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Synthetic Study of Azaspiracid-1: Synthesis of the EFGHI-Ring Fragment

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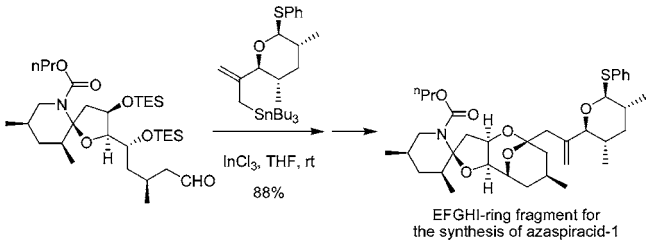
ABSTRACT



Here, we report a synthesis of the lower half C_{21}–C_{40} fragment of the shellfish toxin, azaspiracid-1. The C_{28}–C_{40} fragment was synthesized by a coupling between the C_{28}–C_{35} epoxide and the C_{36}–C_{40} dithioacetal anion, followed by the HI-ring spiroaminal formation. An aldehyde corresponding to the C_{28}–C_{40} fragment was then coupled with the C_{21}–C_{27} allylic stannane by using $InCl_3$. Finally, the FG-ring was constructed by HF·pyridine to accomplish the synthesis of the suitably protected C_{21}–C_{40} fragment.

Azaspiracid-1 (**1**) is a causative toxin for a new type of shellfish poisoning syndrome named azaspiracid poisoning (AZP), which has prevailed since November 1995 at a coastal region in Europe.[1] The structure was first elucidated spectroscopically by a group led by Yasumoto and Satake at Tohoku University in 1998 by using contaminated Irish mussels, *Mytilus edulis*.[2] The structural characteristics are (1) a C_6−C_{17} bisspiroketal fused to a C_{17}−C_{20} tetrahydrofuran and (2) an unusual C_{33}−C_{40} azaspiro ring fused with C_{28}−C_{40} 2,9-dioxabicyclo[3.3.1]nonane. Four congeners, azaspiracids-2−5, were found by the same group thereafter,[3] and the lethality against mice (LD_{50}) was found to be nearly comparable to that of **1** (0.2 mg/kg) for azaspiracids-2−4 (0.11−0.47 mg/kg) and less toxic for azaspiracid-5 (>1 mg/kg).[4]

Since the first publication on **1**,[2] synthetic studies have been actively developed by many groups, and the first total synthesis and the structural revision of **1** were made by the Nicolaou group in 2004 by a strategy synthesizing possible combinations of partial structures.[5] Recently, the Nicolaou group also synthesized azaspiracid-2 and -3, confirming the revised structure for these congeners.[6] We have been working toward the total synthesis of **1** to clarify the molecular mechanism of AZP toward humans.[7] Here, we describe our

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(4) Ofuji, K.; Satake, M.; Oshima, Y.; Naoki, H.; Yasumoto, T. In *43rd Symposium on the Chemistry of Natural Products*; Osaka, 2001; p 353.

(5) For the whole story for the structural revision of **1**, see: (a) Freemantle, M. *Chem. Eng. News* **2004**, *82*, 27. (b) Nicolaou, K. C.; Vyskocil, S.; Koftis, T. V.; Yamada, Y. M. A.; Ling, T. T.; Chen, D. Y. K.; Tang, W. J.; Petrovic, G.; Frederick, M. O.; Li, Y. W.; Satake, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4312. (c) Nicolaou, K. C.; Koftis, T. V.; Vyskocil, S.; Petrovic, G.; Ling, T. T.; Yamada, Y. M. A.; Tang, W. J.; Frederick, M. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 4318. (d) Nicolaou, K. C.; Pihko, P. M.; Bernal, F.; Frederick, M. O.; Qian, W. Y.; Uesaka, N.; Diedrichs, N.; Hinrichs, J.; Koftis, T. V.; Loizidou, E.; Petrovic, G.; Rodriquez, M.; Sarlah, D.; Zou, N. *J. Am. Chem. Soc.* **2006**, *128*, 2244. (e) Nicolaou, K. C.; Chen, D. Y. K.; Li, Y. W.; Uesaka, N.; Petrovic, G.; Koftis, T. V.; Bernal, F.; Frederick, M. O.; Govindasamy, M.; Ling, T. T.; Pihko, P. M.; Tang, W. J.; Vyskocil, S. *J. Am. Chem. Soc.* **2006**, *128*, 2258. (f) Nicolaou, K. C.; Koftis, T. V.; Vyskocil, S.; Petrovic, G.; Tang, W. J.; Frederick, M. O.; Chen, D. Y. K.; Li, Y. W.; Ling, T. T.; Yamada, Y. M. A. *J. Am. Chem. Soc.* **2006**, *128*, 2859.

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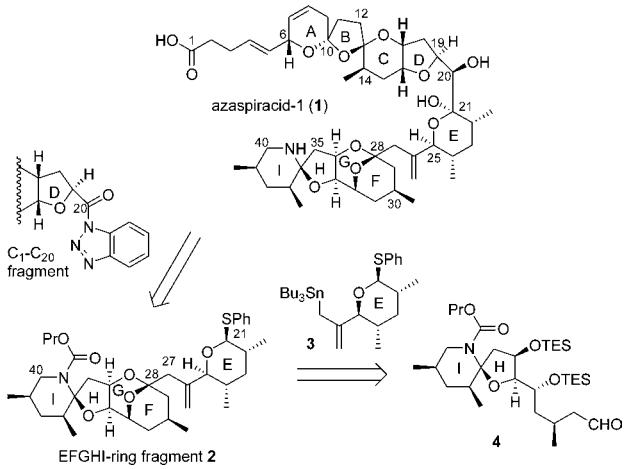
efforts toward this goal and the synthesis of the $C_{21}-C_{40}$ EFGHI-ring fragment **2**, the lower half of **1**.[8]

Our synthetic plan toward azaspiracid-1 (**1**) is shown in Scheme 1. Retrosynthetic disconnection at the $C_{20}-C_{21}$ bond

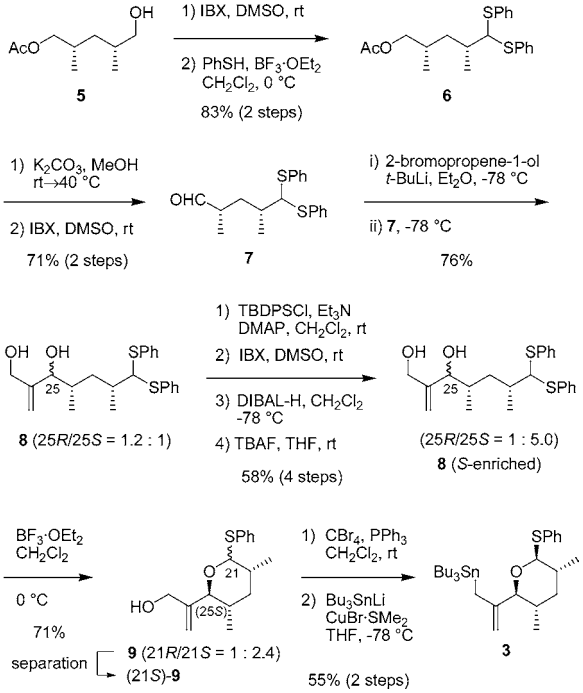
Scheme 1. Synthetic Strategy for Azaspiracid-1 (**1**)



generates two C_1-C_{20} and $C_{21}-C_{40}$ (**2**) fragments. These fragments would be assembled by the coupling between a benzotriazole amide and a sulfonylpyran, also employed in the synthesis of altohyrtin C by Evans et al.[9] The $C_{21}-C_{40}$ EFGHI-ring fragment **2** was further divided retrosynthetically into two fragments, corresponding to the $C_{21}-C_{27}$ E-ring (**3**) and the $C_{28}-C_{40}$ FGHI-ring (**4**) domains, which were to be coupled by the reaction between an aldehyde and an allylic stannane under mild conditions.

The synthesis of the E-ring allylic stannane **3** started with the known optically active acetoxyalcohol **5**, which can be readily prepared from methylmalonic acid ester over six steps via the key enzymatic desymmetrization of *meso*-2,4-dimethyl-1,5-pentanediol (Scheme 2).[10] At first, the hydroxy group was oxidized with IBX[11] and the aldehyde was treated with thiophenol and $BF_3{\cdot}OEt_2$ to give the dithioacetal **6** in 83% yield. Removal of the acetyl group followed by oxidation provided aldehyde **7**, which in turn was reacted with the vinylic anion, generated from 2-bromopropen-1-ol[12] and *t*-BuLi. The reaction proceeded quite smoothly at

Scheme 2. Preparation of the E-Ring Allylic Stannane **3**



−78 °C to give diol **8** in 76% yield as a chromatographically inseparable, diastereomeric mixture (*R*/*S* = 1.2:1). We then enriched the desired (25*S*)-isomer. Thus, the four-step transformation, including (1) the temporary protection of the primary hydroxy group, (2) oxidation of the remaining hydroxy group by IBX, (3) stereoselective reduction of the ketone by using DIBAL-H, and (4) the removal of the TBDPS protecting group by TBAF, allowed enrichment of the diastereomeric ratio of **8** to *R*/*S* = 1:5.0.[13] To construct the E-ring tetrahydropyran, **8** was next treated with $BF_3{\cdot}OEt_2$ in CH_2Cl_2. The (25*S*)-isomer preferentially reacted to give **9** in 71% yield as a diastereomeric mixture at the acetal carbon center (21*R*/21*S* = 1:2.4), and the dithioacetal with unnatural (*R*) stereochemistry at the C_{25} position was recovered and recycled for the same transformation again. Careful chromatographic separation of the alcohol **9** gave the pure (21*S*)-isomer, which was finally converted to the allylic stannane (21*S*)-**3**, the E-ring fragment, by bromination followed by stannylation (Bu_3SnLi, CuBr).[14]

The synthesis of the FGHI-ring fragment, corresponding to the $C_{28}-C_{40}$ position, was next explored. The stereoselective construction of the unique azaspiro HI-ring is the major concern in this synthesis. Our first-generation synthesis employed the Boc protecting group for the C_{40}−amino functionality.[7] However, a partial decomposition of the Boc group was observed under the conditions for the HI-ring formation using $Yb(OTf)_3$. Here, we decided to explore an

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(7) Sasaki, M.; Iwamuro, Y.; Nemoto, J.; Oikawa, M. *Tetrahedron Lett.* **2003**, *44*, 6199.

(8) For synthetic studies on the E-, F-, G-, H-, and/or I-ring domain of **1**, see: (a) Carter, R. G.; Weldon, D. J. *Org. Lett.* **2000**, *2*, 3913. (b) Aiguade, J.; Hao, J. L.; Forsyth, C. J. *Org. Lett.* **2001**, *3*, 979. (c) Aiguade, J.; Hao, J. L.; Forsyth, C. J. *Tetrahedron Lett.* **2001**, *42*, 817. (d) Forsyth, C. J.; Hao, J. L.; Aiguade, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 3663. (e) Zhou, X. T.; Carter, R. G. *Chem. Commun.* **2004**, 2138. (f) Zhou, X. T.; Carter, R. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 1787. (g) Nguyen, S.; Xu, J.; Forsyth, C. J. *Tetrahedron* **2006**, *62*, 5338.

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(10) (a) Lin, G. Q.; Xu, W. C. *Tetrahedron* **1996**, *52*, 5907. (b) Fujita, K.; Mori, K. *Eur. J. Org. Chem.* **2001**, 493.

(11) Frigerio, M.; Santagostino, M. *Tetrahedron Lett.* **1994**, *35*, 8019.

(12) Li, K. Q.; Du, W. S.; Que, N. L. S.; Liu, H. W. *J. Am. Chem. Soc.* **1996**, *118*, 8763.

(13) The stereochemistry of these products was determined by the modified Mosher ester analysis of undesired (*R*)-**8**, which was obtained by recrystallization.

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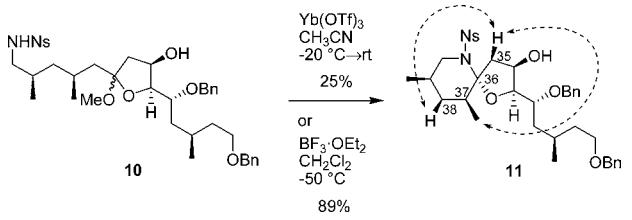
alternative, stable protecting group, which also supports the stereoselective cyclization of the azaspiro ring.

The 2-nitrobenzensulfonyl (nosyl, Ns) group[15] was at first employed because it is electron withdrawing and hence is expected to promote the spiroaminal formation smoothly (Scheme 3). However, the reaction on **10**[16] by using Yb-

Scheme 3. Spiroaminal Formation Leading to an Unnatural Isomer[a]

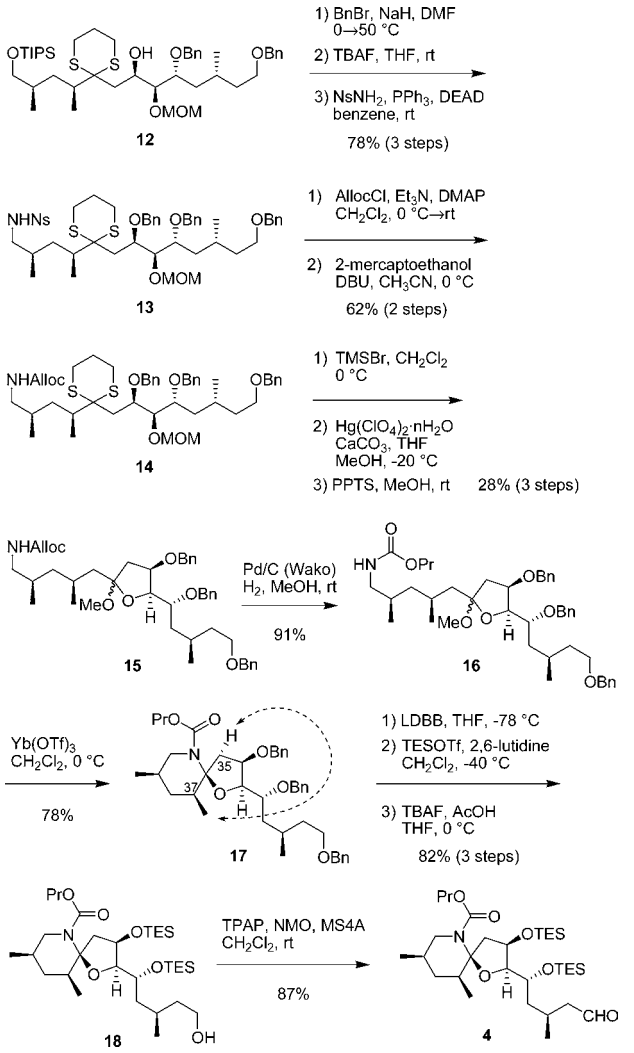


[a] Dashed arrows indicate the NOESY correlations observed.

$(OTf)_3$ was found to be very slow at room temperature, and the stereochemistry of the product **11**, obtained in 25% as the sole product, was inconsistent with that of the natural azaspiracid-1 (**1**). That is, from the NOEs observed at H_{35}-(pro-*S*)/C_{37}−Me and H_{35}(pro-*S*)/H_{38}ax, the stereochemistry at C_{36} was determined to be *R*, which is not desired. When BF_3·OEt_2 was used for the Lewis acid, the cyclization proceeded quite smoothly in 89% yield, but the stereoselectivity was not improved.[17]

We next examined the alkylcarbamate protecting group, which is more stable than the Boc group previously used for the C_{40}−amino functionality.[7] The alcohol **12**[7] was at first protected, and a 2-nosylamino group was introduced to the C_{40} position by desilylation with TBAF and the Mitsunobu reaction,[18] to afford **13** in 78% overall yield (Scheme 4). After the amide was allyloxycarbonylated (Alloc), the Ns group was removed by the Fukuyama protocol using 2-mercaptoethanol to give **14**. Then, the H-ring moiety was constructed by successive deprotection of the MOM[19] and the dithiane groups,[20] followed by the furanose formation in MeOH to give **15** in 28% yield (47% based on recovered materials) for three steps. At this time, the Alloc protecting group was hydrogenated with a less acidic catalyst[21] to provide the propyl carbamate **16** in 91% yield, ready for the next crucial cyclization. The reduction was necessary for the selective removal of the benzyl groups at the C_{28}, C_{32}, and C_{34} positions later.[22] With the cyclization precursor **16** in hand, Yb(OTf)$_3$-catalyzed spiroaminal formation[8d] was at-

Scheme 4. Preparation of the C_{28}−C_{40} FGHI-Ring Domain **4** with Correct Spiroaminal Stereochemistry[a]



[a] Dashed arrow indicates the NOESY correlations observed.

tempted in CH_2Cl_2 at 0 °C. As expected, the cyclization proceeded smoothly in 1 h, to give spiroaminal **17** in 78% yield. The cyclization was highly stereoselective, and the stereochemistry at the spirocenter was unambiguously determined to be identical to that of the natural **1** by the NOE at H_{35}(pro-*R*)/C_{37}−Me. The spiroaminal **17** was further converted to alcohol **18** by protecting group manipulations, which was then oxidized by tetrapropylammonium perruthenate (TPAP)[23] to give aldehyde **4** for the coupling with the E-ring fragment **3** in 71% yield over four steps.

With the key fragments **3** and **4** in hand, their coupling reactions leading to the desired EFGHI-ring domain were next explored. In our first-generation synthesis, where simple

(15) (a) Fukuyama, T.; Jow, C. K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373. (b) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 353.

(16) Prepared from **12** in 54% yield over five steps.

(17) The stereoselectivity observed in the spiroaminal formation was unexpected but was reasonably explained by calculation at the MMFF94S force field. The details will be reported in the full account of this work.

(18) Henry, J. R.; Marcin, L. R.; McIntosh, M. C.; Scola, P. M.; Harris, G. D.; Weinreb, S. M. *Tetrahedron Lett.* **1989**, *30*, 5709.

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(20) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287.

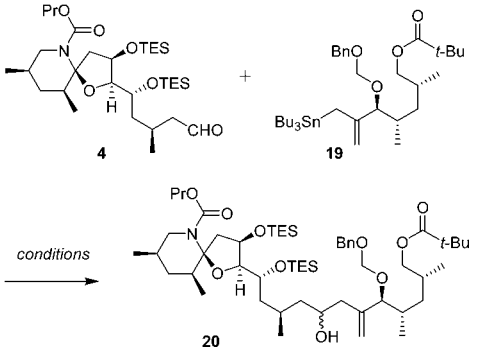
(21) Sajiki, H.; Ikawa, T.; Hirota, K. *Tetrahedron Lett.* **2003**, *44*, 7407.

(22) An alkyl carbamate protecting group can be generally removed with various nucleophiles; see: (a) Tius, M. A.; Kerr, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 5959. (b) Wenkert, E.; Hudlicky, T.; Showalter, H. D. H. *J. Am. Chem. Soc.* **1978**, *100*, 4893. (c) Corey, E. J.; Weigel, L. O.; Floyd, D.; Bock, M. G. *J. Am. Chem. Soc.* **1978**, *100*, 2916.

(23) Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc. Chem. Commun.* **1987**, 1625.

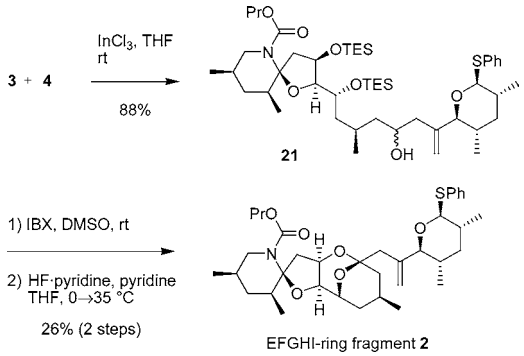
methallyl stannane was used as a preliminary study, $MgBr_2 \cdot OEt_2$ was employed for this coupling.[7] However, it was found from another preliminary model study using more complex allylic stannane **19**[24] that neither $MgBr_2 \cdot OEt_2$ nor $LiClO_4$ worked for this coupling reaction, only recovering the substrates (Table 1, runs 1 and 2). More reactive $BF_3 \cdot OEt_2$ induced decomposition even at −78 °C (run 3). From the ^1H NMR spectra of the product mixture, the decomposition was supposed to occur at the HI-ring azaspiro moiety. To avoid the decomposition, we investigated another Lewis acid, which would hopefully work in a Lewis basic solvent. It was then found that, when $InCl_3$ was used in acetone, the allylic stannane **19** reacted with acetone cleanly (run 4).[25] We therefore explored other solvents, and THF was finally found to be satisfactory to give the desired product **20** in a quantitative yield (run 5). With this protocol using $InCl_3$ (3.0 equiv) in THF, the coupling reaction between the key fragments **3** (5.6 equiv) and **4** (1.0 equiv) also proceeded smoothly at room temperature to give **21**, which has the $C_{21}-C_{40}$ entire chain of azaspiracid (**1**) with complete functionalities, in 88% yield (Scheme 5). After mild oxidation with IBX, the FG-ring was stereoselectively constructed upon exposure to HF·pyridine, which induced desilylation and intramolecular acetalization, to give the desired EFGHI-ring fragment **2** of azaspiracid-1 (**1**) in 26% yield. The stereochemistry was unambiguously confirmed by NOESY experiments (see the Supporting Information).

Table 1. Preliminary Model Studies for the Coupling of **4** with Allylic Stannane **19**



run	conditions	yield for **20** (%)/ recovery of **4** (%)
1	$MgBr_2 \cdot OEt_2$ (4.0 equiv), CH_2Cl_2, −20 °C	0/56
2	$LiClO_4$ (3.0 equiv), Et_2O, rt	0/95
3	$BF_3 \cdot OEt_2$ (2.0 equiv), MS4A, CH_2Cl_2, −78 °C	0/<30
4	$InCl_3$ (3.0 equiv), acetone, rt	0/88
5	$InCl_3$ (3.0 equiv), THF, rt	100/−

Scheme 5. Synthesis of the EFGHI-Ring Fragment of Azaspiracid-1 (**1**)



In summary, we have successfully synthesized the suitably protected, lower half $C_{21}-C_{40}$ fragment **2** of azaspiracid-1 (**1**) in 0.025% yield for the longest linear pathway from D-glutamic acid (37 steps). The synthesis features (1) stereoselective construction of the HI-ring spiroaminal and (2) high-yield coupling of the $C_{21}-C_{27}$ and $C_{28}-C_{40}$ fragments by allylic stannane chemistry. Work is in progress toward the total synthesis of **1** in our laboratory.

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Supporting Information Available: Experimental details and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) The allylic stannane **19** was prepared from 2-bromo-3-trimethylsilylpropene in 37% yield over nine steps. See the Supporting Information. The details will be given in the full account of this work.

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