Synthesis of an Advanced Intermediate Bearing Two Hydroxy Groups for (−)-Tetrodotoxin and Its Analogs

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A new intermediate for tetrodotoxin and its analogs was synthesized from a bicyclic lactam intermediate, which was prepared from a known enone compound by a novel neighboring group participation as a key step.

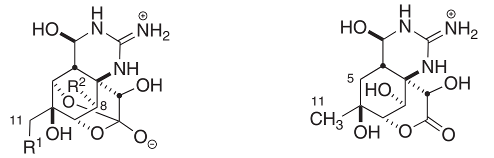

tetrodotoxin (**1**) $R^1 = R^2 = OH$
11-deoxytetrodotoxin (**2**) $R^1 = H$, $R^2 = OH$
8,11-dideoxytetrodotoxin (**3**) $R^1 = R^2 = H$
5,11-dideoxytetrodotoxin (**4**)

Figure 1. Structures of tetrodotoxin and its analogs.

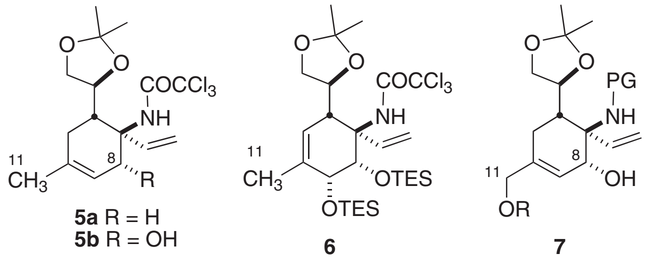
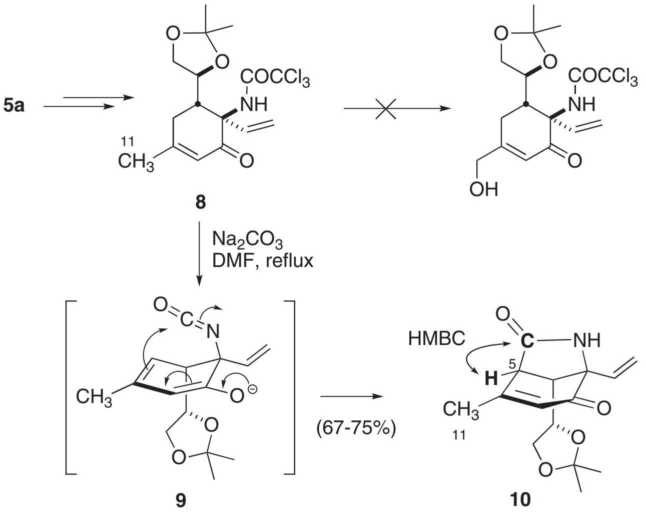

5a R = H
5b R = OH
6
7

Figure 2. The synthetic intermediates for tetrodotoxin and its analogs.

In the course of our synthetic studies on tetrodotoxin (**1**), a well-known marine natural product,[1,2] we have employed a simple compound **5a** as a common key intermediate[3] for the synthesis of 11-deoxy analogs of tetrodotoxin such as 11-deoxytetrodotoxin (**2**),[4] 8,11-dideoxytetrodotoxin (**3**),[5] and 5,11-dideoxytetrodotoxin (**4**)[6] (Figures 1 and 2). Fortunately, the intermediate **5a** could also be used for total synthesis of tetrodotoxin (**1**),[7] for which additional hydroxy group was introduced at the C-11 position by allylic oxidation with SeO_2 of the olefinic methyl group of an intermediate **6** prepared from **5a**. However, similar allylic oxidation of the other substrates, such as **5a** and **5b**, proved to be difficult. Our recent interest in elucidating the biosynthetic and metabolic pathway of tetrodotoxin prompted us to develop a more general and flexible synthetic route for tetrodotoxin analogs, and particularly for 11-hydroxylated analogs of tetrodotoxin. For this purpose, we designed an advanced intermediate as **7** possessing two hydroxy groups at the C-11 and C-8 positions. This short article describes the synthesis of compound **7** from a known intermediate **5a** by using a novel neighboring group participation of the trichloroacetamide.

The common intermediate **5a** was transformed to enone **8** by a method developed in our laboratory,[6] and hydroxylation at the C-11 position was then attempted. Direct allylic oxidation of the methyl group with SeO_2 gave a complex mixture. Two-step hydroxylation including formation of vinyl silyl ether from **8** followed by oxidation with MCPBA was also unsuccessful. The regioselectivity of the enolization might be one of the reasons. During examination of conditions for the enolization, we were surprised to find that treatment of enone **8** with Na_2CO_3 in refluxing DMF gave bicyclic lactam **10** in moderate yield (67–75%). The condition has been employed for generation of isocyanate from trichloroacetamide,[8] therefore involvement of an isocyanate intermediate **9** would promote the intramolecular trapping by dienolate emerging from the enone to result in affording **10**. The structure of **10** was determined by extensive NMR analysis; the newly formed C–C bond was confirmed by HMBC correlation between the methine proton at the C-5 position and the lactam carbonyl carbon as shown in Scheme 1. Since the bicyclic structure of the product **10** allows exclusive enolization at the C-11 position, hydroxylation of the C-11 position was attempted again by the above-mentioned procedure. The lactam of **10** was protected with a Boc group, and then the product **11** was used for preparation of the corresponding vinyl silyl ether (Scheme 2). Upon treatment of **11** with TBSOTf in the presence of Et_3N, the desired vinyl silyl ether **12** was obtained as an unstable product, which was therefore immediately epoxidized with MCPBA for this hydroxylation. We found that the oxidation was best carried out



Scheme 1. Synthesis of bicyclic γ-lactam intermediate **10**.

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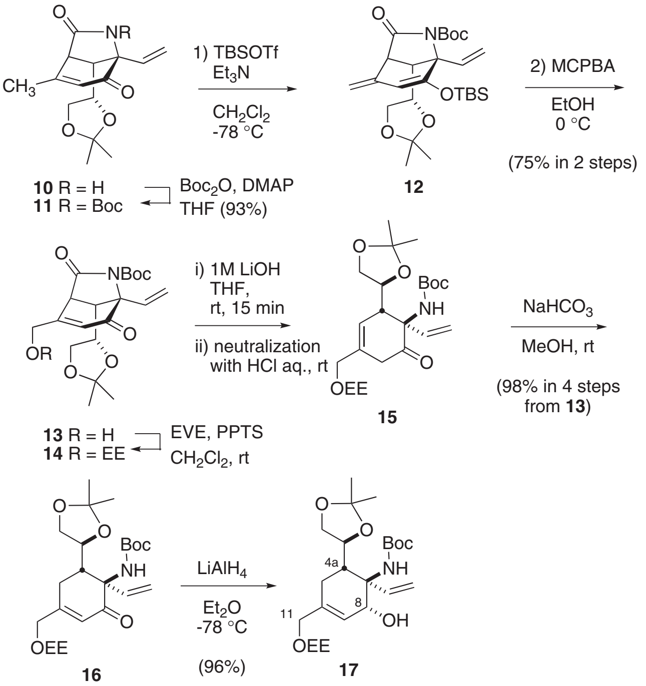

Scheme 2. Synthesis of the new intermediate **17**.

in ethanol solvent for spontaneous hydrolysis to give the desired allylic alcohol **13** in a good yield. After protection of the alcohol as 1-ethoxyethyl (EE) ether, the lactam ring of the resulting **14** was cleaved with aqueous lithium hydroxide. Subsequent decarboxylation occurred upon careful neutralization to yield β,γ-unsaturated ketone **15**, which was treated with $NaHCO_3$ in methanol to afford α,β-unsaturated ketone **16** in an excellent overall yield. Reduction of the unsaturated ketone **16** with $LiAlH_4$ in ether as a solvent proceeded stereoselectively to furnish **17** (**7**: R = EE) in a high yield (96% as a single product). The configuration of the C-8 position was determined by observing the NOESY correlation between the protons of the C-8 and the C-4a positions.

In summary, we have developed a route for the synthesis of the advanced intermediate **17** from enone **8**. The salient feature of this route is utilization of the bicyclic intermediate, which was incidentally obtained from **8**. In our previous synthetic studies on tetrodotoxin, the trichloroacetyl group has been used not only as a protective group for the amino function, but also for other purposes such as guanidine synthesis and hydroxylation through a neighboring group participation. The synthesis described in this short article demonstrates a new use for the trichloroacetamide group. The compound **17** possessing two hydroxy groups at the C-8 and -11 positions should play a crucial role for expeditious synthesis of a variety of tetrodotoxin derivatives for biochemical experiments.

Experimental

Bicyclic Lactam 10. To a solution of the enone **8** (17.5 g, 44.3 mmol) in DMF (500 mL) was added Na_2CO_3 (9.40 g, 88.6 mmol). After stirring at 170 °C for 15 min, the reaction mixture was poured into an ice-cold sat. NH_4Cl solution. The mixture was extracted with AcOEt (×3), and the combined organic layer was washed with H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (SiO_2 gel 250 g, AcOEt:hexane = 1:1) to afford bicyclic lactam **10** (8.30 g, 67%) as yellow crystals. Mp 165.7–166.5. $[\alpha]_D^{24}$ -371 (*c* 1.00, $CHCl_3$). IR (KBr): ν_{max} 3306, 3084, 2987, 2936, 2879, 2814, 1722, 1682, 1622, 1435, 1380, 1070 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, s, CH_3 of acetonide), 1.36 (3H, s, CH_3 of acetonide), 2.11 (3H, d, $J = 1.5$ Hz, $CH{=}CCH_3$), 2.80 (1H, dd, $J = 9, 4$ Hz, NH–C–C*H*), 2.87 (1H, dd, $J = 4, 0.5$ Hz, $CH{=}CCH_3CH$), 3.56 (1H, dd, $J = 8, 6.5$ Hz, O–CH_AH_B–CH–O), 3.97 (1H, dd, $J = 8, 6$ Hz, O–CH_AH_B–CH–O), 4.21 (1H, dt, $J = 9, 6$ Hz, O–CH_2–C*H*–O), 5.36 (1H, d, $J = 11$ Hz, $CH{=}CH_CH_D$), 5.39 (1H, d, $J = 17.5$ Hz, $CH{=}CH_CH_D$), 5.66 (1H, br s, NH), 5.90 (1H, m, $CH{=}CCH_3$), 6.45 (1H, dd, $J = 17.5, 11$ Hz, $CH{=}CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 25.6, 26.6, 52.2, 61.3, 68.4, 69.0, 72.5, 110.4, 115.2, 124.0, 133.9, 156.6, 175.0, 191.9. Anal. Calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05%. Found: C, 64.98; H, 6.83; N, 5.31%.

***N*-Boc-bicyclic Lactam 11.** A solution of bicyclic lactam **10** (7.10 g, 25.6 mmol), Boc_2O (6.5 mL, 28.2 mmol), and DMAP (312 mg, 2.56 mmol) in THF (200 mL) was stirred at reflux temperature for 30 min. The reaction mixture was diluted with Et_2O, washed with H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (SiO_2 gel 300 g, AcOEt:hexane = 1:2) to afford *N*-Boc-bicyclic lactam **11** (9.0 g, 93%) as yellow crystals. Mp 128.9–129.6. $[\alpha]_D^{25}$ -331 (*c* 0.74, $CHCl_3$). IR (KBr): ν_{max} 2987, 1787, 1698, 1624, 1372, 1298 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.27 (3H, s, CH_3 of acetonide), 1.34 (3H, s, CH_3 of acetonide), 1.42 (9H, s, *t*-butyl), 2.10 (3H, d, $J = 1.5$ Hz, $CH{=}CCH_3$), 2.82 (1H, dd, $J = 8, 4$ Hz, NBoc–C–C*H*), 2.96 (1H, dd, $J = 4, 1$ Hz, $CH{=}CCH_3CH$), 3.55 (1H, dd, $J = 8, 6.5$ Hz, O–CH_AH_B–CH–O), 3.94 (1H, dd, $J = 8, 6$ Hz, O–CH_AH_B–CH–O), 4.11 (1H, dt, $J = 8, 6.5$ Hz, O–CH_2–C*H*–O), 5.34 (1H, d, $J = 18$ Hz, $CH{=}CH_CH_D$), 5.36 (1H, d, $J = 11$ Hz, $CH{=}CH_CH_D$), 5.90 (1H, m, $CH{=}CCH_3$), 6.70 (1H, dd, $J = 18, 11$ Hz, $CH{=}CH_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.8, 25.5, 26.4, 27.7, 52.2, 58.6, 68.3, 71.4, 72.0, 84.6, 110.3, 115.1, 124.5, 133.7, 148.6, 154.9, 170.9, 189.8. Anal. Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71%. Found: C, 63.44; H, 7.20; N, 3.88%.

11-Hydroxy-*N*-Boc-bicyclic Lactam 13. A flask was charged with *N*-Boc-bicyclic lactam **11** (310 mg, 0.822 mmol) and connected to a vacuum/nitrogen line. The flask was evacuated and then filled with nitrogen. The substrate was dissolved in dry CH_2Cl_2 (9.3 mL). This solution was cooled to −78 °C and treated with Et_3N (0.57 mL, 4.11 mmol) and TBSOTf (0.57 mL, 2.47 mmol). After stirring at −78 °C for 3 h 10 min, the reaction mixture was diluted with Et_2O. The mixture was quenched with sat. $NaHCO_3$ solution and extracted with Et_2O (×3). The combined organic extracts were washed with sat. NH_4Cl solution (×2), sat. $NaHCO_3$ solution (×2), H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. This crude vinyl silyl ether **12** (577 mg) was used for the next step without further purification. To a stirred solution of MCPBA (213 mg, 1.23 mmol) in EtOH (10 mL) cooled at 0 °C was added a solution of the crude vinyl silyl ether **12** (577 mg) in EtOH (7 mL) cooled at 0 °C. After stirring for 17.5 h, the reaction mixture was poured into sat. Na_2SO_3 solution and sat. $NaHCO_3$ solution. The mixture was extracted with Et_2O (×3), and the combined organic layer was washed with H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (SiO_2 gel, 9 g, AcOEt:hexane = 1:1) to afford lactam **13** (243 mg, 75% in 2 steps) as white crystals. Mp 139.7–

140.1. $[\alpha]_D^{25}$ −316 (c 0.995, $CHCl_3$). IR (KBr): ν_{max} 3524, 2985, 2874, 1782, 1698, 1372, 1307, 1156 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.26 (3H, s, CH_3 of acetonide), 1.34 (3H, s, CH_3 of acetonide), 1.41 (9H, s, t-butyl), 2.86 (1H, dd, $J = 8.5$, 4 Hz, NBoc–C–CH), 3.08 (1H, dd, $J = 4$, 0.5 Hz, CH=CCH$_2$OHCH), 3.56 (1H, dd, $J = 8$, 6.5 Hz, O–CH_AH$_B$–CH–O), 3.98 (1H, dd, $J = 8$, 6 Hz, O–CH$_A$$H_B$–CH–O), 4.11 (1H, dt, $J = 8.5$, 6 Hz, O–CH$_2$–CH–O), 4.32 (1H, dd, $J = 16.5$, 2 Hz, CH_CH$_D$OH), 4.40 (1H, dd, $J = 16.5$, 1.5 Hz, CH$_C$$H_D$OH), 5.35 (1H, d, $J = 18$ Hz, CH=CH_CH$_D$), 5.36 (1H, d, $J = 11$ Hz, CH=CH$_C$$H_D$), 6.12 (1H, m, C$H$=CCH$_2$OH), 6.71 (1H, dd, $J = 18$, 11 Hz, CH=CH$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.5, 26.4, 27.8, 47.8, 58.6, 63.5, 68.2, 71.9, 72.2, 84.9, 110.4, 115.1, 122.1, 133.7, 148.6, 156.2, 171.4, 189.7. Anal. Calcd for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.92; N, 3.56%. Found: C, 61.05; H, 6.87; N, 3.69%.

11-Protected-Hydroxy-*N*-Boc Enone 16. To a solution of **13** (2.47 g, 6.28 mmol) in CH_2Cl_2 (70 mL) were added ethyl vinyl ether (1.80 mL, 18.9 mmol) and pyridinium p-toluenesulfonate (2.37 g, 9.42 mmol). After stirring at rt for 15 min, the reaction mixture was diluted with Et_2O, washed with H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. The crude product **14** (2.95 g) was dissolved in THF (88 mL), and 1 M LiOH solution (12.6 mL, 12.6 mmol) was added. After vigorous stirring at rt for 15 min, the reaction mixture was carefully neutralized to pH 7 by slow addition of 1 M HCl into cold reaction mixture with stirring, and the stirring was continued at rt for 14 h 40 min. The mixture was diluted with AcOEt, washed with H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. The crude product **15** (2.81 g) was dissolved in MeOH (84 mL), and $NaHCO_3$ (791 mg, 9.42 mmol) was added. After vigorous stirring at rt for 21.5 h, the reaction mixture was quenched with sat. NH_4Cl solution. The mixture was extracted with AcOEt (×3), and the combined organic layer was washed with H_2O (×2) and brine (×1), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (SiO_2 gel 55 g, AcOEt:hexane = 1:2) to afford enone **16** (2.7 g, 98% in 4 steps) as a colorless amorphous solid. $[\alpha]_D^{24}$ +7.3 (c 1.08, $CHCl_3$). IR (KBr): ν_{max} 3398, 2984, 2873, 1718, 1496, 1369 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.19 (3/2H, t, $J = 7$ Hz, OCH$_2$CH_3), 1.20 (3/2H, t, $J = 7$ Hz, OCH$_2$CH_3), 1.33 (3H, d, $J = 5$ Hz, OCHCH_3), 1.36 (3H, s, CH_3 of acetonide), 1.39 (3H, s, CH_3 of acetonide), 1.44 (9H, s, t-butyl), 2.19 (2H, m, CH=CCH_2CH), 3.25 (1H, q, $J = 8$ Hz, CH$_2$CHCNBoc), 3.42–3.54 (1H, m, OCH_AH$_B$CH$_3$), 3.56–3.68 (1H, m, OCH$_A$$H_BCH_3$), 3.66 (1H, t, $J = 8$ Hz, O–CH_CH$_D$–CH–O), 4.00–4.28 (4H, m, O–CH$_C$$H_D$–CH–O, O–CH$_2$–C$H$–O, C$H_2$OEE), 4.76 (1H, q, $J = 5$ Hz, O–CH–O), 5.09 (1H, br s, NH), 5.29 (1H, dd, $J = 17.5$, 1 Hz, CH=CH_EH$_F$), 5.35 (1H, d, $J = 11$ Hz, CH=CH$_E$$H_F$), 5.88 (1H, ddd, $J = 17.5$, 11, 1.5 Hz, CH=CH$_2$), 6.27 (1H, m, CH=CCH$_2$OEE). Anal. Calcd for $C_{23}H_{37}NO_7$: C, 62.85; H, 8.48; N, 3.19%. Found: C, 62.85; H, 8.61; N, 3.19%.

Allylic Alcohol 17. A flame-dried flask was charged with $LiAlH_4$ (207 mg, 5.47 mmol), and evacuated and then filled with nitrogen. Dry Et_2O (20 mL) was added, and the suspension was cooled to −78 °C. To this flask was added the enone **16** (1.20 g, 2.73 mmol) in dry Et_2O (16 mL). After stirring at −78 °C for 1 h, the reaction was quenched with H_2O. The mixture was stirred with AcOEt, H_2O, and aqueous potassium sodium tartrate for an additional 10 min, and then extracted with AcOEt (×3). The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography (SiO_2 gel 36 g, AcOEt:hexane = 1:3 → 1:1) to afford allylic alcohol **17** (1.16 g, 96%) as a colorless amorphous solid. $[\alpha]_D^{25}$ −2.0 (c 1.03, $CHCl_3$). IR (KBr): ν_{max} 3362, 2984, 2864, 1699, 1538, 1370, 1286 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.19 (3H, t, $J = 7.5$ Hz, OCH$_2$CH_3), 1.30 (3H, d, $J = 5.5$ Hz, OCHCH_3), 1.38 (3H, s, CH_3 of acetonide), 1.41 (3H, s, CH_3 of acetonide), 1.44 (9H, s, t-butyl), 1.55–1.72 (1H, m, CH=CCH_AH$_B$CH), 1.78–1.88 (1H, m, CH=CCH$_A$$H_B$CH), 1.89–2.01 (1H, m, CH$_2$CHC-NBoc), 3.45 (1/2H, q, $J = 7$ Hz, OCH_CH$_D$CH$_3$), 3.48 (1/2H, q, $J = 7$ Hz, OCH_CH$_D$CH$_3$), 3.57–3.67 (2H, m, OCH$_C$$H_DCH_3$, O–C$H_EH_F$–CH–O), 3.83–4.07 (4H, m, CH_2OEE, O–CH$_E$$H_F$–CH–O, O–CH$_2$–C$H$–O), 4.44 (1H, br s, C$H$OH), 4.68 (1H, q, $J = 5.5$ Hz, O–CH–O), 5.29 (1H, br d, $J = 17.5$ Hz, CH=CH_IH$_J$), 5.41 (1H, br d, $J = 11$ Hz, CH=CH$_I$$H_J$), 5.63 (1H, s, C$H$=CCH$_2$OEE), 5.79 (1H, ddd, $J = 17.5$, 11, 1 Hz, CH=CH$_2$), 6.72 (1H, s, OH), 7.52 (1H, s, NH). Anal. Calcd for $C_{23}H_{39}NO_7$: C, 62.56; H, 8.90; N, 3.17%. Found: C, 62.56; H, 9.17; N, 3.28%.

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Supporting Information

Copies of ^1H NMR and ^{13}C NMR spectra of all new compounds. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

References

1 a) T. Goto, Y. Kishi, S. Takahashi, Y. Hirata, *Tetrahedron* **1965**, *21*, 2059. b) K. Tsuda, S. Ikuma, M. Kawamura, R. Tachikawa, K. Sakai, C. Tamura, O. Amakasu, *Chem. Pharm. Bull.* **1964**, *12*, 1357. c) R. R. Woodward, *Pure Appl. Chem.* **1964**, *9*, 49.

2 For reviews on tetrodotoxin, see: a) Issue of Tetrodotoxin, Saxitoxin, and the Molecular Biology of the Sodium Channel: *Ann. N.Y. Acad. Sci.* **1986**, *479*. doi:10.1111/j.1749-6632.1986.tb15556.x b) T. Noguchi, O. Arakawa, *Mar. Drugs* **2008**, *6*, 220. c) T. Narahashi, *Proc. Jpn. Acad., Ser. B* **2008**, *84*, 147.

3 T. Nishikawa, M. Asai, N. Ohyabu, N. Yamamoto, Y. Fukuda, M. Isobe, *Tetrahedron* **2001**, *57*, 3875.

4 T. Nishikawa, M. Asai, M. Isobe, *J. Am. Chem. Soc.* **2002**, *124*, 7847.

5 a) T. Nishikawa, D. Urabe, K. Yoshida, T. Iwabuchi, M. Asai, M. Isobe, *Org. Lett.* **2002**, *4*, 2679. b) T. Nishikawa, D. Urabe, K. Yoshida, T. Iwabuchi, M. Asai, M. Isobe, *Chem.—Eur. J.* **2004**, *10*, 452.

6 a) T. Nishikawa, M. Asai, N. Ohyabu, N. Yamamoto, M. Isobe, *Angew. Chem., Int. Ed.* **1999**, *38*, 3081. b) M. Asai, T. Nishikawa, N. Ohyabu, N. Yamamoto, M. Isobe, *Tetrahedron* **2001**, *57*, 4543.

7 a) T. Nishikawa, D. Urabe, M. Isobe, *Angew. Chem., Int. Ed.* **2004**, *43*, 4782. b) D. Urabe, T. Nishikawa, M. Isobe, *Chem. Asian J.* **2006**, *1*, 125.

8 a) D. Urabe, K. Sugino, T. Nishikawa, M. Isobe, *Tetrahedron Lett.* **2004**, *45*, 9405. b) T. Nishikawa, D. Urabe, M. Tomita, T. Tsujimoto, T. Iwabuchi, M. Isobe, *Org. Lett.* **2006**, *8*, 3263.