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Published on Web 02/02/2006

Total Synthesis and Structural Elucidation of Azaspiracid-1. Final Assignment and Total Synthesis of the Correct Structure of Azaspiracid-1

K. C. Nicolaou,* Theocharis V. Koftis, Stepan Vyskocil, Goran Petrovic, Wenjun Tang, Michael O. Frederick, David Y.-K. Chen, Yiwei Li, Taotao Ling, and Yoichi M. A. Yamada

Contribution from the Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093

Received August 15, 2005; E-mail: kcn@scripps.edu

Abstract: The molecular structure of azaspiracid-1, a neurotoxin isolated from mussels, has been elucidated by total synthesis which also enriched its supplies. The degradatively derived fragments of this marine biotoxin, compounds **5** (EFGHI), **6** (FGHI), and **40** (ABCD), were matched with synthetic materials, thus confirming their structural identities. Based on this detective work, a new structure of azaspiracid-1 (i.e., **1**) was proposed and constructed by total synthesis. The final strategy for the total synthesis of azaspiracid-1 featured a dithiane anion ($C_{21}-C_{27}$ fragment) reacting with a pentafluorophenol ester (C_1-C_{20} fragment) followed by a Stille-type union of an advanced allylic acetate substrate (C_1-C_{27} fragment) with a vinyl stannane as the main coupling processes to assemble the carbon skeleton of the molecule. In addition to the total synthesis of azaspiracid-1 (**1**), the syntheses of its C_1-C_{20} epimer (**2**) and of several truncated analogues for biological investigations are described.

Introduction

In two previous articles[1,2] we described our pursuits toward the originally proposed[3] structures of azaspiracid-1 (**1a** and **1b**, Figure 1) and how we came to realize that they did not represent the true structure of the natural substance. In this paper we report our investigations that led to the structural elucidation and total synthesis of this notorious marine neurotoxin (**1**, Figure 1) and its C_1-C_{20} epimer (**2**, Figure 1).

Results and Discussion

After synthesizing several isomers of the originally proposed structure of azaspiracid-1 and failing to pinpoint the correct architecture or the precise location of the error, we adopted a more systematic plan of action directed toward identifying the site(s) at which the structural discrepancy resided. Specifically, we suggested to Professor Satake[3] that a scission of the $C_{20}-C_{21}$ diol system within the molecule of azaspiracid-1 might separate the two major domains of the structure and thus facilitate the identification of the problem. Indeed, the Satake group was able to utilize the little precious sample of the natural substance they had to obtain compounds **3**−**6** (Figure 2) and supply us with ^{1}H NMR spectra of their samples.[4] These spectra were equally precious to us, for we could now compare them with those of our synthetic samples and derive useful information as we conducted our investigations, which would turn out to read more like a most adventurous and fascinating detective story. Although the spectral data of our synthetic materials up to this stage of the campaign strongly suggested structural errors in the ABCD domain of the molecule of azaspiracid-1, we decided to synthesize both the ABCD and the EFGHI fragments of the originally proposed structures (**1a** and **1b**, Figure 1) for the sake of thoroughness in our intended structural elucidation studies.

1. Determination of the Relative and Absolute Stereochemistry of the EFGHI Domains. Our first priority was to determine the relative stereochemistry between ring E and the rest of the polycyclic framework within the EFGHI lactone **5**. To this end, enantiomer **7**[1] of the $C_{21}-C_{27}$ fragment was converted to the two diastereoisomers of the EFGHI lactone, compounds **20** and **5**, by a sequence that incorporated the two enantiomers of the FHI ring system (**11** and *ent*-**11**, respectively),[1] as shown in Scheme 1. Thus, diol **7** was selectively acetylated at the primary position (Ac_2O, 2,4,6-collidine, 85% yield), and the resulting hydroxy acetate (**8**) was converted to acetoxy lactol **9** by exposure to $PhI(OCOCF_3)_2$ (81% yield),

(1) Nicolaou, K. C.; Pihko, P. M.; Bernal, F.; Frederick, M. O.; Qian, W.; 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*, 2244−2257.
(2) Nicolaou, K. C.; Chen, D. Y.-K.; Li, Y.; Uesaka, N.; Petrovic, G.; Koftis, T. V.; Bernal, F.; Frederick, M. O.; Govindasamy, M.; Ling, T.; Pihko, P. M.; Tang, W.; Vyskocil, S. *J. Am. Chem. Soc.* **2006**, *128*, 2258−2267.
(3) Satake, M.; Ofuji, K.; Naoki, H.; James, K. J.; Furey, A.; McMahon, T.; Silke, J.; Yasumoto, T. *J. Am. Chem. Soc.* **1998**, *120*, 9967.
(4) (a) Oofuji, K. Ph.D. Thesis, Kou8595, Tohoku University, March 25, 2002. (b) Nicolaou, K. C.; Vyskocil, S.; Koftis, T. V.; Yamada, Y. M. A.; Ling, T.; Chen, D. Y.-K.; Tang, W.; Petrovic, G.; Frederick, M. O.; Li, Y.; Satake, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 4312.

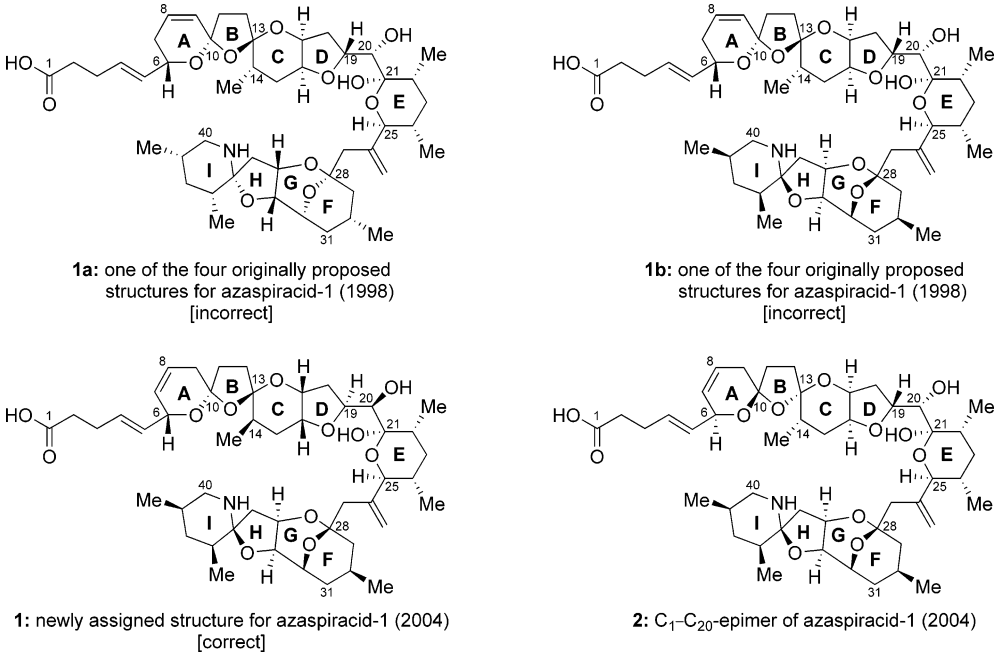


Figure 1. Two of the four originally proposed structures of azaspiracid-1 (**1a** and **1b**), proven to be incorrect, and structures of azaspiracid-1 (**1**) and its C_1-C_{20} epimer (**2**), proven to be correct by total synthesis.

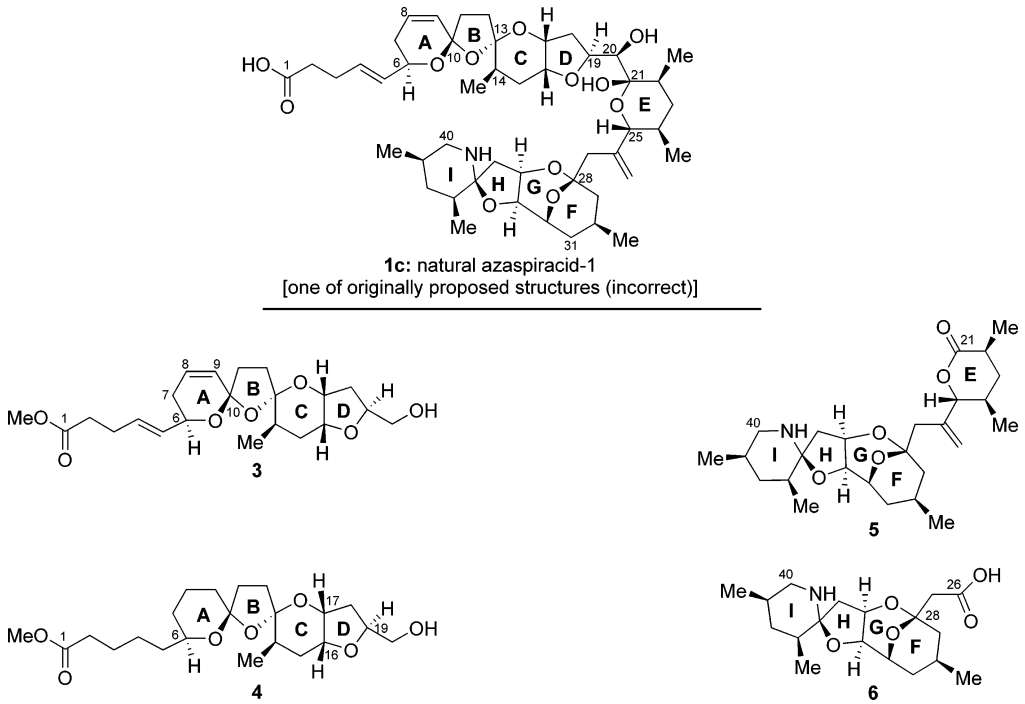
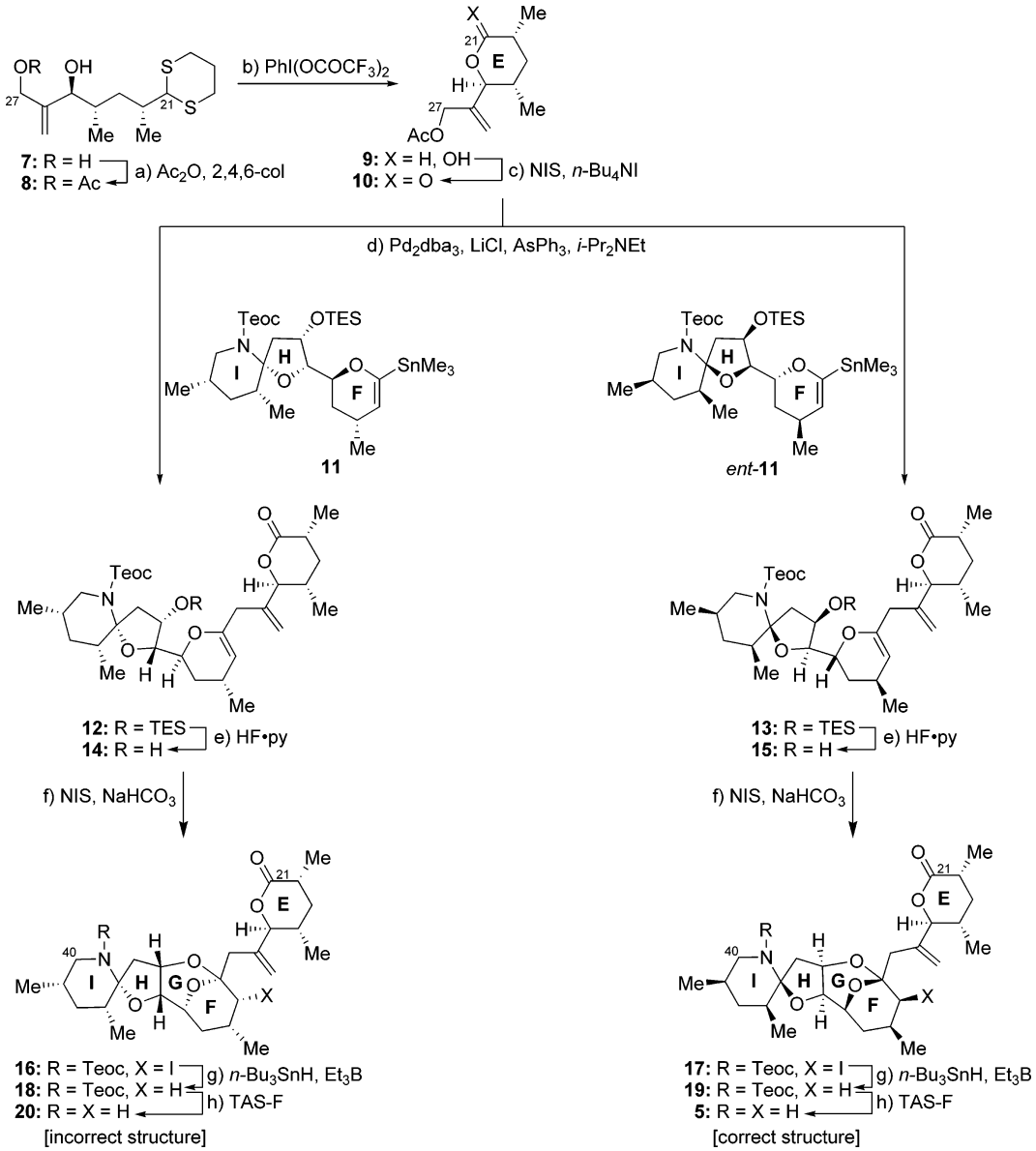


Figure 2. Proposed structures of fragments **3**−**6** obtained from degradation of natural azaspiracid-1 (**1c**) by Satake et al.

conditions that induced dithiane cleavage and allowed the spontaneous ring closure to take place. This lactol (**9**) was then oxidized with NIS (for abbreviations of reagents and protecting groups see legends in schemes) and *n*-Bu_4NI (74% yield) to afford the desired Stille coupling partner, allylic acetate lactone **10**. The coupling of allylic acetate lactone **10** with the FHI stannane **11** proceeded smoothly in the presence of Pd_2dba_3, $AsPh_3$, LiCl, and *i*-Pr_2NEt to afford product **12**, whose TES group was removed by the action of HF·py in THF:py (1:1), leading to the hydroxy tetrahydropyran system **14**. The latter compound (**14**) was then subjected to iodoetherification (NIS, $NaHCO_3$, THF, 0 °C) to generate iodoether **16** in 26% overall yield for the three steps from **10**. Reductive removal of the iodide residue from **16** with excess *n*-Bu_3SnH in toluene in the presence of Et_3B resulted in the formation of **18**, from which the Teoc protecting group was cleaved through the action of TAS-F, furnishing the desired EFGHI lactone **20** in 70% yield for the two steps. In a similar manner, and in approximately the same yields, allylic acetate **10** was united with stannane *ent*-**11** and the product was advanced to EFGHI lactone **5** as depicted in Scheme 1. From the two synthetic samples, only diastereoisomer **5** matched perfectly by ^1H NMR spectroscopy

Scheme 1. Synthesis of $C_{21}-C_{40}$ Lactones **5** and **20** and Determination of Relative and Absolute Stereochemistry within the EFGHI Domain[a]

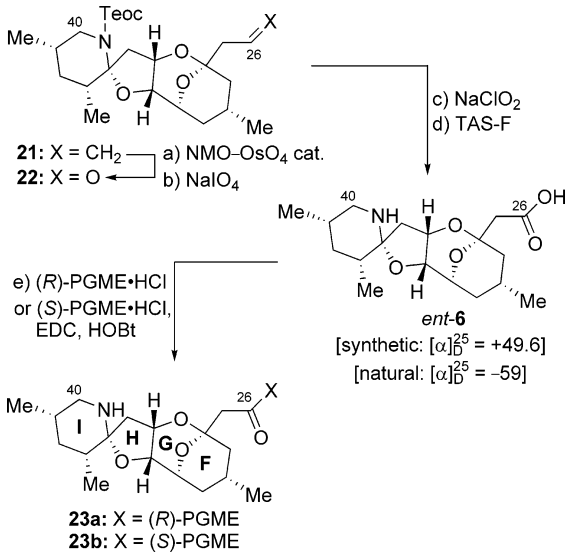


[a] Reagents and conditions: (a) Ac_2O, 2,4,6-collidine, CH_2Cl_2, 25 °C, 16 h, 85%; (b) $PhI(OCOCF_3)_2$ (1.5 equiv), MeCN:pH 7 buffer (4:1), 0 °C, 10 min, 81%; (c) NIS (10 equiv), *n*-Bu_4NI (2.0 equiv), CH_2Cl_2, 25 °C, 40 min, 74%; (d) **10** (3.0 equiv), Pd_2dba_3 (0.9 equiv), $AsPh_3$ (0.9 equiv), LiCl (18 equiv), *i*-Pr_2NEt (12 equiv), NMP, then **11** or *ent*-**11** (0.03 M in THF, syringe pump addition), 45 °C, 4 h; (e) HF•py (excess), THF:py (1:1), 0 → 25 °C, 2 h; (f) NIS (10 equiv), $NaHCO_3$ (30 equiv), THF, 0 °C, 16 h, 26% for **16**, 38% for **17** over three steps; (g) Et_3B (0.1 equiv, 1.0 M in hexanes), *n*-Bu_3SnH:toluene (1:2), 0 °C, 5 min; (h) TAS-F (5.0 equiv), DMF, 0 °C, 20 h, 70% for **20**, 74% for **5** over two steps. Abbreviations: NIS, *N*-iodosuccinimide; dba, dibenzylideneacetone; NMP, *N*-methylpyrrolidine; TAS-F, tris(dimethylamino)sulfur (trimethylsilyl)difluoride.

the degradatively derived material (**5**, Figure 2), thus establishing the relative stereochemistry of the EFGHI domain of azaspiracid-1 as that represented by structure **5**. However, since we had no rotation for the naturally derived lactone (**5**), we could not deduce its absolute stereochemistry. This determination, therefore, had to await the synthesis of the FGHI carboxylic acid **6** for which the Satake group had already secured a measurable optical rotation in their degradative studies.

Starting with the previously synthesized FGHI terminal alkene fragment **21**, the illustrated enantiomer of the carboxylic acid fragment (*ent*-**6**) was prepared as depicted in Scheme 2. Thus, dihydroxylation of **21** with NMO and OsO_4 catalyst, followed by $NaIO_4$ cleavage of the resulting diol, generated aldehyde **22** in 79% yield for the two steps. This aldehyde (**22**) was then oxidized by the standard $NaClO_2$ protocol, and from the so-obtained carboxylic acid the Teoc group was removed by the action of TAS-F, to afford the targeted amino acid (*ent*-**6**) in 35% overall yield. Much to our delight, the ^1H NMR spectrum of synthetic *ent*-**6** was identical to that of the degradatively derived material. Its optical rotation $\{[\alpha]^{25}_D = +49.6$ ($c = 0.4$, MeOH)}, however, was opposite in sign from that of the sample derived from natural azaspiracid-1 $\{[\alpha]^{25}_D = -59.0$ ($c = 0.016$, MeOH)}, leading to the assignment of the absolute stereochemistry of the FGHI domain as shown for **6** in Figure 2 (opposite of that shown for *ent*-**6** in Scheme 2). This assignment was further confirmed by comparing the ^1H NMR spectra of the (*R*)- and (*S*)-phenyl glycine methyl ester (PGME) derivatives (**23a** and **23b**, respectively) with those derived from the natural

Scheme 2. Synthesis of FGHI Amino Acid *ent*-**6** and Its PGME Derivatives[a]



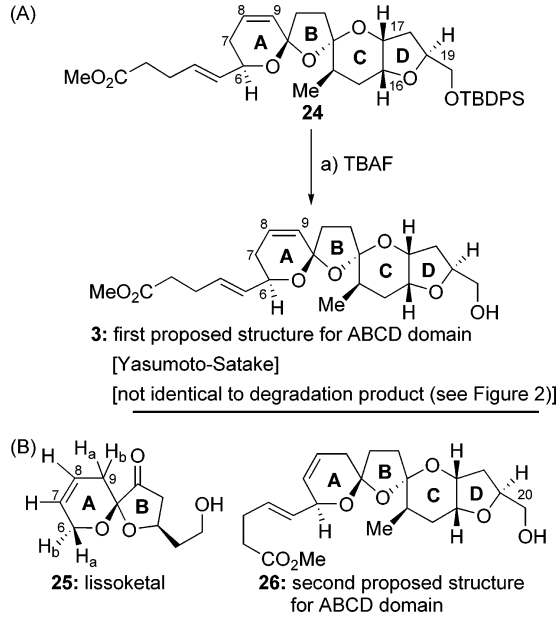
[a] Reagents and conditions: (a) OsO_4 (0.1 equiv), NMO (3.0 equiv), acetone:H_2O (3:1), 25 °C, 3 h, 92%; (b) $NaIO_4$ (3.0 equiv), MeOH:pH 7 buffer (2.5:1), 25 °C, 1 h, 87%; (c) $NaClO_2$ (10 equiv), NaH_2PO_4 (10 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 30 min, 93%; (d) TAS-F (5.0 equiv), DMF, 0 °C, 16 h, 38%; (e) (*R*)-PGME or (*S*)-PGME (5.0 equiv), EDC (5.0 equiv), HOBt (5.0 equiv), $NaHCO_3$ (10 equiv), DMF, 25 °C, 16 h, 75% for **23a** and 63% for **23b**. Abbreviations: NMO, *N*-methylmorpholine *N*-oxide; PGME, phenyl glycine methyl ester; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole.

material. Indeed, the synthetically derived (*R*)-PGME exhibited a ^{1}H NMR spectrum identical to that (provided by Professor Satake) of the degradatively derived (*S*)-PGME. Taken together, these studies provided a complete picture for the structure of the EFGHI domain of azaspiracid-1 (as depicted in structure **5**) and left only the ABCD segment of the molecule as the suspect region for the structural error.

2. Elucidation of the Structure of the ABCD Domain. Comparison of the ^{1}H NMR spectrum of synthetic ABCD methyl ester fragment **3** (obtained from the previously synthesized **24**[1] by desilylation with TBAF in 88% yield, as shown in Scheme 3) with that of the degradatively derived material confirmed what already had become abundantly clear by then, namely that this structure did not represent the true architecture of that region of the molecule of azaspiracid-1. But what was the real structure of this site of the molecule? The first clue to the solution of the puzzle came from lissoketal (**25**, Scheme 3), a marine-derived natural product of modest complexity reported by John D. Faulkner and his group in 1997.[5] Particularly revealing were the weak heteronuclear multiple-bond correlation between C-10 and H-7 and a weak COSY correlation between H-6 and H-9 of **25** which were reminiscent of and consistent with similar effects exhibited by the molecule of natural azaspiracid-1. In addition, the chemical shift of H-6 in synthetic **3** was at considerably higher field (500 MHz, $CDCl_3$, δ = 4.50 ppm) than the corresponding chemical shift in the degradatively derived material (500 MHz, $CDCl_3$, δ = 4.79 ppm). On the basis of these observations, we proposed structure **26** (Scheme 3), in which the endocyclic double bond was shifted by one position for the C_1-C_{20} fragment of azaspiracid-1, and,

(5) Hopmann, C.; Faulkner, D. J. *Tetrahedron Lett.* **1997**, *38*, 169.

Scheme 3. Synthesis of ABCD Alcohol **3** for Comparison with Degradatively Derived Material (A) and Second Proposed Structure (**26**) for the ABCD Domain Based on ^{1}H NMR Spectroscopic Comparisons with Lissoketal (**25**) (B)[a]



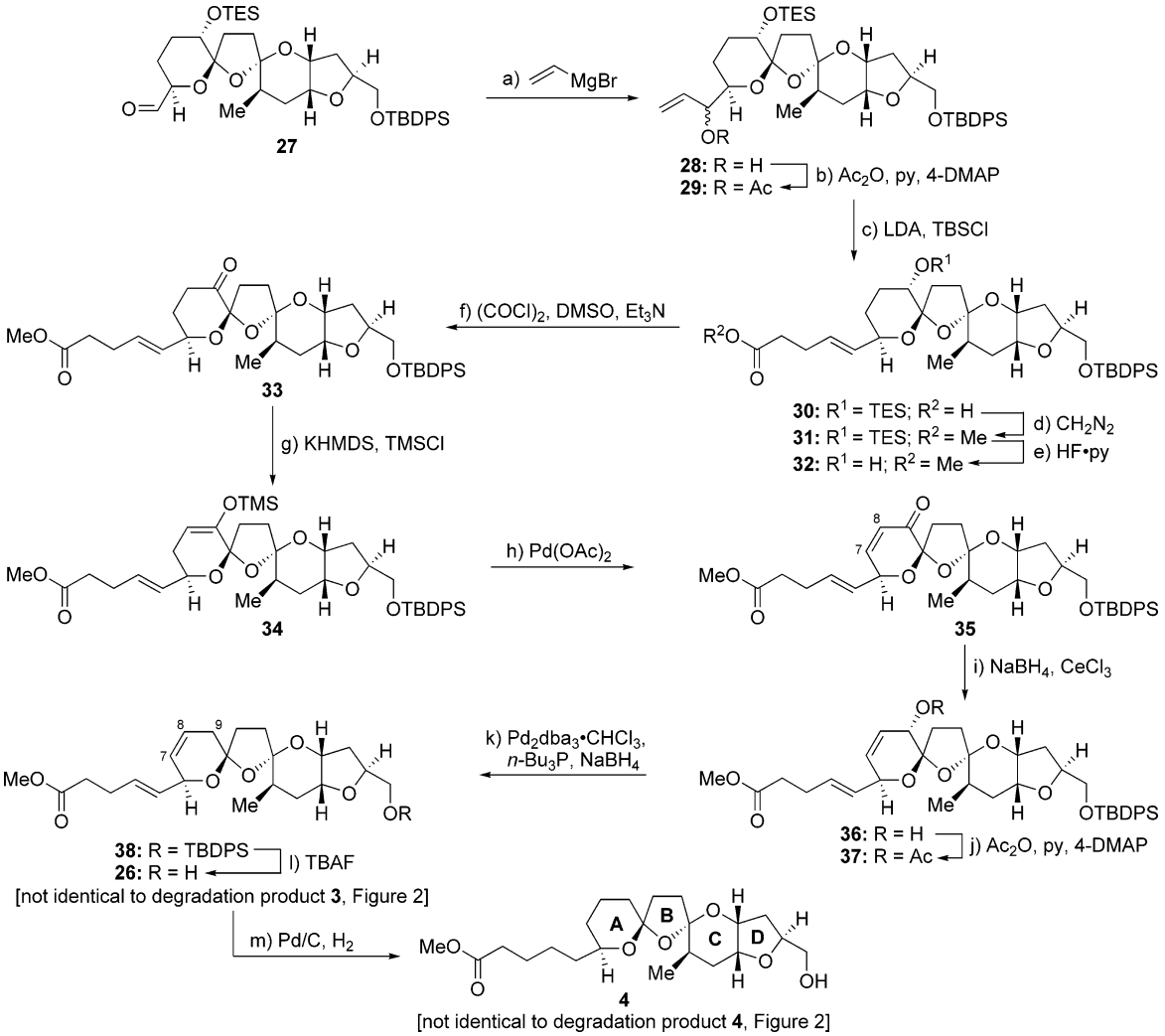
[a] Reagents and conditions: (a) TBAF (2.0 equiv), THF, 0 °C, 2 h, 88%. TBAF, tetra-*n*-butylammonium fluoride.

without delay, set out to synthesize it. Since optical rotation measurements with the minute amounts of the naturally derived material were not possible, we chose randomly to target the indicated enantiomer.

The synthesis of the second proposed structure (**26**) for the ABCD domain began with the previously synthesized aldehyde **27**[1] and proceeded as summarized in Scheme 4. Thus, nonstereoselective vinylmagnesium bromide addition to **27**, followed by acetylation (Ac_2O, py, 4-DMAP) of the resulting hydroxy compounds (**28**), furnished acetate **29** as a 1:1 mixture of diastereomers in 65% overall yield for the two steps. Sequential treatment of a THF solution of this mixture (**29**) with LDA (−78 °C), followed by addition of TBSCl and HMPA (−78 → 25 °C), promoted the intended Ireland−Claisen rearrangement,[6] leading to carboxylic acid **30**, which was methylated with CH_2N_2 to afford methyl ester **31** (52% yield for the two steps). This compound (**31**) was then converted to ketone **33** by selective removal of the TES group through the action of HF·py (85% yield) and Swern oxidation [$(COCl)_2$, DMSO; Et_3N] of the resulting secondary alcohol (**32**) (90% yield). TMS enol ether formation (KHMDS−TMSCl), followed by exposure to $Pd(OAc)_2$, then generated the desired enone **35**,[7] via **34**, in 55% overall yield. This enone was then reduced with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ to produce allylic alcohol **36** as a single isomer, a substance that was transformed into its acetate (**37**) (Ac_2O, py, 4-DMAP) in 71% overall yield for the two steps. The acetate group was then reductively excised from substrate **37** by treatment with $NaBH_4$ in the presence of $Pd_2dba_3 \cdot CHCl_3$ and *n*-Bu_3P[8] to afford bis-olefin **38** as a separable 2:1 mixture with its $\Delta^{8,9}$ regioisomer (**24**), in favor

(6) Ireland, R. E.; Mueller, R. H.; Willard, A. K. *J. Am. Chem. Soc.* **1976**, *98*, 2868.
(7) Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011.
(8) Nakamura, H.; Ono, M.; Shida, Y.; Akita, H. *Tetrahedron: Asymmetry* **2002**, *13*, 705.

Scheme 4. Synthesis of the Second Proposed Structure (**26**) of the ABCD Domain of Azaspiracid-1[a]



[a] Reagents and conditions: (a) CH_2═$CHMgBr$ (1.6 equiv), Et_2O, 0 → 25 °C, 1 h, 76%; (b) Ac_2O (5.0 equiv), py (10 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 3 h, 85%; (c) LDA (1.5 equiv), THF, −78 °C, 10 min, TBSCl (1.5 equiv), HMPA (1.5 equiv), −78 → 25 °C, 24 h; (d) CH_2N_2 (excess), Et_2O, 25 °C, 30 min, 52% over the two steps; (e) HF•py (10 equiv), py:THF (1:1), 0 °C, 2 h, 85%; (f) $(COCl)_2$ (5.0 equiv), DMSO (11 equiv), CH_2Cl_2, −78 °C, 30 min, −60 °C, 1 h, then Et_3N (22 equiv), −78 → −25 °C, 90%; (g) KHMDS (1.5 equiv), THF, −78 °C, 1 h, TMSCl (1.6 equiv), −78 °C, 30 min; (h) $Pd(OAc)_2$ (5.0 equiv), DMSO, 72 h, 55% over the two steps; (i) $NaBH_4$ (3.0 equiv), $CeCl_3 \cdot 7H_2O$ (1.0 equiv), MeOH, −50 °C, 1 h; (j) Ac_2O (10 equiv), py (20 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 → 25 °C, 12 h, 71% over the two steps; (k) $Pd_2dba_3 \cdot CHCl_3$ (0.2 equiv), *n*-Bu_3P (0.4 equiv), $NaBH_4$ (10 equiv), dioxane:water (9:1), 0 → 25 °C, 12 h, 65%, **38**:**24** 2:1; (l) TBAF (3.0 equiv), THF, 0 → 25 °C, 3 h, 98%; (m) 10% Pd/C (10% w/w), H_2, EtOAc, 25 °C, 4 h, 95%. Abbreviations: TES, triethylsilyl; LDA, lithium diisopropylamide; KHMDS, potassium bis(trimethylsilyl)amide.

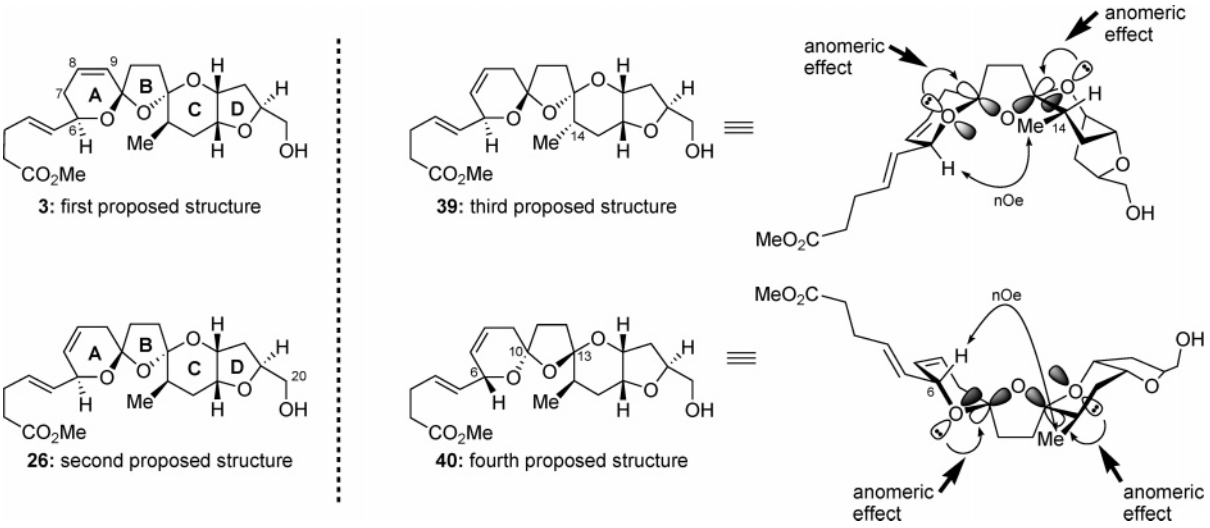


Figure 3. Third (**39**) and fourth (**40**) proposed structures for the ABCD domain of azaspiracid-1 with expected anomeric effects and nOes.

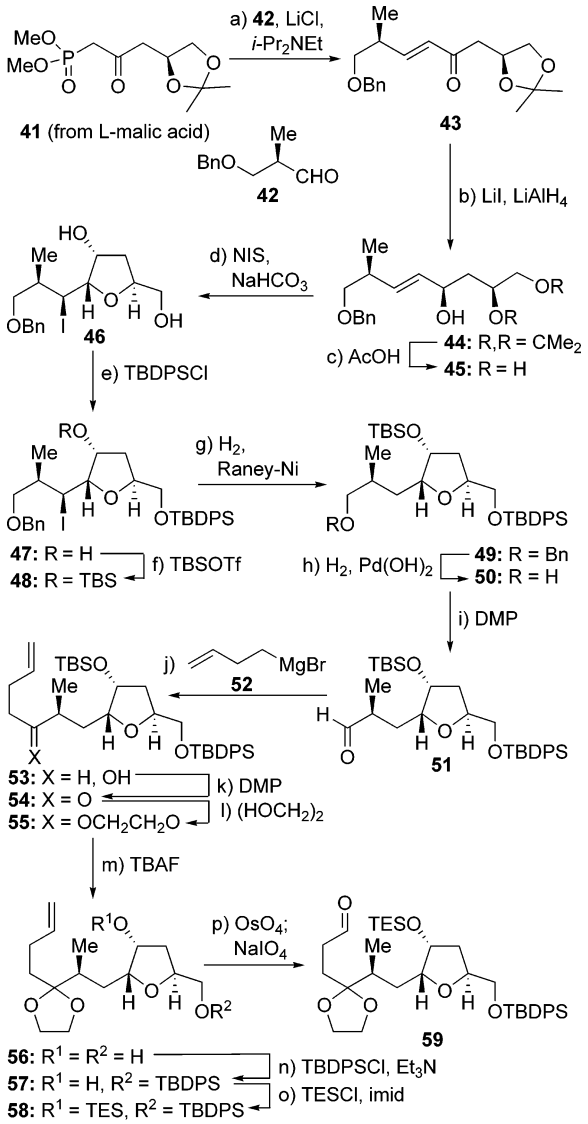
of the desired $\Delta^{7,8}$ isomer (**38**). No optimization was attempted at this point, neither for the installation of the endocyclic double bond nor for the deoxygenation, since we were only one step away from the targeted molecule. Indeed, desilylation of **38** through the action of TBAF afforded the desired ABCD fragment **26**, which was further elaborated to the fully saturated compound **4** by catalytic hydrogenation (H_2, 10% Pd/C) in 95% yield. NMR spectroscopic comparison of synthetic **26** with its naturally derived counterpart suggested that the A regions of the two molecules were closer, but still not identical with each other. Furthermore, the hydrogenated product **4** differed in its ^{1}H NMR spectrum from the naturally derived material, reconfirming the nonidentity of the natural and synthetic ABCD domains and underscoring the fact that something more than the double bond location was wrong with the proposed structure.

Figure 3 summarizes the state of affairs at this stage of the azaspiracid-1 project. The first and second proposed structures of the ABCD domain, as represented by fragments **3** and **26**, respectively, were proven wrong by synthesis. However, those studies led us to believe that the endocyclic double bond was at the C_7-C_8 site, rather than the C_8-C_9 site as originally proposed. Our reality check had also pointed to a thermodynamically most stable structure, as both the natural azaspiracid-1 and its ABCD fragment **3** (derived by degradation of the natural product by Satake et al.[4]) were stable under acidic conditions, in contrast to our synthetic ABCD fragments, whose fleeting nature under such conditions was in line with their lower thermodynamic stability. In addition to this information, we also had knowledge of an NOE exhibited by both the natural azaspiracid-1 and its degradatively derived ABCD fragment **3** between H-6 and the C-14 methyl group. This intelligence allowed us, through manual molecular modeling, to narrow down the most likely structures for the ABCD domain from the 128 (2^7) possible diastereomers to the two shown in Figure 3 (i.e., **39** and **40**). Thus, both of these structures are thermodynamically favored by two anomeric effects, and they should, by virtue of space proximity, exhibit the obligatory NOE effect between H-6 and the C-14 methyl group protons as indicated on their structures, **39** and **40** (see Figure 3). The first to be targeted for synthesis was isomer **39**.

The construction of **39** began with ketophosphonate **41** (derived from L-malic acid as previously described[9]) and aldehyde **42** and proceeded as summarized in Schemes 5 and 6. The sequence leading to aldehyde **59** (Scheme 5) is similar to that described elsewhere[9] for the preparation of a close relative of this compound and, therefore, will not be described here in more detail.

Scheme 6 describes the advancement of aldehyde **59** to ABCD fragment **67**, beginning with the addition of the lithium anion derived from dithiane **60** and *n*-BuLi to afford a mixture of diastereomeric alcohols (**61**). Oxidation of this mixture with DMP led to the cyclization precursor, ketone **62** (93% yield), whose exposure to TMSOTf in CH_2Cl_2 at −78 °C, followed by warming to −30 °C, furnished the ABCD tetracycle **63** as the major product (70% yield). NMR spectroscopic analysis of **63**, however, showed that the stereocenter at C-13 had a configuration opposite to that of the desired structure. This was further confirmed by analyzing the spectral data of a descendant

Scheme 5. Synthesis of Aldehyde **59**, a Precursor for the Third Proposed Structure of the ABCD Domain of Azaspiracid-1[a]

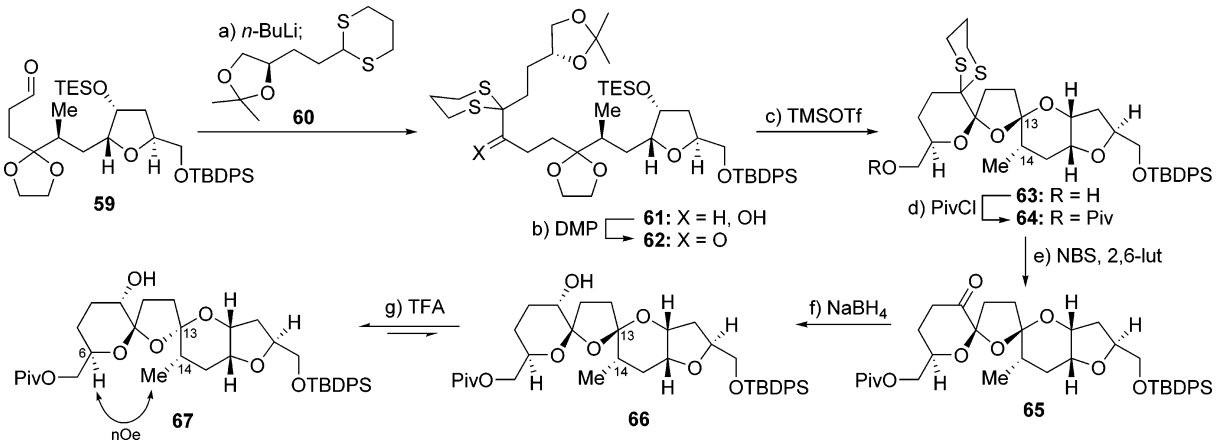


[a] Reagents and conditions: (a) **41** (0.67 equiv), **42** (1.0 equiv), LiCl (1.3 equiv), *i*-Pr_2NEt (1.0 equiv), MeCN, 25 °C, 12 h, 86% based on **41**; (b) $LiAlH_4$ (10 equiv), LiI (8.0 equiv), Et_2O, −100 °C, 30 min, 98%; (c) AcOH:H_2O (2:1), 25 °C, 5 h, 97%; (d) NIS (5.0 equiv), $NaHCO_3$ (10 equiv), THF, 0 °C, 2.5 h, 70%; (e) TBDPSCl (1.4 equiv), Et_3N (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, −10 → 0 °C, 3 h, 90%; (f) TBSOTf (1.6 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2, −10 °C, 30 min, 100%; (g) H_2, Raney-Ni (30 equiv), EtOH, 25 °C, 1 h, 99%; (h) H_2, 20% $Pd(OH)_2$/C (25% w/w), EtOH, 25 °C, 3 h, 88%; (i) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 2 h, 99%; (j) **52** (6.0 equiv), THF, −78 → −10 °C, 3 h, 92%; (k) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 2 h, 98%; (l) $(HOCH_2)_2$ (7.0 equiv), triethyl orthoformate (3.0 equiv), *p*-TsOH (0.1 equiv), 55 °C, 98%; (m) TBAF (1.0 M in THF, 4.0 equiv), THF, 25 °C, 48 h, 96%; (n) TBDPSCl (1.4 equiv), Et_3N (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 97%; (o) TESCl (1.5 equiv), imidazole (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 30 min, 98%; (p) OsO_4 (0.03 equiv), NMO (2.0 equiv), *t*-BuOH:THF:H_2O (10:2:1), 25 °C, 12 h, then $NaIO_4$ (5.0 equiv), pH 7 buffer, 25 °C, 5 h, 89%.

compound (**67**), which was prepared as follows. Reaction of **63** with PivCl, py, and 4-DMAP furnished pivaloate ester **64** (95% yield), from which the dithiane group was removed (NBS, 2,6-lutidine, 80% yield) to afford ketone **65**. The reduction of **65** with $NaBH_4$ in MeOH proceeded stereoselectively, leading to hydroxy pivaloate **66** in 81% yield. Finally, exposure of **66** to TFA in CH_2Cl_2 at 25 °C for 4 h resulted in an equilibrium mixture[9] in which the C-13 epimer **67** predominated in a 55:45

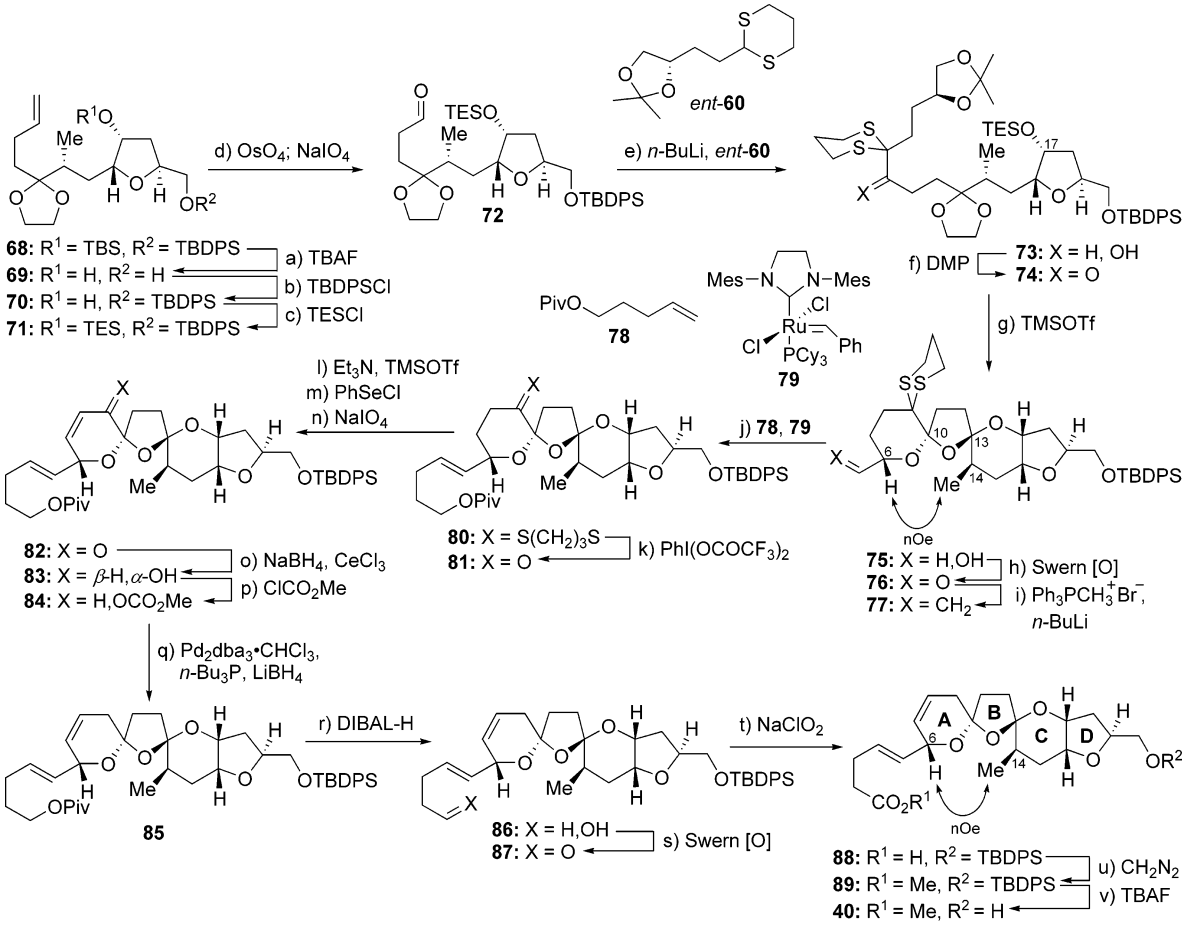
(9) Nicolaou, K. C.; Qian, W.; Bernal, F.; Uesaka, N.; Pihko, P. M.; Hinrichs, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 4068.

Scheme 6. Cyclization and Epimerization of a Precursor (**67**) of the Third Proposed Structure for the ABCD Domain of Azaspiracid-1[a]



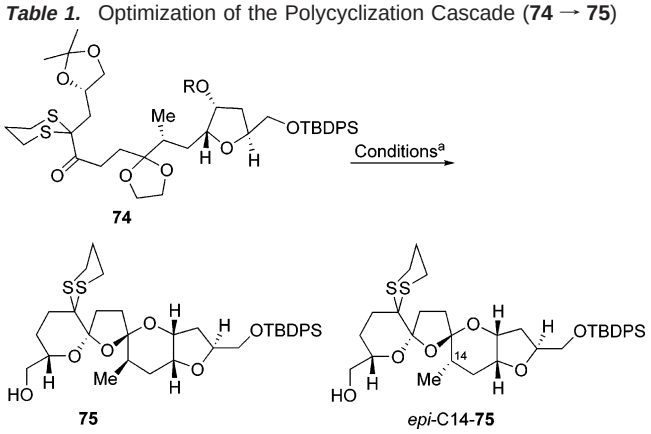
[a] Reagents and conditions: (a) *n*-BuLi (1.6 M in hexanes, 2.6 equiv), **60** (2.6 equiv), THF, −20 °C, 89%; (b) DMP (2.0 equiv), CH_2Cl_2, 25 °C, 1 h, 93%; (c) TMSOTf (3.0 equiv), CH_2Cl_2, −78 → −30 °C, 1 h, 70%; (d) PivCl (3.0 equiv), py (10 equiv), 4-DMAP (0.1 equiv), 25 °C, 3 h, 95%; (e) NBS (8.0 equiv), 2,6-lutidine (16 equiv), MeCN, 25 °C, 2 h, 80%; (f) $NaBH_4$ (1.0 equiv), MeOH, −5 °C, 5 min, 81%; (g) TFA (3.0 equiv), CH_2Cl_2, 25 °C, 4 h, 55% (45% recovered **66**).

Scheme 7. Construction of the Fourth Proposed Structure (**40**) for the ABCD Domain of Azaspiracid-1[a]



[a] Reagents and conditions: (a) TBAF (1.0 M in THF, 3.0 equiv), THF, 25 °C, 48 h, 95%; (b) TBDPSCl (1.2 equiv), Et_3N (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 12 h, 82%; (c) TESCl (1.5 equiv), imidazole (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 30 min, 95%; (d) OsO_4 (0.03 equiv), NMO (2.0 equiv), *t*-BuOH:THF:H_2O (10:2:1), 25 °C, 12 h, then $NaIO_4$ (5.0 equiv), pH 7 buffer, 25 °C, 5 h, 95%; (e) *n*-BuLi (1.6 M in hexanes, 3.5 equiv), *ent*-**60** (3.5 equiv), THF, −30 °C, 30 min, 87%; (f) DMP (2.0 equiv), py (10 equiv), CH_2Cl_2, 0 °C, 1 h, 92%; (g) TMSOTf (4.0 equiv), CH_2Cl_2, −90 °C, 30 min, 89%; (h) $(COCl)_2$ (5.0 equiv), DMSO (11 equiv), CH_2Cl_2, −78 → −60 °C, 1.5 h, then Et_3N (22 equiv), −25 °C; (i) $Ph_3PCH_3^+Br^-$ (5.0 equiv), *n*-BuLi (4.0 equiv), THF, −78 → 0 °C, 1 h, then −78 °C, then **76**, −78 → 0 °C, 30 min, 81% from **75**; (j) **78** (3.0 equiv), **79** (0.1 equiv), CH_2Cl_2, 40 °C, 12 h, 60% *E*:*Z* (ca. 10:1); (k) $PhI(OCOCF_3)_2$ (3.0 equiv, slow addition), MeCN:pH 7 buffer (4:1), 0 °C, 30 min, 90%; (l) Et_3N (44 equiv), TMSOTf (18 equiv), CH_2Cl_2, −20 → 0 °C, 12 h; (m) PhSeCl (1.5 equiv), CH_2Cl_2, −78 → 0 °C, 3 h; (n) $NaIO_4$ (4.5 equiv), THF:pH 7 buffer (4:1); (o) $NaBH_4$ (3.1 equiv), $CeCl_3 \cdot 7H_2O$ (1.0 equiv), MeOH, −65 °C, 40 min, 67% over the four steps; (p) $ClCO_2Me$ (25 equiv), 4-DMAP (1.0 equiv) CH_2Cl_2:py (2:1), −10 °C, 3 h, 87%; (q) $Pd_2dba_3 \cdot CHCl_3$ (0.125 equiv), *n*-Bu_3P (0.48 equiv), $LiBH_4$ (10 equiv), DME, 0 °C, 1 h, $\Delta^{7,8}$:$\Delta^{8,9}$ (7:1), 82%; (r) DIBAL-H (1.0 M in CH_2Cl_2, 2.0 equiv), CH_2Cl_2, −78 °C, 15 min; (s) $(COCl)_2$ (5.0 equiv), DMSO (11 equiv), CH_2Cl_2, −78 → −60 °C, 1.5 h, then Et_3N (22 equiv), −25 °C; (t) $NaClO_2$ (10 equiv), NaH_2PO_4 (10 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 1 h; (u) CH_2N_2 (excess), Et_2O, 25 °C; (v) TBAF (1.0 M in THF, 2.0 equiv), THF, 25 °C, 1 h, 71% from **85** (five steps).

Table 1. Optimization of the Polycyclization Cascade (**74** → **75**)



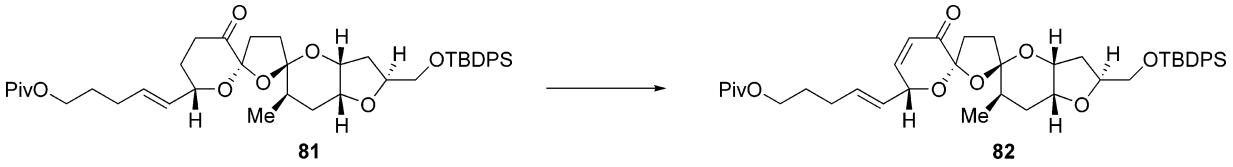
entry	R	temp (°C)	time (h)	yield (%)	**75**:*epi*-C_{14}-**75**[b]
1	TBS (**74′**)	−78	2	78	82:18
2	TBS (**74′**)	−78 → − 30	2	75	75:25
3	TES (**74**)	−78	0.5	85	90:10
4	TES (**74**)	−90	0.5	89	>98:2

[a] Reactions were carried out on a 0.1 mmol scale in CH_2Cl_2 with 5.0 equiv of TMSOTf. [b] Ratios of products were determined by ^{1}H NMR spectroscopy.

ratio. The strong NOE effect between H-6 and the C-14 methyl group protons exhibited by **67** in its ^{1}H NMR spectrum, as expected, supported its structure, but its relative thermodynamic stability with regard to its C-13 epimer (**66**) (i.e., 55:45 mixture) was problematic, since the naturally derived materials were firmly anchored in one stereochemistry and presumed to be the thermodynamically most stable. Furthermore, the chemical shift for the C-14 methyl group in the ^{1}H NMR spectrum of **67** was significantly different from that exhibited by the ABCD fragment **3** (derived from natural azaspiracid). In view of these inconsistencies, the pursuit of the third proposed structure for the ABCD domain (**39**, Figure 3) was abandoned at this stage as one unlikely to be correct.

Having eliminated the third proposed structure, **39**, as a candidate for the long-sought true identity of the ABCD domain, we then turned our attention to the fourth proposed structure, **40** (see Figure 3), as our next synthetic target. Scheme 7 summarizes the construction of the newly targeted compound through a modified sequence that featured a TES protecting group (rather than the TBS that we were using up to this point) at the C-17 hydroxyl group of the cyclization precursor (i.e., **74**, Scheme 7). This protecting group exchange was made in the hope for a higher yield and stereoselectivity in the key cascade reaction leading to the desired ABCD domain, and as we shall see below, it proved beneficial. The starting material for this sequence was the previously synthesized terminal olefin **68**,[8] which was converted to its TES counterpart (**71**) by a standard deprotection to **69** (TBAF, 95% yield), monosilylation to **70** (TBDPSCl, Et_3N, 4-DMAP, 82% yield), and TES attachment (TESCl, imidazole, 4-DMAP, 95% yield). NMO/OsO_4−$NaIO_4$ cleavage of the olefin within the latter compound (**71**) then led to aldehyde **72** (95% overall yield), which was reacted with the lithium anion derived from dithiane *ent*-**60** to afford alcohol **73** (mixture of diastereomers, ca. 1:1). DMP oxidation of **73** led to the cyclization precursor, ketone **74**, in 80% overall yield from aldehyde **72**. With regard to the cyclization event, it was reasoned that, by virtue of its higher lability, the TES group would come off under the Lewis acid catalysis conditions faster and, therefore, allow for both a lower temperature and a cleaner reaction overall. Indeed, and as Table 1 shows, this was the case. Thus, while the TBS-protected substrate **74′** (R = TBS), when exposed to TMSOTf at −78 °C, led to an 82:18 ratio in favor of **75** in 78% combined yield (entry 1), the corresponding TES-protected precursor **74** (R = TES) led, under the same conditions (except for a shorter reaction time), to an improved ratio (**75**:*epi*-C_{14}-**75** 90:10) and a higher yield (85%, entry 3). Furthermore, when the reaction involving the TBS compound (**74′**, R = TBS) was carried out at −78 °C with warming to −30 °C, both the yield and the ratio deteriorated (to 75% and 75:25, respectively, entry 2), but when the TES derivative was used, the temperature could be lowered to −90 °C, allowing for an improved yield of the desired isomer (**75**) (89%, single compound by ^{1}H NMR).

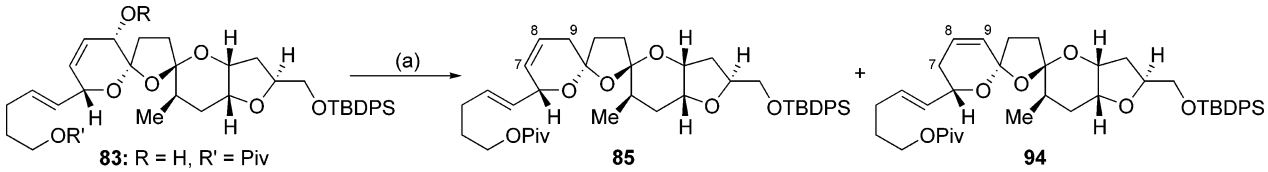
Table 2. Optimization for Enone Formation (**81** → **82**) for the Correct ABCD Domain of Azaspiracid-1



entry	reagents and conditions[a]	temp (°C)	time (h)	yield (%)
1	IBX (1.1 equiv), DMSO	50	1	decomposition
2	(i) LHMDS (1.5 equiv), PhSeCl (1.6 equiv)	−78 → 0	0.5	
	(ii) H_2O_2 (2.0 equiv), py (2.0 equiv), CH_2Cl_2	0	1	decomposition
3	(i) LHMDS (1.5 equiv), PhSeCl (1.6 equiv), THF	−78 → 0	0.5	
	(ii) *m*CPBA (2.0 equiv), CH_2Cl_2	0	0.5	decomposition
4	(i) LHMDS (1.5 equiv), PhSeCl (1.6 equiv), THF	−78 → 0	0.5	
	(ii) $NaIO_4$ (2.0 equiv), THF:MeOH:H_2O (4:1:1)	0 → 25	6	53
5	(i) KHMDS (1.5 equiv), TMSCl (1.6 equiv)	−78	0.5	
	(ii) $Pd(OAc)_2$ (2.0 equiv), DMSO	25	48	55
6	LDA (1.5 equiv), *t*-BuN═S(Cl)Ph (1.6 equiv), THF	−78	0.5	50−81[b]
7	(i) Et_3N (44 equiv), TMSOTf (18 equiv), CH_2Cl_2	−20 → 0	12	
	(ii) PhSeCl (1.5 equiv), CH_2Cl_2	−78 → 0	3	
	(iii) $NaIO_4$ (4.5 equiv), THF; pH 7 buffer (4:1)	25	12	69

[a] Reactions were carried out on a 0.1−0.2 mmol scale. [b] Yields varied depending on the purity of *t*-BuN═S(Cl)Ph (purchased from Tokyo Chemical Industry).

Table 3. Optimization of the Deoxygenation of Allylic Alcohol (**83**) Derivatives to Olefinic Compound **94**



83: R = H, R' = Piv
84: R = CO_2Me, R' = Piv
90: R = CS_2Me, R' = Piv
91: R = Ms, R' = Piv
92: R = Ts, R' = Piv
93: R = Ac, R' = Piv
95: R = R' = H

entry	R	(a) conditions	temp (°C)	time (h)	yield (%)	**85**:**94**
1	CS_2Me (**90**)[a]	*n*-Bu_3SnH (10 equiv), AIBN (1.0 equiv), toluene	110	1	62	1:1
2	CS_2Me (**90**)[a]	Et_3B (1.0 equiv), toluene:*n*-Bu_3SnH (2:1)	25	2	57	1:1
3	Ms (**91**)[b]	$LiAlH_4$ (1.0 M in Et_2O, 2.0 equiv), THF	25	12	(**95**)[e]	N/A
4	Ms (**91**)[b]	$LiBH_4$ (2.0 equiv), THF	25	12	(**95**)[e]	N/A
5	Ms (**91**)[b]	Superhydride (10 equiv), THF	45	12	(**95**)[e]	N/A
6	Ts (**92**)[c]	$LiAlH_4$ (1.0 M in Et_2O, 2.0 equiv), THF	25	12	(**95**)[e]	N/A
7	H (**83**)	$LiClO_4$ (12 equiv), Et_3SiH (3.0 equiv), Et_2O	25	36	no reaction	N/A
8	Ac (**93**)[d]	$LiClO_4$ (12 equiv), Et_3SiH (3.0 equiv), Et_2O	25	36	no reaction	N/A
9	Ac (**93**)[d]	20% $Pd(OH)_2$/C (25% w/w), EtOH:cyclohexene (2:1)	65	26	50	3:1
10	Ac (**93**)[d]	Pd_2dba_3·$CHCl_3$ (0.2 equiv, *n*-Bu_3P (0.4 equiv), $NaBH_4$ (10 equiv), dioxane:H_2O (9:1)	25	12	40	2:1
11	CO_2Me (**84**)	Pd_2dba_3·$CHCl_3$ (0.125 equiv), *n*-Bu_3P (0.48 equiv), $LiBH_4$ (10 equiv), DME	0	1	82	7:1

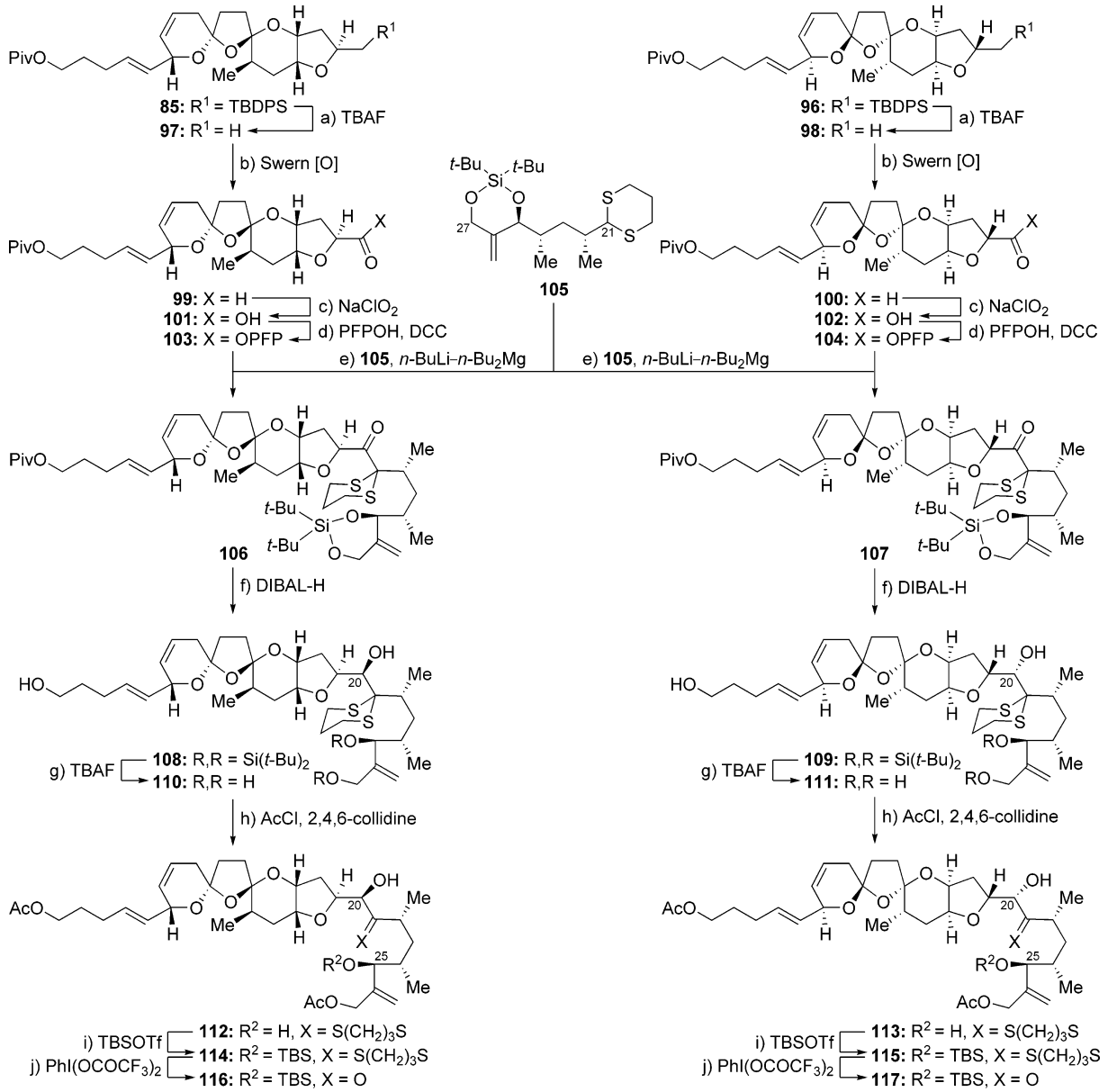
[a] Synthesized by treating **83** with NaH (1.1 equiv), CS_2 (2.0 equiv), and MeI (3.0 equiv), THF, −78 → 25 °C, 1 h, 85%. [b] Synthesized by treating **83** with MsCl (2.0 equiv) and Et_3N (4.0 equiv), CH_2Cl_2, 0 °C, 1.5 h, 90%. [c] Synthesized by treating **83** with TsCl (5.0 equiv), Et_3N (10 equiv), and 4-DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 48 h, 81%. [d] Synthesized by treating **83** with AcCl (10 equiv), py (20 equiv), and 4-DMAP (0.1 equiv), CH_2Cl_2, 25 °C, 24 h, 89%. [e] The only product isolated was alcohol **95**.

With the cascade polycyclization step optimized, the resulting ABCD system **75** was then oxidized under Swern conditions [$(COCl)_2$, DMSO; Et_3N] to the corresponding aldehyde (**76**), which was converted to terminal olefin **77** by the standard Wittig reaction in 81% overall yield for the two steps (Scheme 7). Adopting the previously developed cross-metathesis-based method,[10] the olefinic chain of this compound (**77**) was then extended to afford **80** by reaction with excess alkene **78** in the presence of Grubbs' second generation catalyst **79** (60% yield with 35% recovered **77**, 90% combined yield after three recyclings, *E*:*Z* ca. 10:1 chromatographically separable). The dithiane group was then removed from **80** [$PhI(OCOCF_3)_2$] to give ketone **81** in 90% yield. The conversion of this ketone to the corresponding enone (e.g., **81** → **82**), however, proved rather capricious and challenging. Table 2 summarizes the results of the various conditions employed, leading to the definition of the preferred method for this operation. Thus, while IBX[11] (entry 1) led to decomposition, the two-step procedure involving LDA−PhSeCl[12] followed by H_2O_2, *m*CPBA, or $NaIO_4$ (entries 2−4) succeeded only in the last case, furnishing the enone (**82**) in 53% yield. Good results were also obtained in the cases of KHMDS/TMSCl−$Pd(OAc)_2$[7] (entry 5, 55% yield) and LDA−*t*-BuN═S(Cl)Ph (entry 6, 50−81% yield),[13] although in the latter instance the yield varied depending on the quality of the difficult-to-purify reagent (available from Tokyo Chemical Industry). A more reliable approach was the three-step protocol shown in Table 2, entry 7, whereby the TMS enol ether of ketone **81** was reacted with PhSeCl and the resulting phenylseleno derivative was oxidatively removed with $NaIO_4$, leading to a 69% overall yield of the desired enone (**82**). Furthermore, and as shown in Scheme 7, reduction of the enone (**82**) was accomplished by treatment with $CeCl_3$·$7H_2O$ in MeOH, providing allylic alcohol **83** in 67% yield over the four steps (**81** → **83**) without purification.

The next challenge was the deoxygenation of allylic alcohol **83** without migration of the double bond from the $\Delta^{7,8}$ to the $\Delta^{8,9}$ position, a side reaction that plagued the operation for a while. Table 3 lists some of our attempts to optimize this reaction toward the desired product (**85**). Thus, Barton-McCombie[14] type deoxygenations through the xanthate (**90**, entries 1 and 2), employing *n*-Bu_3SnH-AIBN or *n*-Bu_3SnH-Et_3B, proceeded in 62 and 57% yields, respectively, but the desired $\Delta^{7,8}$ product (**85**) was contaminated with its $\Delta^{8,9}$ isomer (**94**) (ca. 1:1 ratio), making this process unacceptable. We next prepared the corresponding mesylate (**91**) and attempted its reduction with $LiAlH_4$, $LiBH_4$, or superhydride (entries 3−5). In all three cases both hydroxy groups (at C-1 and C-9) were unveiled, giving the corresponding diol (**95**) rather than the desired deoxygenated product. Similarly, treatment of the tosylate (**92**) with $LiAlH_4$ led to diol **95** as the sole product (entry 6). Deoxygenation protocols using $LiClO_4$ and Et_3SiH on either the free allylic alcohol (**83**, entry 7)[15] or its acetate (**93**, entry 8)[12] led to no reaction, while treatment of the acetate (**93**) with 10% $Pd(OH)_2$/C (entry 9)[16] led to deoxygenation in

(10) Nicolaou, K. C.; Koftis, T. V.; Vyskocil, S.; Petrovic, G.; Ling, T.; Yamada, Y. M. A.; Tang, W.; Frederick, M. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 4318.
(11) Nicolaou, K. C.; Zhong, Y.-L.; Baran, P. S. *J. Am. Chem. Soc.* **2000**, *122*, 7596.
(12) Sharpless, K. B.; Lauer, R. F.; Teranishi, A. Y. *J. Am. Chem. Soc.* **1973**, *95*, 6137.
(13) Mukaiyama, T.; Matsuo, J.; Kitagawa, H. *Chem. Lett.* **2000**, 1250.
(14) (a) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574. (b) Lee, E.; Yoon, C. H. *J. Chem. Soc., Chem. Commun.* **1994**, 479.
(15) Wustrow, D. J.; Smith, W. J.; Wise, L. D. *Tetrahedron Lett.* **1994**, *35*, 61.

Scheme 8. Construction of the Diastereomeric C_1-C_{27} Advanced Intermediates **116** and **117**[a]



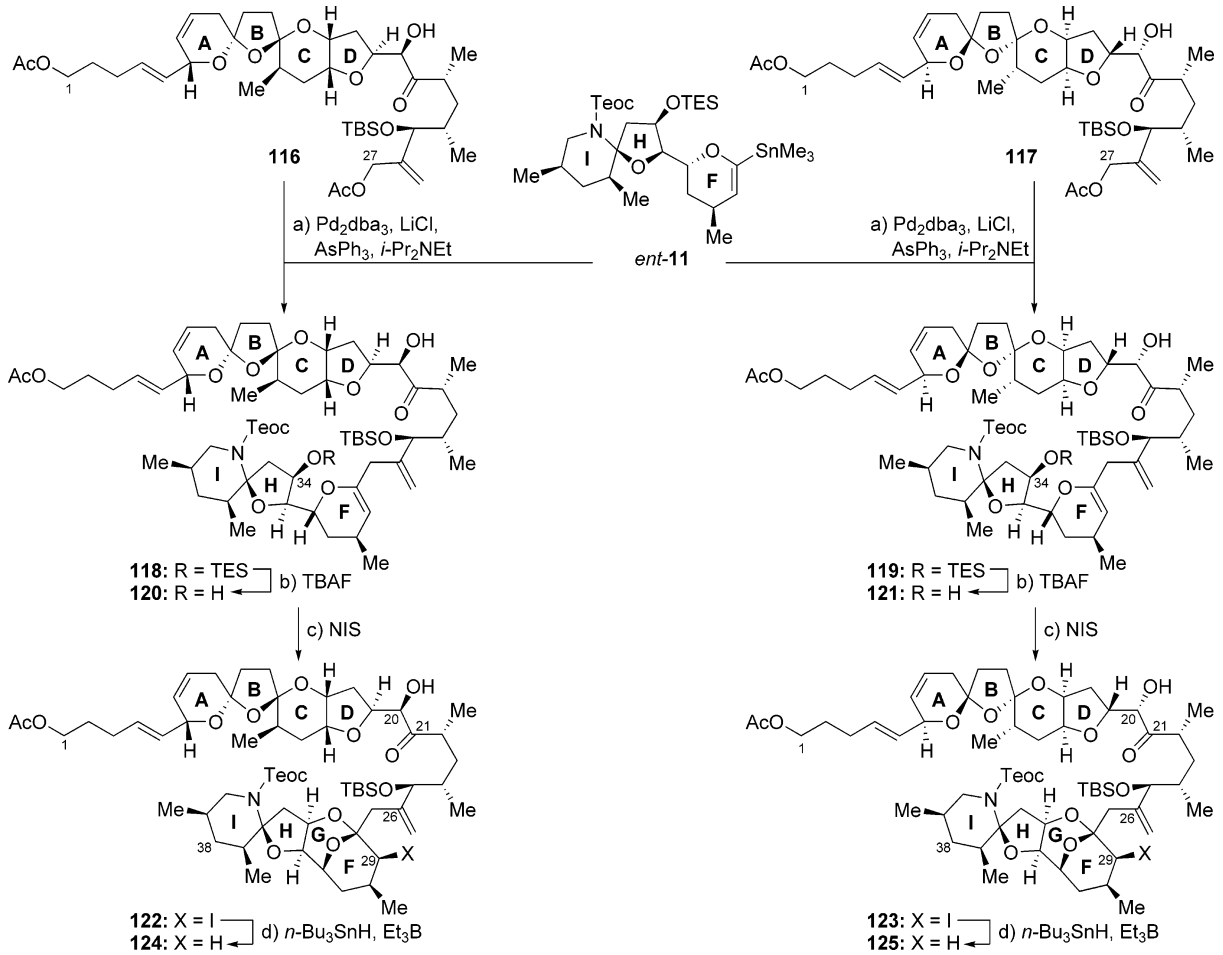
[a] Reagents and conditions: (a) TBAF (1.0 M in THF, 2.0 equiv), THF, 25 °C, 2 h, 98% for **97** and 98% for **98**; (b) $(COCl)_2$ (5.0 equiv), DMSO (11 equiv), CH_2Cl_2, −78 °C, 30 min, then Et_3N (22 equiv), −78 → 0 °C; (c) $NaClO_2$ (6.0 equiv), NaH_2PO_4 (6.0 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 1 h, 90% for **101** and 90% for **102**; (d) PFPOH (1.5 equiv), DCC (2.0 equiv), 25 °C, 2.0 h, 80% for **103** and 82% for **104**; (e) **105** (7.0 equiv), *n*-BuLi−*n*-Bu_2Mg (1.1 M in hexanes, 4.7 equiv), THF, 0 → 25 °C, 1.5 h, then −90 °C, then **103** and **104**, 30 min, 50% for **106**, 48% for **107**; (f) DIBAL-H (1.0 M in CH_2Cl_2, 10 equiv), CH_2Cl_2, −90 °C, 1 h; (g) TBAF (1.0 M in THF, 2.0 equiv), THF, 25 °C, 8 h, 44% for **110**, 39% for **111** over the two steps; (h) AcCl (20 equiv), 2,4,6-collidine (40 equiv), CH_2Cl_2, −78 °C, 6 h, 90% for **112**, 87% for **113**; (i) TBSOTf (10 equiv), 2,6-lutidine (20 equiv), CH_2Cl_2, −78 → 0 °C, 30 min, 86% for **114**, 85% for **115**; (j) $PhI(OCOCF_3)_2$ (2.0 equiv), MeCN:pH 7 buffer (4:1), 0 °C, 68% for **116**, 65% for **117**. Abbreviations: PFPOH, pentafluorophenol; DCC, dicyclohexylcarbodiimide.

50% yield with a ratio of **85**:**94** of ca. 3:1. Reduction of this acetate (**93**) with Pd_2dba_3·$CHCl_3$, *n*-Bu_3P, and $NaBH_4$ (entry 10)[7] gave 40% yield of deoxygenation products in a somewhat less favorable ratio (ca. 2:1, in favor of **85**). Finally, it was found that switching to the methyl carbonate derivative of the allylic alcohol as the reduction substrate and employing Pd_2dba_3·$CHCl_3$ (0.125 equiv), *n*-Bu_3P (0.48 equiv), and $LiBH_4$ (10 equiv) in DME provided, in 82% yield and a 7:1 ratio, the desired $\Delta^{7,8}$ product (**85**) with its $\Delta^{8,9}$ isomer (**94**). Chromatographic separation yielded pure **85**.

With the deoxygenation hurdle satisfactorily overcome, the now readily accessible diolefin **85** was converted to the targeted ABCD fragment **40** by the following sequence and in 71% overall yield: (i) DIBAL-H-induced cleavage of the pivaloate ester; (ii) Swern oxidation to the aldehyde **87** [$(COCl)_2$, DMSO; Et_3N]; (iii) $NaClO_2$ oxidation to the carboxylic acid **88**; (iv) methyl ester formation with CH_2N_2; and (v) TBAF deprotection (Scheme 7). Delightfully, the ^1H NMR spectrum of synthetic **40** was identical with that of the sample obtained from natural azaspiracid-1 by degradation (by Satake et al.).[2] Unfortunately, no rotation of the naturally derived substance corresponding to **40** was available due to the insufficient amounts obtained, so

(16) Bianco, A.; Passacantilli, P.; Righi, G. *Tetrahedron Lett.* **1989**, *30*, 1405.

Scheme 9. Advancement to Octacyclic ABCD−FGHI Systems **124** and **125**[a]



[a] Reagents and conditions: (a) **116** and **117**, Pd_2dba_3 (0.3 equiv), $AsPh_3$ (0.3 equiv), LiCl (6.0 equiv), *i*-Pr_2NEt (12 equiv), then *ent*-**11** (3.0 equiv, 0.03 M in THF, syringe pump addition), NMP, 40 °C, 1 h, 55% for **118**, 55% for **119**; (b) TBAF (1.0 M in THF, 1.2 equiv), THF, 0 °C, 20 min, 80% for **120**, 81% for **121**; (c) NIS (2.0 equiv), $NaHCO_3$ (10 equiv), THF, 0 °C, 12 h, 62% for **122**, 60% for **123**; (d) Et_3B (1.0 M in hexanes, 0.2 equiv), *n*-Bu_3SnH: toluene (1:2), 0 °C, 5 min, 86% for **124**, 85% for **125**. Abbreviations: NIS, *N*-iodosuccinimide; NMP, *N*-methylpyrrolidone.

we were left with yet another unknown to be determined by chemical synthesis, namely the absolute stereochemistry of the ABCD system. The only way to do that was to synthesize both enantiomers and combine them with the EFGHI domain whose relative and absolute stereochemistry was by then well known to us.

3. Coupling of Key Building Blocks and Completion of the Synthesis. Having secured the ABCD (both enantiomers, **85** and **96**), E (**105**), and FHI (*ent*-**11**) key building blocks, we proceeded to join them together by the appropriate sequence as already developed,[2] thus hoping to arrive at azaspiracid-1 (and its C_1-C_{20} diastereomer **2**). These endeavors are summarized in Schemes 8−10. Scheme 8 depicts the conversion of enantiomeric ABCD (C_1-C_{20}) fragments **85** and **96** to the diastereomeric C_1-C_{27} advanced intermediates **116** and **117**, respectively, by attachment of the $C_{21}-C_{27}$ segment (**105**). It should be noted that, as it happened, it was the wrong enantiomer (i.e., **96**) that reached the end first, even though the two sequences are presented in these schemes in parallel and with details only for the sequence leading from the correct enantiomer (i.e., **85**) to azaspiracid-1. Thus, TBAF-induced desilylation of **85** led to primary alcohol **97** (98% yield), whose sequential Swern [$(COCl)_2$, DMSO; Et_3N] and $NaClO_2$ oxidations furnished carboxylic acid **101** (90% yield over the two steps). Coupling of **101** with pentafluorophenol (PFPOH) in the presence of DCC then generated activated ester **103** in 80% yield. The addition of the stabilized dithiane anion derived from **105** and *n*-BuLi−*n*-Bu_2Mg afforded ketone **106** (50% yield), whose diastereoselective reduction with DIBAL-H in CH_2Cl_2 at −90 °C produced alcohol **108**. Removal of the silicon group from the latter compound (**108**) by the action of TBAF led to tetraol **110** in 44% yield for the two steps. The stereochemistry at C-20 was assumed to be as shown, by analogy to our previous results.[17] It should be noted, however, that the degradation studies erased this stereochemistry (C-20), so, other than the NMR spectroscopic evidence in the original report of the azaspiracid-1 structure, we did not have any further support for the depicted configuration at this stage, nor were we absolutely sure of its true configuration in the natural product. In any event, we could take some comfort in the fact that we could invert this (C-20) stereocenter, if needed, as we had already demonstrated with the originally proposed structures.[2]

From the two previously developed routes to the final azaspiracid-like structures,[2] we chose to follow the one involving a TBS (rather than an acetate) protecting group for the C-25

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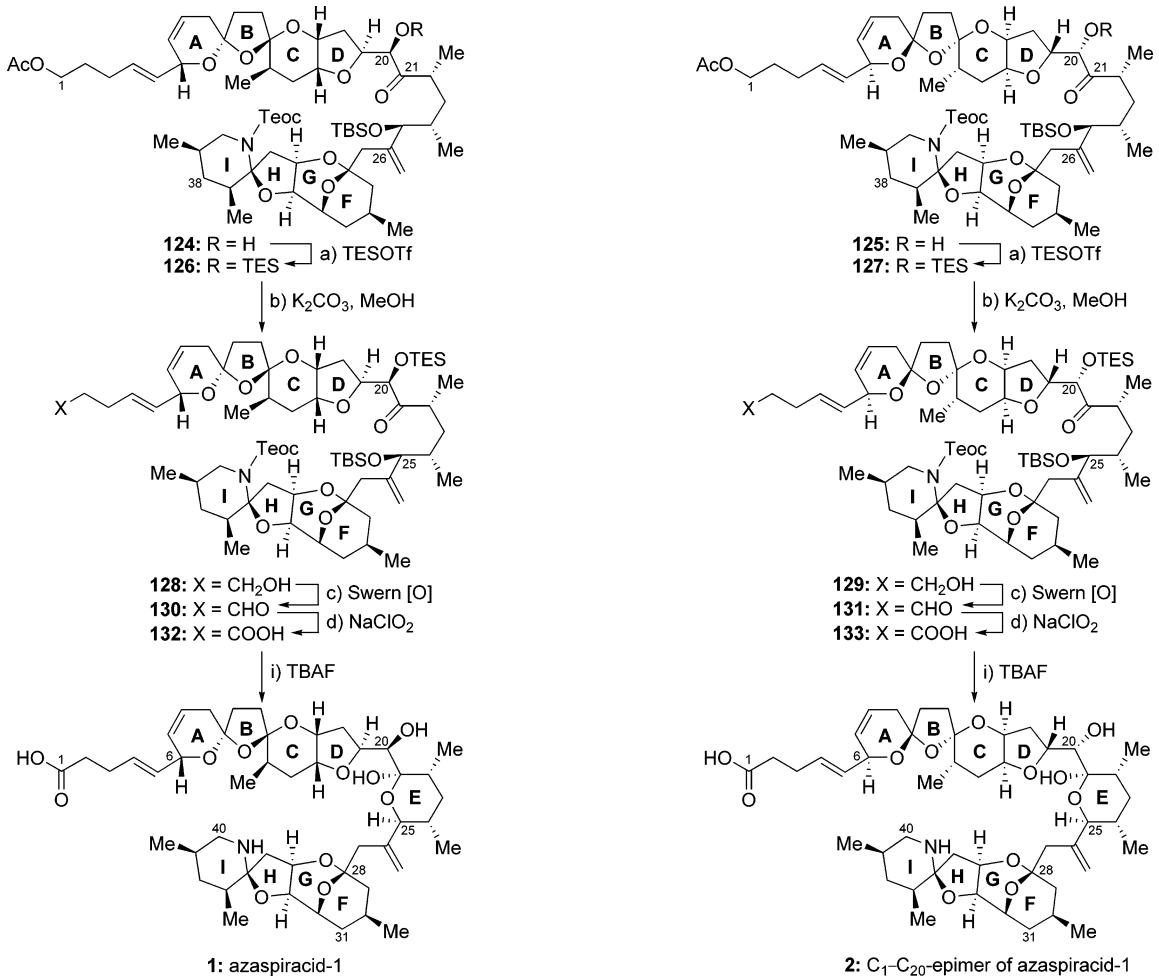
Scheme 10. Total Synthesis of Azaspiracid-1 (**1**) and Its C_1-C_{20} Epimer (**2**)[a]



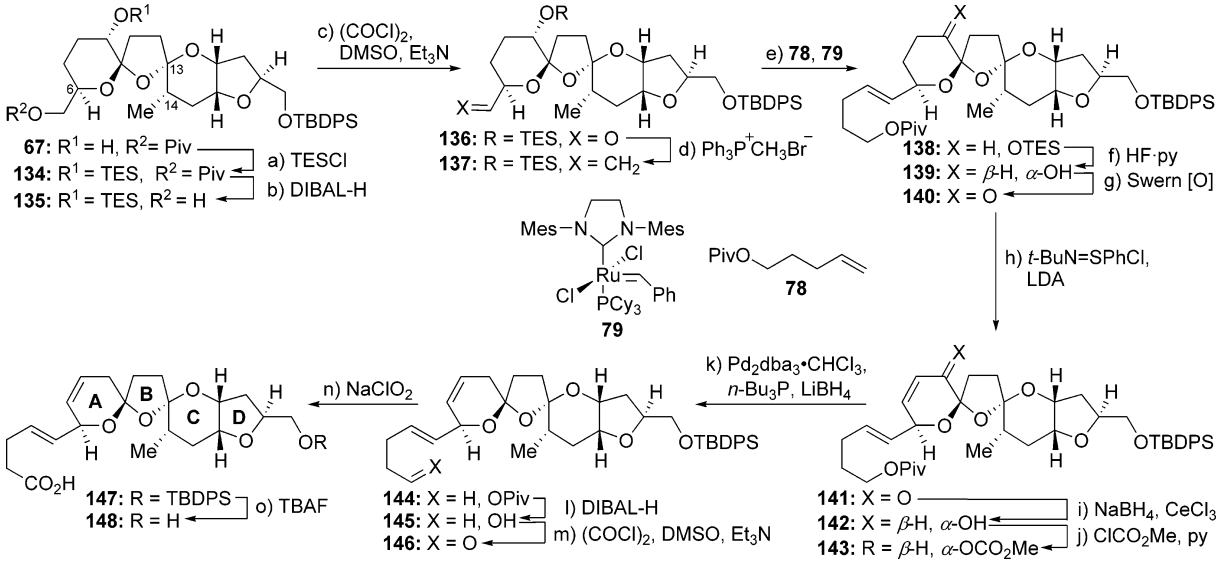
[a] Reagents and conditions: (a) TESOTf (10 equiv), 2,6-lutidine (20 equiv), CH_2Cl_2, −78 → 0 °C, 10 min, 91% for **126** and 90% for **127**; (b) K_2CO_3 (1.0 equiv), MeOH, 25 °C, 2 h, 81% for **128** and 84% for **129**; (c) $(COCl)_2$ (10 equiv), DMSO (20 equiv), CH_2Cl_2, −78 °C, 1 h, then Et_3N (50 equiv), −78 → −20 °C, 30 min; (d) $NaClO_2$ (10 equiv), NaH_2PO_4 (10 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 30 min, 70% for **132** and 65% for **133** over the two steps; (e) TBAF (5.0 equiv), THF, 25 °C, 15 h, 60% for **1** and 55% for **2**. Teoc, 2-(trimethylsilyl)ethoxycarbonyl.

hydroxyl group due to the advantage it bestowed on the molecule for a global deprotection step at the end. Thus, the tetraol **110** (Scheme 8) was sequentially and selectively converted to allylic acetate **116** via intermediates **112** and **114** by (i) reaction with AcCl in CH_2Cl_2 at −78 °C in the presence of 2,4,6-collidine (90% yield), (ii) silylation of the resulting compound with TBSOTf in CH_2Cl_2 in the presence of 2,6-lutidine (−78 → 0 °C, 86% yield), and (iii) dithiane removal with $PhI(OCOCF_3)_2$ (68% yield). By the same route, and in similar yields, enantiomer **96** was transformed to diastereomer **117** through intermediates **98**, **100**, **102**, **104**, **107**, **109**, **111**, **113**, and **115** (Scheme 8).

The anticipated Stille coupling of the so-obtained allylic acetates **116** and **117** was carried out with the single enantiomer of the FHI stannane *ent*-**11**, and the coupling products were advanced to the octacyclic ABCD−FGHI ring systems **124** and **125** as shown in Scheme 9. Thus, and as previously developed,[2] addition of stannane *ent*-**11** (3.0 equiv in THF, syringe pump) to a heated (40 °C) mixture of allylic acetate **116**, Pd_2dba_3, LiCl, and *i*-Pr_2NEt furnished compound **118** (55% yield), containing all carbon atoms needed for the azaspiracid-1 structure. Selective removal of the TES group from **118** with controlled amounts of TBAF at 0 °C then provided dihydroxy compound **120** (80% yield), setting the stage for the pending intramolecular iodoetherification, a process that was brought about by NIS in THF at 0 °C in the presence of $NaHCO_3$, furnishing iodoether **122** (62% yield). With the G ring closed, the next task was to remove the superfluous iodide residue from **122**, an operation that was successfully carried out with *n*-Bu_3SnH in toluene (ca. 1:2) in the presence of catalytic amounts of Et_3B as the radical initiator. The desired product, octacyclic system **124**, was obtained from this operation in 86% yield. The same sequence was followed (in similar yields) to provide diastereomeric compound **125** from **117** through intermediates **119**, **121**, and **123** (Scheme 9).

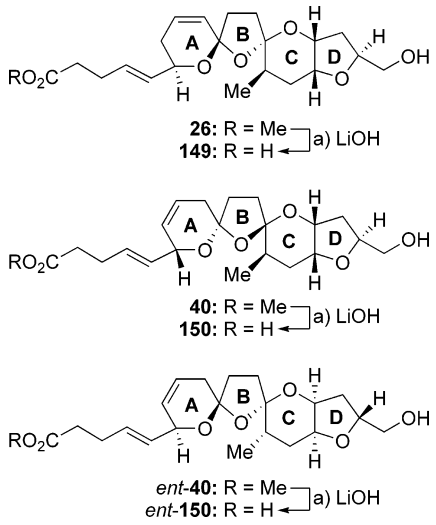
Scheme 10 summarizes the final stages of the drive toward azaspiracid-1 and its ABCD diastereomer. Thus, the C-20 hydroxyl group of the advanced intermediate **124** was silylated with TESOTf and 2,6-lutidine to afford the TES ether **126** (91% yield), from which the acetate group at C-1 was removed by treatment with K_2CO_3 in MeOH, furnishing primary alcohol **128** (81% yield). This compound was then taken to carboxylic acid **132** via aldehyde **130** by sequential oxidation, first under Swern conditions [$(COCl)_2$, DMSO; Et_3N] and then with $NaClO_2$, in 70% overall yield for the two steps. In principle, only one operation stood between compound **132** and the targeted azaspiracid-1 structure, that of global deprotection and

Scheme 11. Synthesis of Truncated ABCD Carboxylic Acid **148** for Biological Testing[a]



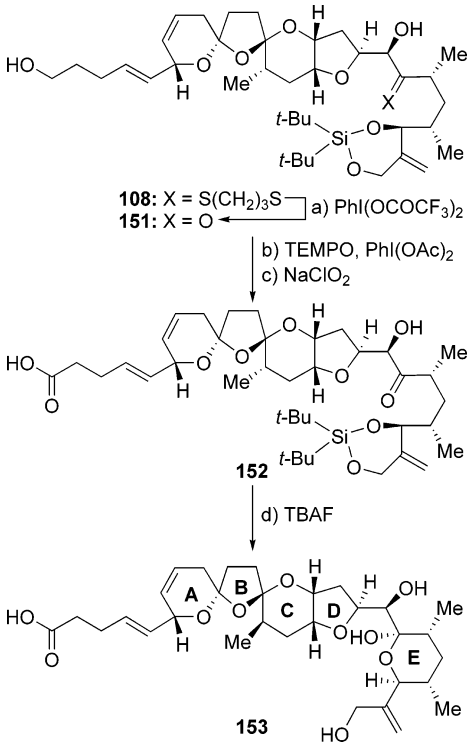
[a] Reagents and conditions: (a) TESCl (3.0 equiv), imidazole (5.0 equiv), CH_2Cl_2, 0 °C, 30 min, 97%; (b) DIBAL-H (1.0 M in CH_2Cl_2, 3.0 equiv), CH_2Cl_2, −78 °C, 1 h, 96%; (c) $(COCl)_2$ (5.0 equiv), DMSO (10 equiv), CH_2Cl_2, −78 °C, 1.5 h, then Et_3N (22 equiv), −78 → 0 °C, 1 h, 91%; (d) $Ph_3PCH_3^+Br^-$ (6.0 equiv), *n*-BuLi (1.6 M in THF, 5.0 equiv), THF, −78 → 0 °C, 1 h, then **136**, −78 → 0 °C, 2 h, 84%; (e) **79** (0.1 equiv), **78** (3.0 equiv), CH_2Cl_2, 40 °C, 12 h, 55% (45% recovered **137**); (f) HF·py (3.0 equiv), THF:py (1:1), 0 °C, 2 h, 86%; (g) $(COCl)_2$ (5.0 equiv), DMSO (10 equiv), CH_2Cl_2, −78 °C, 1.5 h, then Et_3N (22 equiv), −78 → 0 °C, 1 h, 88%; (h) LDA (1.5 equiv), *t*-BuN═SPhCl (1.6 equiv), THF, −78 °C, 40 min, 71%; (i) $NaBH_4$ (3.1 equiv), $CeCl_3·7H_2O$ (1.0 equiv), MeOH, −65 °C, 40 min, 82%; (j) $ClCO_2Me$ (25 equiv), 4-DMAP (1.0 equiv), CH_2Cl_2:py (2:1), −10 °C, 3 h, 82%; (k) $Pd_2dba_3·CHCl_3$ (0.125 equiv), *n*-Bu_3P (0.48 equiv), $LiBH_4$ (10 equiv), DME, 0 °C, 1 h, 78%, $\Delta^{7,8}$:$\Delta^{8,9}$ = (6:1); (l) DIBAL-H (1.0 M in CH_2Cl_2, 2.0 equiv), CH_2Cl_2, −78 °C, 15 min, 72%; (m) $(COCl)_2$ (5.0 equiv), DMSO (10 equiv), CH_2Cl_2, −78 °C, 1.5 h, then Et_3N (22 equiv), −78 → 0 °C, 1 h, 80%; (n) $NaClO_2$ (10 equiv), NaH_2PO_4 (10 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 1 h, 82%; (o) TBAF (1.0 M in THF, 2.0 equiv), THF, 25 °C, 12 h, 67%.

Scheme 12. Synthesis of Truncated Azaspiracid ABCD Analogues **149**, **150**, and *ent*-**150**[a]



[a] Reagents and conditions: (a) LiOH, MeOH:THF:H_2O (4:1:1), 25 °C, 78% for **149**, 82% for **150**, 83% for *ent*-**150**.

Scheme 13. Synthesis of Truncated ABCDE Azaspiracid-1 **152**[a]



[a] Reagents and conditions: (a) $PhI(OCOCF_3)_2$ (1.5 equiv), MeCN:pH 7 buffer (4:1), 0 °C, 10 min, 68%; (b) TEMPO (0.2 equiv), $PhI(OAc)_2$ (3.0 equiv), CH_2Cl_2, 25 °C, 7 h; (c) $NaClO_2$ (5.0 equiv), NaH_2PO_4 (5.0 equiv), 2-methyl-2-butene (10 equiv), *t*-BuOH:H_2O (4:1), 25 °C, 2 h, 65% over the two steps; (d) TBAF (1.0 M in THF), THF, 25 °C, 12 h, 72%. TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl.

spontaneous casting of the missing ring E. Indeed, exposure of **132** to TBAF in THF at ambient temperature, followed by preparative thin-layer chromatography, gave a single compound, in 60% yield, whose ^1H NMR spectrum matched that of the naturally derived azaspiracid-1 (**1**). All physical data collected for synthetic **1** (R_f, $[\alpha]^{25}_D$, IR, ^1H and ^{13}C NMR, and MS) were in agreement with those reported for the natural product. The C_1−C_{20}-*epi*-azaspiracid-1 (**2**) was synthesized from **125** by the same route and in similar yields through intermediates **127**, **129**, **131**, and **133**, as shown in Scheme 10.

4. Synthesis of Truncated Azaspiracids for Biological Investigations. Cognizant of the importance of biological studies in this area and as part of our azaspiracid program, we synthesized, in addition to azaspiracid-1 (**1**) and its ABCD dia-

stereomer (**2**), a number of truncated azaspiracids. Schemes 11−13 summarize these constructions starting from intermediates already encountered in our path. Thus, the truncated azaspiracid-1 **148** (pertaining to the third proposed structure of the ABCD domain of azaspiracid-1) was constructed from intermediate **67** (for preparation of **67**, see Schemes 5 and 6) by the developed chemistry as summarized in Scheme 11. Similarly, the truncated azaspiracids **149**, **150**, and *ent*-**150**, containing only the ABCD domain of azaspiracid-1, were prepared from their corresponding methyl esters through the action of LiOH as shown in Scheme 12. Finally, the ABCDE truncated azaspiracid-1 **152** was synthesized from **108** through a three-step sequence as shown in Scheme 13. Thus, oxidative removal of the dithiane of **108** with $PhI(OCOCF_3)_2$ to the corresponding ketone (68% yield), followed by selective oxidation of the primary alcohol of **151** with TEMPO and $PhI(OAc)_2$[18] to the corresponding aldehyde, and $NaClO_2$ oxidation provided carboxylic acid **152** (65% yield for the two steps), whose TBAF deprotection furnished the coveted structure **153** in 72% yield. The biological properties of these truncated azaspiracid analogues as well as of synthetic **1**, ABCD diastereomer **2**, and truncated isomers **5**, **6**, **23**, and **24** will be reported elsewhere.[19]

Conclusion

The initial phase of the campaign to synthesize the notorious neurotoxin found in mussels, azaspiracid-1, led to the realization that the originally reported structure of this natural product was in error. This recognition set in motion a second wave of investigations directed at determining the true structure of azaspiracid-1, an objective that was considerably aided by degradative studies of the minute amounts of the natural substance available as carried out by the Satake group. These studies, together with our synthetic work, identified the correct structures of all three domains of the natural product corresponding to rings ABCD, FGHI, and E, including relative and absolute stereochemistry, except for that of the ABCD domain, whose absolute structure remained elusive due to insufficient amounts of the naturally derived intermediate for optical rotation measurements. That final structural determination had to await the total synthesis of the two diastereomeric structures proposed for the natural product, an accomplishment that, indeed, defined the true structure of azaspiracid-1 as **1**. Like several other total syntheses, this endeavor demonstrated once again the continued role of chemical synthesis in structural elucidation of natural products[20] and its indispensable nature as a source of scarce, but highly valuable, substances for biological investigations.

Acknowledgment. We thank Drs. D. H. Huang, G. Siuzdak, and R. Chadha for NMR spectroscopic, mass spectrometric, and X-ray crystallographic assistance, respectively. Financial support for this work was provided by The Skaggs Institute for Chemical Biology, the National Institutes of Health (USA), predoctoral fellowships from the National Science Foundation (to M.O.F.) and Eli Lilly (to Y.L.), postdoctoral fellowships from the Ernst Schering AG Foundation (to S.V.) and the Uehara Memorial Foundation (to Y.M.A.Y.), and grants from Abbott, Amgen, ArrayBiopharma, Boehringer-Ingelheim, Glaxo Smith Kline, Hoffmann-LaRoche, DuPont, Merck, Novartis, Pfizer, and Schering Plough.

Supporting Information Available: Experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

JA054750Q

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