) Yasumoto, T.; Murata, M. *Chem. Rev.* **1993**, *93*, 1897. (b) Scheuer, P. J. *Tetrahedron* **1994**, *50*, 3. (c) Lehane, L. *Med. J. Aust.* **2000**, *172*, 176.
2. (a) Murata, M.; Legrand, A.-M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1990**, *112*, 4380. (b) Satake, M.; Morohashi, A.; Oguri, H.; Oishi, T.; Hirama, M.; Harada, N.; Yasumoto, T. *J. Am. Chem. Soc.* **1997**, *119*, 11325.
3. Satake, M.; Murata, M.; Yasumoto, T. *Tetrahedron Lett.* **1993**, *34*, 1975.
4. (a) Yasumoto, T.; Fukui, M.; Sasaki, K.; Sugiyama, K. *JAOAC Intern.* **1995**, *78*, 574. (b) Hokama, Y.; Takenaka, W. E.; Nishimura, K. L.; Ebesu, J. S. M. *JAOAC Intern.* **1998**, *81*, 727. (c) Pauillac, S.; Sasaki, M.; Inoue, M.; Naar, J.; Branaa, P.; Chinain, M.; Tachibana, K.; Legrand, A.-M. *Toxicon* **2000**, *38*, 669.
5. Oguri, H.; Tanaka, S.; Hishiyama, S.; Oishi, T.; Hirama, M.; Tsumuraya, T.; Tomioka, Y.; Mizugaki, M. *Synthesis* **1999**, 1431.
6. Carbon numbering corresponding to that of ciguatoxin has been used throughout this paper.
7. (a) Oguri, H.; Hishiyama, S.; Oishi, T.; Hirama, M. *Synlett* **1996**, 1252. (b) Oguri, H.; Hishiyama, S.; Sato, O.; Oishi, T.; Hirama, M.; Murata, M.; Yasumoto, T.; Harada, N. *Tetrahedron* **1997**, *53*, 3057. (c) Oguri, H.; Sasaki, S.; Oishi, T.; Hirama, M. *Tetrahedron Lett.* **1999**, *40*, 5405.
8. For other synthetic studies of the ABC(D)-ring fragment, see: (a) Hosokawa, S.; Isobe, M. *J. Org. Chem.* **1999**, *64*, 37. (b) Oka, T.; Fujiwara, K.; Murai, A. *Tetrahedron* **1998**, *38*, 21. (c) Sasaki, M.; Fuwa, H.; Ishikawa, M.; Tachibana, K. *Org. Lett.* **1999**, *1*, 1075. (d) Leeuwenburgh, M. A.; Kulker, C.; Overkleeft, H. S.; vander Marel, G. A.; van Boom, J. H. *Synlett* **1999**, 1945. (e) Eriksson, L.; Guy, S.; Perlmutter, P. *J. Org. Chem.* **1999**, *64*, 8396.
9. Marzabadi, C. H.; Spilling, C. D. *J. Org. Chem.* **1993**, *58*, 3761.
10. Oguri, H.; Tanaka, S.; Oishi, T.; Hirama, M. *Tetrahedron Lett.* **2000**, *41*, 975.
11. (a) Maeda, K.; Oishi, T.; Oguri, H.; Hirama, M. *Chem. Commun.* **1999**, 1063. (b) Maruyama, M.; Maeda, K.; Oishi, T.; Oguri, H.; Hirama, M. *Heterocycles* **2001**, *54*, 93.
12. Hori, N.; Matsukura, H.; Matsuo, G.; Nakata, T. *Tetrahedron Lett.* **1999**, *40*, 2811.
13. Shoyama, Y.; Tanaka, H.; Fukuda, N. *Cytotechnology* **1999**, *31*, 9.
14. (a) Reinartz, H. W.; Quinn, J. G.; Zaenker, K.; O'Kennedy, R. *Analyst* **1996**, *121*, 767. (b) Seifert, M.; Haindl, S.; Hock, B. *Adv. Exp. Med. Biol.* **1998**, *444*, 113. (c) Sakai, G.; Nakata, S.; Uda, T.; Miura, N.; Yamazoe, N. *Electrochimica Acta* **1999**, *44*, 3849.
15. (a) Tsuji, H.; Hong, J. C.; Kim, Y. S.; Ikehara, Y.; Narimatsu, H.; Irimura, T. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 374. (b) Regnault, V.; Arvieux, J.; Vallar, L.; Lecompte, T. *Immunology* **1999**, *97*, 400. (c) van Remoortere, A.; Hokke, C. H.; van Dam, G. J.; van Die, I.; Deelder, A. M.; van den Eijnden, D. H. *Glycobiology* **2000**, *10*, 601.
16. The kinetic rate constants were calculated by fitting bivalent analyte model, see: *BIAevaluation Software Handbook Version 3.0*: 1997, Appendix C.
17. Selected examples, see: (a) Karlsson, R. *Anal. Biochem.* **1994**, *221*, 142. (b) Karlsson, R.; Stahlberg, R. *Anal. Biochem.* **1995**, *228*, 274. (c) MacKenzie, C. R.; Hirama, T.; Deng, S.; Bundle, D. R.; Narang, S. A.; Young, N. M. *J. Biol. Chem.* **1996**, *271*, 1527. (d) Stachan, G.; Grant, S. D.; Learmonth, D.; Longstaff, M.; Porter, A. J.; Harris, W. J. *Biosens. Bioelectron.* **1998**, *13*, 665. (e) Visser, N. V.; Smit-Kingma, I. E. *Spectrochemica Acta Part A* **1999**, *55*, 2271. (f) Adamczyk, M.; Moore, J. A.; Yu, Z. G. *Methods* **2000**, *20*, 319.