Supporting Information

Synthesis-Based Approach Toward Direct Sandwich Immunoassay for Ciguatoxin CTX3C

Hiroki Oguri,[†] Masahiro Hirama,*[,†] Takeshi Tsumuraya,[‡] Ikuo Fujii,*[,‡] Megumi Maruyama,[†] Hisatoshi Uehara,[†] and Yoko Nagumo[†].Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan

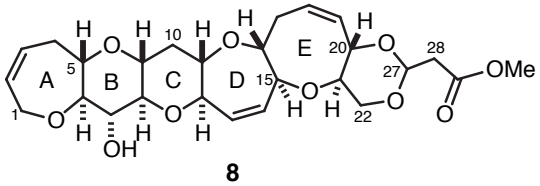
Biomolecular Engineering Research Institute, Osaka 565-0874, Japan

hirama@ykbsc.chem.tohoku.ac.jp; fujii@beri.or.jp

General methods: Safety precautions for highly toxic materials should be used when working with toxins such as CTX3C; all operations should be carried out in the hood and protective gloves, glasses, and mask should be worn. All reactions sensitive to air were carried out under argon or nitrogen under anhydrous conditions, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, dichloromethane (CH_2Cl_2), 1,2-dichloroethane and toluene from calcium hydride, and DMF from calcium hydride under reduced pressure. Phosphate buffered saline (PBS: Na_2HPO_4-KH_2PO_4, pH 7.4) was prepared by using Dulbecco's PBS powder (Wako Pure Chemical Industries. LTD). All other reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F254 precoated plates. Column chromatography was performed using 100-210 μm Silica Gel 60N (Kanto Chemicals Co., Inc.), and 40-50 μm Silica Gel 60N (Kanto Chemicals Co., Inc.) was used for flash column chromatography. ^{1}H NMR were recorded on a Varian INOVA 500 (500 MHz) spectrometer and referenced to residual $CHCl_3$ [^{1}H-NMR (7.26)] as an internal standard. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) spectra were recorded on a PerSeptive Biosystem Voyager DE STR SI-3 instrument.

Synthesis of 8: The ABCDE ring fragment (**5**) was synthesized as reported previously.[1] A solution of **5** (6.2 mg, 8.2 μmol) in 1,2-dichloroethane (2 ml) and H_2O (100 μl) was treated with DDQ (8.0 mg, 35.2 μmol), and stirred for 5 h at room temperature. The mixture was cooled to 0 °C, diluted with Et_2O and saturated aqueous $Na_2S_2O_3$. The organic layer was separated and washed with H_2O. After being dried over Na_2SO_4, the mixture was concentrated under reduced pressure. The residue

was purified by silica gel column chromatography to give 2.3 mg (3.5 μmol, 42%) of **6** and 2.8 mg (recovered 45%) of **5**. Multiple runs were used to generate the larger amount. A solution of **6** (4.0 mg, 6.0 μmol) in THF (2 ml) was treated with TBAF (18 μL, 1.0 M in THF, 18 μmol) and stirred for 30 min at room temperature. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 2.5 mg (5.9 μmol, 98%) of **7**. A solution of **7** (2.8 mg, 6.6 μmol) in DMF (300 μl) was treated with methyl 3,3-dimethoxypropionate (10 μl, 70 μmol), $TsOH{\cdot}H_2O$ (0.5 mg, 3 μmol). After stirring for 1.5 h at room temperature, toluene (1 ml) was added. The reaction mixture was concentrated under reduced pressure (120 mbar/hPa) and kept the pressure for 1 h. The residue was purified by silica gel column chromatography to give 1.9 mg of **8** (3.8 μmol, 57%) as a diastereomeric mixture (27*R*:27*S* = 2:1).



8

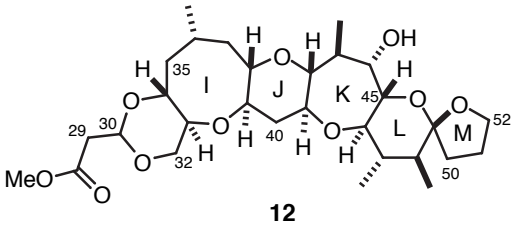
8: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.53 (1H, m, H10ax), 2.28 (1H, dt, *J* = 12.0, 4.5 Hz, H10eq), 2.32 (1H, m, H17), 2.37 (1H, m, H4), 2.62 (1H, m, H4), 2.64 (1H, m, H17), 2.67 (1H, dd, *J* = 15.5, 5.5 Hz, H28major), 2.69 (1H, dd, *J* = 15.5, 5.5 Hz, H28major), 2.84 (1H, dd, *J* = 14.5, 6.0 Hz, H28minor), 2.91 (1H, dd, *J* = 14.5, 6.0 Hz, H28minor), 3.01 (1H, t, *J* = 9.0 Hz, H8), 3.11 (1H, ddd, *J* = 11.0, 9.0, 4.5 Hz, H9), 3.22-3.26 (2H, m, H5, H11), 3.34 (1H, m, H21major), 3.43 (1H, t, *J* = 10.5 Hz, H22major), 3.44 (1H, m, H21minor), 3.58-3.64 (3H, m, H16, H7, H6), 3.64 (1H, m, H22minor), 3.70 (3H, s, OMe), 3.80 (1H, ddd, *J* = 9.0, 4.0, 2.5 Hz, H12), 3.94 (1H, dd, *J* = 15.5, 6.0 Hz, H22minor), 4.02 (1H, ddt, *J* = 15.0, 4.0, 2.5 Hz, H1), 4.11 (1H, dd, *J* = 10.5, 5.5 Hz, H22major), 4.12 (1H, m, H15), 4.24 (1H, m, H20major), 4.32 (1H, dd, *J* = 15.0, 6.0 Hz, H1), 4.48 (1H, m, H20minor), 4.93 (1H, t, *J* = 5.5 Hz, H27major), 5.41 (1H, t, *J* = 6.0 Hz, H27minor), 5.63 (1H, brdt, *J* = 12.5, 2.5 Hz, H14), 5.70 (1H, m, H13), 5.73 (1H, dd, *J* = 10.5, 5.0 Hz, H19), 5.80 (1H, m, H18), 5.83 (1H, m, H3), 5.92 (1H, m, H2). MALDI-TOF MS: Calcd for $C_{26}H_{34}O_{10}Na$ 529.205 ($M+Na^+$); Found 529.185.

Preparation of KLH-conjugate 9: A solution of **8** (2.5 mg, 4.9 μmol) in *t*-BuOH (0.5 ml) and H_2O (125 μl) was treated with $LiOH{\cdot}H_2O$ (2.8 mg, 68 μmol) and stirred for 1 h at room temperature. The reaction mixture was acidified with $KHSO_4$ (18.6 mg, 136 μmol) and diluted with EtOAc (40 ml). After being dried over $MgSO_4$, the mixture was concentrated under reduced pressure to give **3**,

which was dissolved in DMF (200 μl) and treated with *N*-hydroxysuccine imide (5.6 mg, 49 μmol) and EDC·HCl (4.8 mg, 25 μmol). The mixture was stirred for 12 h at room temperature and diluted with EtOAc (40 ml). The organic layer was washed with H_2O and dried over Na_2SO_4. The mixture was concentrated under reduced pressure, and the resulting activated ester was dissolved in DMF (100 μl), which was used for conjugation with carrier proteins. To a solution of KLH (5.0 mg) in PBS (1.0 ml) was added the DMF solution of the activated ester (50 μl, ca. 2.4 μmol). After 24 hour, the KLH conjugate **9** was dialyzed against PBS (pH 7.4, 1000 ml x 3).

Preparation of BSA-conjugate 10: To a solution of BSA (5.0 mg) in PBS (1.0 ml) was added the DMF solution of the activated ester (50 μl, ca. 2.4 μmol). After 24 hour, the BSA conjugate **10** was dialyzed against PBS (pH 7.4, 1000 ml x 3). MALDI-TOF MS analysis revealed approximately 8 haptens per molecule of BSA.

Synthesis of 12: The IJKLM ring fragment (**11**) was synthesized as reported previously.[2] A solution of **11** (4.2 mg, 9.0 μmol) in CH_2Cl_2 (300 μl) was treated with methyl 3,3-dimethoxypropionate (10 μl, 70 μmol), $TsOH·H_2O$ (0.5 mg, 3 μmol). After stirring for 1.5 h at room temperature, toluene (1 ml) was added. The reaction mixture was concentrated under reduced pressure (120 mbar/hPa) and kept the pressure for 1 h. The residue was diluted with EtOAc and washed with $NaHCO_3$ and brine. After being dried over $MgSO_4$, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 4.7 mg of **12** (8.5 μmol, 94%) as a diastereomeric mixture (30*R*:30*S* = 1:3).



12

12: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.89 (3H, d, *J* = 6.5Hz, Me48), 1.03 (3H, d, *J* = 6.0Hz, Me47), 1.06 (3H, d, *J* = 7.0Hz, Me36), 1.15 (3H, d, *J* = 7.5Hz, Me43), 1.39 (1H, brq, *J* = 11.5Hz, H40ax), 1.50~1.62 (4H, m, H48, H37, H35, H37), 1.77-1.88 (4H, m, H51, H50, H36, H35, H37), 1.92-1.97 (2H, m, H50, H51), 2.02 (1H, qdd, *J* = 7.5, 5.0, 3.0Hz, H43), 2.21 (brdt, *J* = 11.5, 5.0Hz, H40eq), 2.62 (2H, *J* = 5.0Hz, H29) 2.85(1H, dd, *J* = 9.5, 5.0Hz, H42), 2.96 (1H, brtd, *J* = 9.5, 3.0Hz, H38), 3.13 (1H, brddd, *J* = 11.5, 9.5, 5.0Hz, H39), 3.24 (1H, t, *J* = 9.5Hz, H46), 3.29 (1H, brtd, *J* = 9.5, 4.5Hz, H33), 3.32 (1H, t, *J* = 9.5Hz, H32), 3.41 (1H, brtd, *J* = 9.5, 3.0Hz, H34), 3.64 (1H, dd, *J* =

9.5, 1.5 Hz, H45), 3.69 (3H, s, OMe), 3.68-3.72 (2H, m, H41, H44), 3.78 (1H, brq, *J* = 7.5Hz, H52), 3.88 (1H, m, H52), 4.01 (1H, dd, *J* = 9.5, 4.5Hz, H32), 4.87 (1H, t, *J* = 5.0 Hz, H30). MALDI-TOF MS: Calcd for $C_{29}H_{46}O_{10}Na$ 577.299 ($M+Na^+$); Found 577.244.

Preparation of KLH-conjugate 13: A solution of **12** (4.7 mg, 8.5 μmol) in *t*-BuOH (0.5 ml) and H_2O (125 μl) was treated with $LiOH \cdot H_2O$ (2.8 mg, 68 μmol) and stirred for 1 h at room temperature. The reaction mixture was acidified with $KHSO_4$ (18.6 mg, 136 μmol) and diluted with EtOAc (10 ml). After being dried over $MgSO_4$, the mixture was concentrated under reduced pressure to give **3**, which was dissolved in DMF (200 μl) and treated with *N*-hydroxysuccine imide (9.7 mg, 85 μmol) and EDC·HCl (8.1 mg, 43 μmol). The mixture was stirred for 12 h at room temperature and diluted with EtOAc (10 ml). The organic layer was washed with H_2O and dried over Na_2SO_4. The mixture was concentrated under reduced pressure, and the activated ester was dissolved in DMF (100 μl), which was used for conjugation with carrier proteins. To a solution of KLH (7.0 mg) in PBS (2.0 ml) was added the DMF solution of the activated ester (50 μl, ca. 4.2 μmol). After 24 h, the KLH conjugate **13** was dialyzed against PBS (pH 7.4, 1000 ml x 3).

Preparation of BSA-conjugate 14: To a solution of BSA (7.0 mg) in PBS (2.0 ml) was added the DMF solution of the activated ester (50 μl, ca. 4.2 μmol). After 24 hour, the BSA conjugate **14** was dialyzed against PBS (pH 7.4, 1000 ml x 3). MALDI-TOF MS analysis revealed approximately 10 haptens per molecule of BSA.

References

1. Maruyama, M.; Inoue, M.; Oishi, T.; Oguri, H.; Ogasawara, N.; Shindo, Y.; Hirama, M. *Tetrahedron* **2002**, *58*, 1835.
2. Uehara, H.; Oishi, T.; Inoue, M.; Shoji, M.; Nagumo, Y.; Kosaka, M.; Le Brazidec, J.-Y.; Hirama, M. *Tetrahedron* **2002**, *58*, 6493.