Structural Revision and Total Synthesis of Azaspiracid-1, Part 1: Intelligence Gathering and Tentative Proposal

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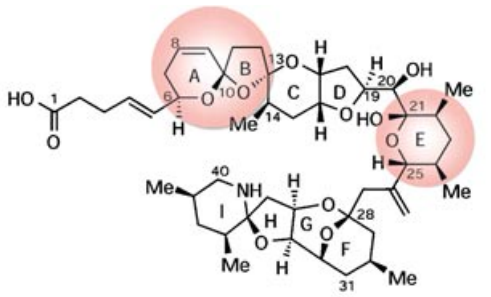
Structural Revision and Total Synthesis of Azaspiracid-1, Part 1: Intelligence Gathering and Tentative Proposal**

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

In memory of D. John Faulkner

Structure **1-i** (Figure 1, absolute stereochemistry and relative stereochemistry between ABCDE and FGHI domains unknown) was proposed in 1998, on the basis of NMR spectroscopic analysis, for a poisonous substance called azaspiracid-1, which was isolated from the mussel family *Mytilus edulis*.[1] A recent total synthesis[2,3] of **1-i** and its FGHI epimer proved that this assignment was incorrect.[4] Herein we report synthetic and degradation studies that led to the revised structure **1-c** or ABCD-*epi*-**1-c** as the most likely depiction of the molecular architecture of this biotoxin (Figure 1). The only doubt that remained was the absolute configuration of the ABCD domain of the molecule, and this question was resolved by total synthesis, as is described in the following paper in this issue.[5]

Upon realizing that the originally proposed structure **1-i** of azaspiracid-1 was in error,[2,3] our plan for determining its true structure called for the degradation of the natural material into smaller fragments, whose chemical synthesis may narrow down the location of the structural discrepancy between the synthesized (originally proposed) and actual



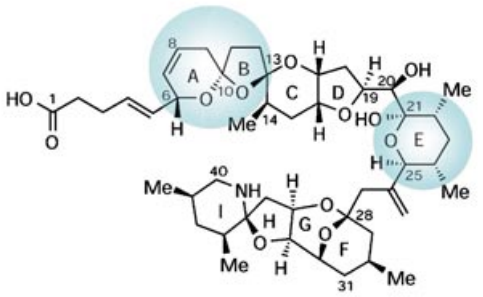


Figure 1. Originally proposed structure **1-i** (relative stereochemistry between ABCDE (C1–C27) and FGHI (C28–C40) domains and absolute stereochemistry unknown) and newly proposed structure **1-c** or ABCD-*epi*-**1-c** of azaspiracid-1.

structures. To this end, azaspiracid-1 was derivatized by exposure to $TMSCHN_2$ in MeOH, and the methyl ester obtained was treated with $NaIO_4$ in aqueous MeOH at ambient temperature, resulting in the cleavage of its C20–C21 bond and in the formation of the corresponding aldehyde **3** (C1–C20 fragment) and lactone **4** (C21–C40 fragment) in quantitative yield (Scheme 1). Compound **3** was further elaborated by treatment with $NaBH_4$ to afford, supposedly, hydroxy methyl ester **5** (≈90% yield), followed by hydrogenation to give the fully saturated compound **6** (≈90% yield). On the other hand, cleavage of the exocyclic olefinic bond within **4** with O_3–Me_2S to the corresponding ketone, followed by saponification of the E-ring lactone with aqueous NaOH in MeOH and esterification of the resulting carboxylic acid with $TMSCHN_2$ gave methyl ester **7** (≈90% yield). Finally, treatment of compound **7** with $NaIO_4$ furnished amino acid **8** in high yield (≈90%). These degradatively derived intermediates (i.e. **4**–**6** and **8**, Scheme 1) were to serve several purposes. First, spectroscopic comparisons with synthetic materials would immediately pinpoint any errors in each domain of the molecule. Second, comparison of amino lactone **4** with the two synthetic FGHI epimers of the same structure (i.e. **4**) would clarify the relative stereochemistry between the ABCDE and FGHI domains of azaspiracid-1. Third, optical rotation comparisons of the degradative and synthetic samples may reveal the absolute configuration of each fragment and, consequently, of the entire structure.

One drawback of the adopted degradation scheme was the loss of stereochemical information regarding the C20 and

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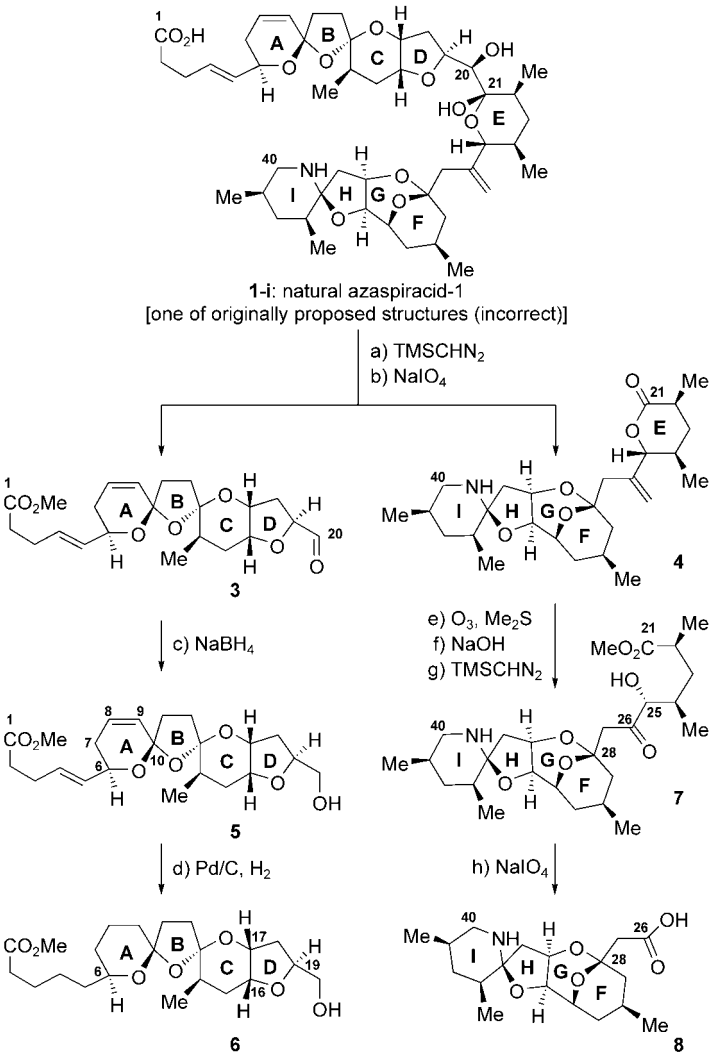


Scheme 1. Chemical degradation and derivatization of azaspiracid-1 (**1-i**: originally proposed structure) to C1–C20 alcohol **6**, C21–C40 lactone **4**, and C26–C40 carboxylic acid **8**. Reagents and conditions: a) $TMSCHN_2$, MeOH, 25 °C, 1 h; b) $NaIO_4$, MeOH/H_2O (4:1), 25 °C, 1 h, ≈100% over two steps; c) $NaBH_4$, MeOH, 25 °C, ≈90%; d) Pd/C (5%), H_2, MeOH, 25 °C, ≈90%; e) O_3, Me_2S, MeOH, −78 °C; f) NaOH (0.1 N), MeOH/H_2O (4:1), 25 °C, 2 h; g) $TMSCHN_2$, MeOH, 25 °C, 15 min, ≈90% over three steps; h) $NaIO_4$, MeOH/H_2O (4:1), 25 °C, 30 min, ≈90%. TMS = trimethylsilyl.

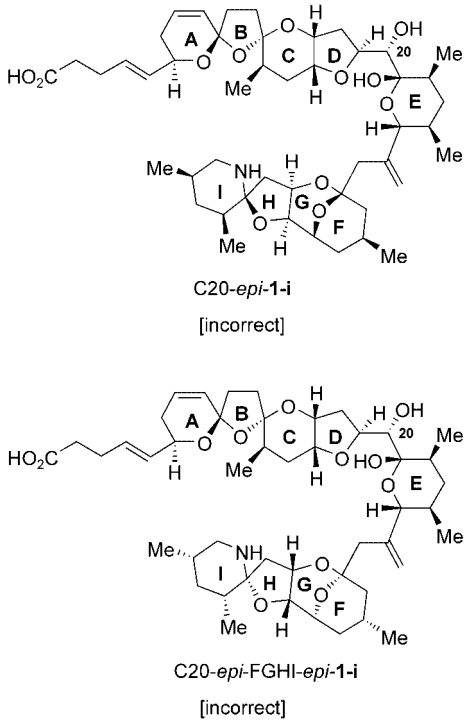
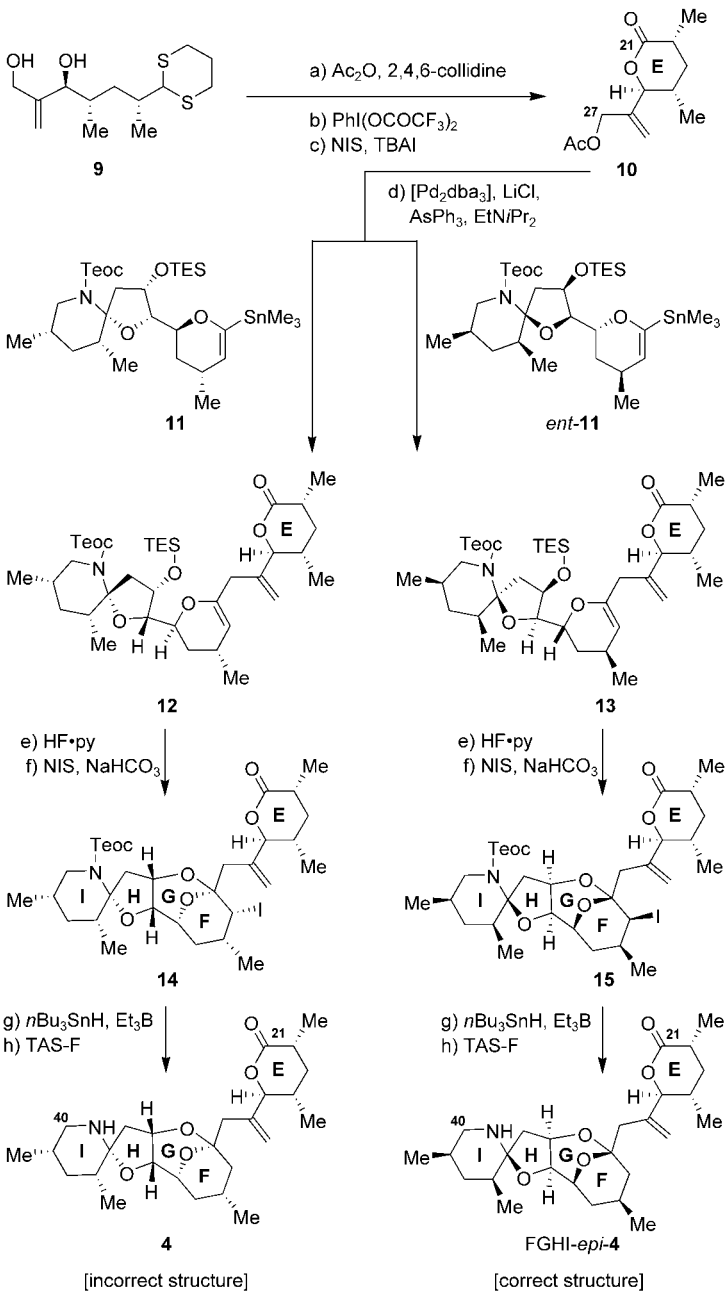


Figure 2. Structures of C20-*epi*-**1-i** and C20-FGHI-*epi*-**1-i** (synthesized and proven incorrect).

C21 hydroxy-bearing centers. However, if we assume that the C21 hemiketal functionality would rest in its thermodynamically most stable conformation, the only stereocenter in question would be that at C20. As **1-i** had already been eliminated as the true structure, the C20-*epi*-azaspiracid-1 (C20-*epi*-**1-i**) structure and its FGHI epimer (C20-*epi*-FGHI-*epi*-**1-i**) as logical targets moved to the front as possible alternatives (see Figure 2). Their total syntheses along the same lines as those described for **1-i**,[2,3] however, also proved them to be incorrect.[6]

Having established that the azaspiracid-1 structural problem did not reside exclusively at the C20 stereocenter, the focus of our investigation shifted next to the synthesis of the two diastereomeric EFGHI amino lactones **4** and FGHI-*epi*-**4** (Scheme 2). Thus, the previously synthesized dihydroxy dithiane **9**[2] was selectively monoacetylated (at the primary position) with Ac_2O in the presence of 2,4,6-collidine (85% yield), and the resulting hydroxy dithiane intermediate was deprotected by exposure to $PhI(OCOCF_3)_2$[7] to afford the corresponding lactol, whose oxidation with NIS–TBAI[8] completed the synthesis of the required allylic acetate lactone **10**. The latter compound, **10**, underwent Stille coupling with stannanes **11** and *ent*-**11** according to a previously described procedure[3] to afford compounds **12** and **13**, respectively. These substrates were then smoothly transformed into the targeted amino lactones **4** and FGHI-*epi*-**4**, respectively, via intermediate compounds **14** (for **4**) and **15** (for FGHI-*epi*-**4**) according to our previous sequence[3] and as summarized in Scheme 2: 1) HF·py-induced selective TES removal; 2) NIS-initiated ring closure; 3) nBu_3SnH–Et_3B-facilitated reductive deiodination; and 4) TAS-F-mediated desilylation. The only deviation from the previously employed sequence[3] was the use of TAS-F[9] instead of TBAF, as the former reagent afforded superior results in the final deprotection step. Most gratifyingly, the ^1H NMR spectral data of one of these synthetic materials, namely FGHI-*epi*-**4**, matched those of the sample derived from natural azaspiracid-1 (Scheme 1), whereas its diastereomeric amino lactone **4** exhibited slightly different and diagnostically distinct signals to those of the naturally derived substance. Based on these results, we concluded that the relative stereochemical arrangement for the EFGHI domain of azaspiracid-1 is that depicted by structure FGHI-*epi*-**4** (absolute stereochemistry unknown).[10]

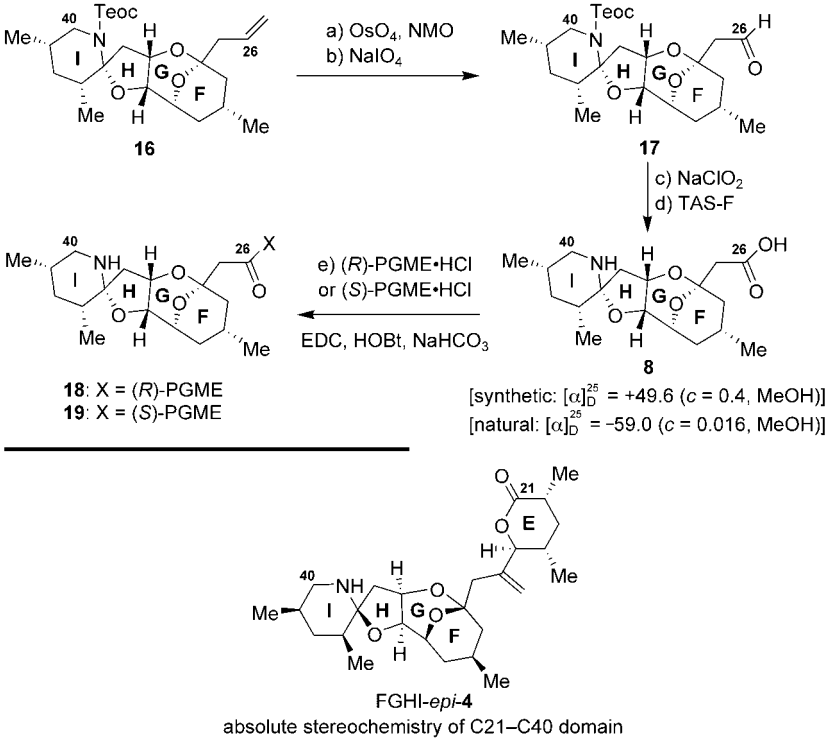
The next goal was to establish the absolute stereochemistry of azaspiracid-1, and as we had confirmed the structural



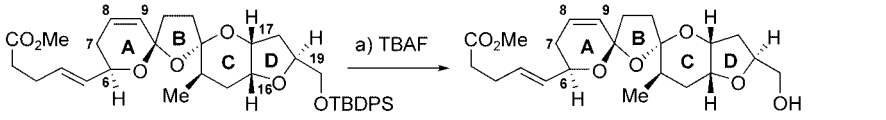
Scheme 2. Chemical synthesis of C21–C40 lactone **4** and its FGHI epimers, FGHI-*epi*-**4** and determination of the relative stereochemistry within the EFGHI domain. Reagents and conditions: a) Ac_2O (5.0 equiv), 2,4,6-collidine (10.0 equiv), CH_2Cl_2, 25 °C, 16 h, 85 %; b) $PhI(OCOCF_3)_2$ (1.5 equiv), MeCN/pH 7 buffer (4:1), 0 °C, 10 min, 81 %; c) NIS (10.0 equiv), TBAI (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), $EtNiPr_2$ (12 equiv); then **11** or *ent*-**11** (0.03 M in THF, syringe pump addition), NMP, 45 °C, 4 h; e) HF·py (excess), THF/py (1:1), 0→25 °C, 2 h; f) NIS (10.0 equiv), $NaHCO_3$ (30 equiv), THF, 0 °C, 16 h, 26 % for **14** and 38 % for **15** over three steps; g) Et_3B (1.0 M in hexanes, 0.2 equiv), nBu_3SnH/toluene (1:2), 0 °C, 5 min; h) TAS-F (5.0 equiv), DMF, 0 °C, 20 h, 70 % for **4** and 74 % for FGHI-*epi*-**4** over two steps. NIS = *N*-iodosuccinimide, NMP = *N*-methylpyrrolidinone, TBAI = tetra-*n*-butylammonium iodide, dba = dibenzylideneacetone, TAS-F = tris(dimethylamino)sulfur (trimethylsilyl)difluoride.

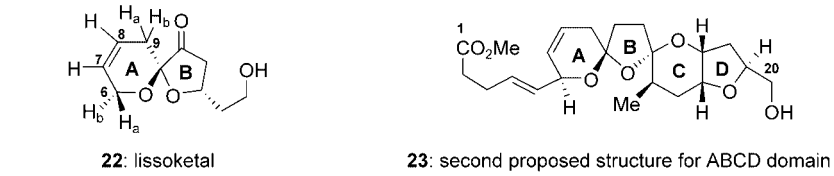
integrity of the FGHI domain **8** of the molecule and had secured its optical rotation from degradation studies (see Scheme 1), we decided to synthesize the carboxylic acid **8** for comparison purposes. This objective was met expeditiously, starting with the previously prepared intermediate **16**,[2] as shown in Scheme 3. Thus, a two-step oxidative protocol involving dihydroxylation (NMO–OsO_4 cat.) of the terminal olefin of **16**, followed by $NaIO_4$-induced cleavage of the resulting 1,2-diol, provided aldehyde **17** in 80 % overall yield. Oxidation of this aldehyde (**17**) with $NaClO_2$, followed by TAS-F-mediated desilylation resulted in the formation of the desired amino acid **8** in 35 % yield over the two steps. As expected, the NMR spectroscopic data of synthetic **8** matched those of the degradatively obtained material. Furthermore, the two samples exhibited comparable rotations, but with opposite signs (synthetic: $[\alpha]_D^{25} = +49.6$ ($c = 0.4$, MeOH); natural: $[\alpha]_D^{25} = -59.0$ ($c = 0.016$, MeOH)). Furthermore, the ^{1}H NMR spectrum of the (*R*)-PGME (phenyl glycine methyl ester) derivative **18** (see Scheme 3 for preparation of the PGME derivatives) of synthetic carboxylic acid **8** was identical to the (*S*)-PGME derivative of the naturally derived acid **8** (and (*S*)-PGME derivative **19** derived from synthetic **8** was identical to the (*R*)-PGME derivative of the naturally derived **8**), thereby establishing the absolute stereochemistry of the FGHI domain of azaspiracid-1 as the antipode of compound **8** that we had prepared as shown in Scheme 3. By virtue of the chemistry depicted in Scheme 2, the absolute stereochemistry of the entire EFGHI domain of azaspiracid-1 could now be shown as that in Scheme 3 (structure FGHI-*epi*-**4**).

With the establishment of both the relative and absolute stereochemistries of the EFGHI domain of azaspiracid-1, our attention was then turned to the remaining segment, namely the ABCD (C1–C20) framework of the natural product, in which we had, by now, suspected the structural discrepancy to be embedded. As outlined in Scheme 4, a synthetic sample of **21**, a substance corresponding to the degradatively derived compound from the natural product (structure **5**, Scheme 1) was prepared from the previously synthesized intermediate **20**[2] by desilylation (TBAF, 88 % yield). The spectroscopic data for these two samples (**5** and **21**) differed significantly, thereby confirming our suspicions for the location of the structural discrepancy (see Table 1). Furthermore, the main differences between the two sets of spectra were associated with protons located on ring A, namely 6-H, 7-H, 8-H, and 9-H (see numbering on structure **21**, Scheme 4). Despite these clear differences, the final structural deconvolution of this region of azaspiracid-1 remained elusive, primarily as a result of the very similar 2D NMR spectral data for the naturally derived and synthetic materials whose comparison implied only subtle connectivity and spatial differences. Finally, it was a discovery by C. Hopmann and the late Professor D. John Faulkner that shed light on this issue. Indeed, the structural elucidation of lissoketal (**22**, see Scheme 4),[11] a marine natural product isolated by these workers in 1997, provided the first clue to the azaspiracid-1 puzzle. This secondary metabolite **22** bears a close resemblance to the proposed structure of azaspiracid-1 (**1-i**) except for the fact that the endocyclic double bond in ring A resides between C7 and C8, rather than between C8 and C9. Intriguingly, close examination of the ^{1}H NMR chemical shift values (see Table 1) and the 2D NMR correlation pattern reported for lissoketal (**22**)



Scheme 3. Synthesis of FGHI amino acid **8** and determination of the absolute stereochemistry of EFGHI domain of azaspiracid-1. 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) $NaClO_2$ (10.0 equiv), NaH_2PO_4 (10.0 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 **18** and 63% for **19**. NMO = *N*-methylmorpholine *N*-oxide, PGME = phenyl glycine methyl ester, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole.





Scheme 4. Chemical synthesis of C1–C20 alcohol **21** for comparison with naturally derived material, structure of lissoketal (**22**), and second proposed structure, **23**, for the ABCD domain of azaspiracid-1. Reagents and conditions: a) TBAF (2.0 equiv), THF, 25 °C, 2 h, 88%. TBAF = tetra-*n*-butyl ammonium fluoride.

showed remarkable similarities to those observed for natural azaspiracid-1.[1] In particular, a weak HMBC correlation between C10 and 7-H as well as a weak COSY correlation between 6-H and 9-H observed for the natural azaspiracid-1 are in line with observations reported by Hopmann and Faulkner[11] and, therefore, point to the lissoketal ring A structural arrangement. Furthermore, relocating the endocyclic double bond from C8–C9 to C7–C8, places the 6-H in a doubly allylic environment, thereby providing a possible explanation for the significant downfield shift for this proton exhibited in the ^{1}H NMR spectrum of the naturally derived intermediate **5** (δ = 4.79 ppm) relative to that observed for the synthetic material **21** (δ = 4.51 ppm).[12] With the NMR spectroscopic parameters associated with rings B, C, and D for the naturally derived (**5**) and synthetic (**21**) samples in good agreement, the alternative structure **23** (see Scheme 4) was proposed for the hydroxy methyl ester derived from the natural product through intermediate **3** (see Scheme 1). To test this hypothesis, a chemical synthesis of the newly proposed structure **23** was required.

The synthesis of the newly proposed structure **23** was initiated from the previously described compound **24**[2] and proceeded as outlined in Scheme 5. Thus, the free hydroxy group in **24** was protected as a TES ether (95% yield), the pivaloate ester was cleaved with DIBAL-H (96% yield), and the liberated primary alcohol was oxidized under Swern conditions to afford aldehyde **25** (95% yield). Addition of vinyl Grignard reagent to **25** (76% yield) followed by acetylation (85% yield) yielded allylic acetate **26** as a mixture of diastereoisomers (≈2:1). Ireland–Claisen rearrangement[13] of **26** followed by methylation of the resulting carboxylic acid with diazomethane gave methyl ester **27** in 52% overall yield. Selective removal of the TES group (HF·py, 85% yield) from **27**, Swern oxidation of the resulting alcohol (90% yield) followed by treatment with KHMDS–TMSCl led to TMS enol ether **28**, whose oxidation to the corresponding enone (55% overall yield), reduction with $NaBH_4$, and acetylation (71% overall yield) furnished allylic acetate **29**. Finally, exposure of **29** to catalytic amounts of $[Pd_2dba_3]\cdot CHCl_3$ in the presence of nBu$_3$P and $NaBH_4$,[14] followed by treatment with TBAF led to the targeted intermediate **23** (42% overall yield) through intermediate **30** (which was chromatographically separated from its $\Delta^{8,9}$ isomer that emerged as the minor product during the reduction step (≈2:1)).

Much to our disappointment, however, the spectral data of synthetic **23** did not match those of the degradatively

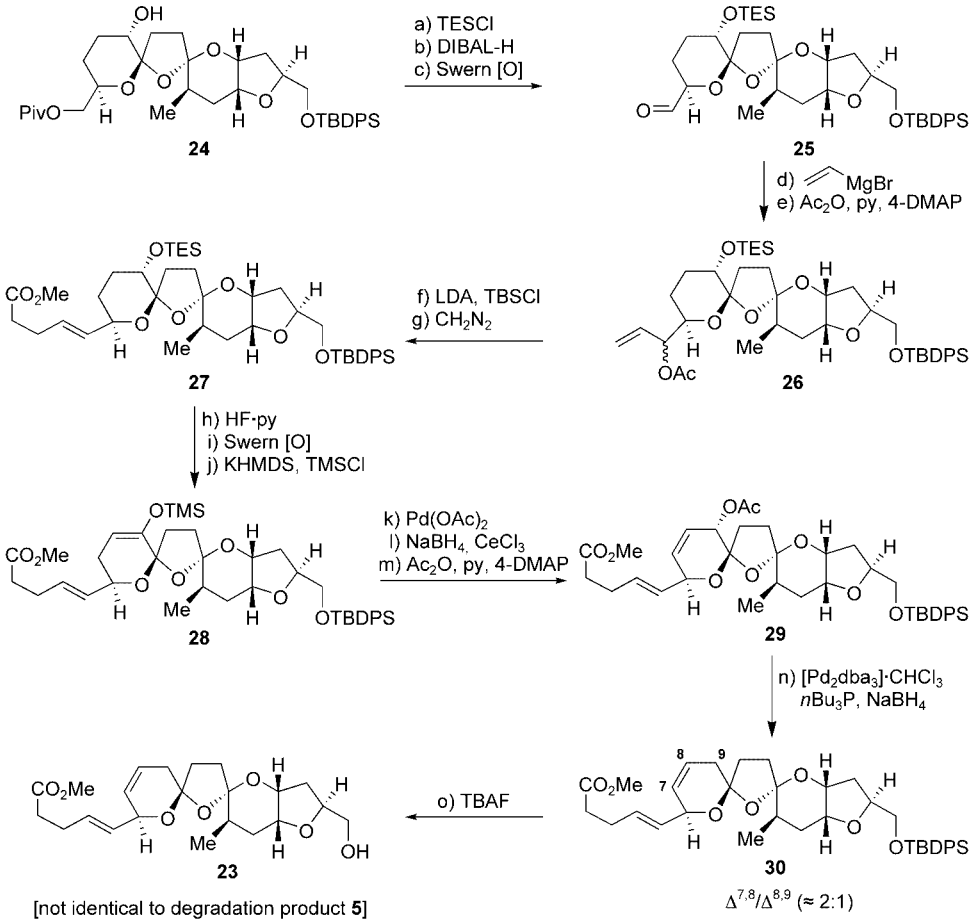
Table 1: Key ^{1}H NMR chemical shifts of compounds **5**, and **21**–**23**.

H	**5**[a] (natural)	**21**	**22**	**5**[b]	**23**
		$\delta(^1H)$ [ppm]			
6-H	4.79	4.50	4.34	4.79	4.81
7-H	2.48	2.06	5.80	5.62	5.61
	2.03	2.04			
8-H	5.74	6.07	5.80	5.74	5.77
9-H	5.62	5.79	2.57	2.48	2.51
			1.91	2.03	2.18
16-H	3.78	3.94	–	3.78	3.84
17-H	4.21	4.27	–	4.21	4.15
19-H	4.34	4.57	–	4.34	4.51
41-H	0.92	0.98	–	0.92	0.95

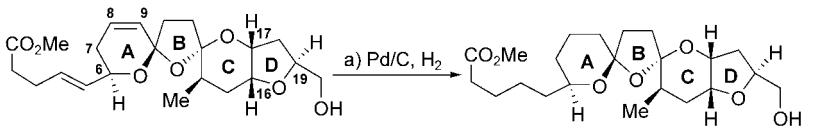
[a] Original incorrect assignments. [b] Revised (correct) assignments for the $\Delta^{7,8}$ isomer at **5** (note that structure was still incorrect).

derived **5** (see Table 1), sending us, one more time, back to the drawing board. Apparently, the location of the double bond in ring A was not the only problem with the originally proposed structure (**1-i**) of azaspiracid-1. A more profound structural change, perhaps having to do with the skeletal stereochemistry, was needed to accommodate the structure of the natural product. Indeed, the fully hydrogenated synthetic product **31** (see Scheme 6) obtained from synthetic **21** (10% Pd/C, H_2, 95% yield) proved to be different from the corresponding hydrogenated fragment **6** obtained from **5**, which was derived by degradation and elaboration of the natural product as already described above (see Scheme 1).

At this juncture, and having pinned down the location of the double bond within ring A, we were still faced with 128 possible structures for the ABCD domain of azaspiracid-1 arising from its seven stereogenic centers. However, our intelligence gathering at this stage extended to an additional piece of information regarding the thermodynamic stability of the degradatively derived ABCD fragments (e.g. **5**) and of the natural product itself. Their ABC double spiroketal bridge



Scheme 5. Chemical synthesis of the second proposed structure **23** for the ABCD domain. Reagents and conditions: a) TESCl (1.5 equiv), imidazole (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0°C, 12 h, 95%; b) DIBAL (2.0 equiv), CH_2Cl_2, −78°C, 1 h, 96%; c) $(COCl)_2$ (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, 95%; d) CH_2=CHMgBr (1.6 equiv), Et_2O, 0→25°C, 1 h, 76%; e) Ac_2O (5.0 equiv), py (10.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0°C, 3 h, 85%; f) LDA (1.5 equiv), THF, −78°C, 10 min, TBSCl (1.5 equiv), HMPA (1.5 equiv), −78→25°C, 24 h; g) CH_2N_2 (excess), Et_2O, 25°C, 30 min, 52% over two steps; h) HF·py (10.0 equiv), py/THF (1:1), 0°C, 2 h, 85%; i) $(COCl)_2$ (10.0 equiv), DMSO (22 equiv), CH_2Cl_2, −78°C, 30 min, −60°C, 1 h; then Et_3N (44 equiv), −78→−25°C, 90%; j) KHMDS (1.5 equiv), THF, −78°C, 1 h, TMSCl (1.6 equiv), −78°C, 30 min; k) $Pd(OAc)_2$ (5.0 equiv), DMSO, 72 h, 55% over two steps; l) $NaBH_4$ (3.0 equiv), $CeCl_3 \cdot 7H_2O$ (1.0 equiv), MeOH, −50°C, 1 h; m) Ac_2O (20 equiv), py (40 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0→25°C, 12 h, 71% over two steps; n) $[Pd_2dba_3]\cdot CHCl_3$ (0.2 equiv), nBu_3P (0.4 equiv), $NaBH_4$ (10.0 equiv), dioxane/H_2O (9:1), 0→25°C, 12 h; o) TBAF (3.0 equiv), THF, 0→25°C, 3 h, $\Delta^{7,8}/\Delta^{8,9}$ (2:1), 42% over two steps. TES = triethylsilyl, DMSO = dimethyl sulfoxide, TBS = *tert*-butyldimethylsilyl, LDA = lithium diisopropylamide, KHMDS = potassium bis(trimethylsilyl)amide.



(synthetic compound from L-malic acid)

Scheme 6. Preparation of fully saturated synthetic ABCD fragment **31**. Reagents and conditions: a) Pd/C (10%), H_2, CH_3CO_2Et, 25 °C, 1 h, 95%.

was noted to be stable under acidic conditions, whereas our corresponding synthetic materials so far revealed their fleeting nature under similar conditions. This intriguing observation, subtle as it was, proved to be crucial in shaping our next hypothesis for solving the mystery of azaspiracid-1, as we will describe in the following communication in this issue.[5]

Experimental Section

4: $R_f = 0.30$ (silica gel, EtOAc/MeOH 9:1); $[\alpha]_D^{25} = +36.4$ (MeOH, $c = 0.14$); IR (film): $\tilde{\nu}_{max} = 3416, 2954, 2919, 1727, 1430, 1261, 1096, 1044, 797, 673\ cm^{-1}$; ^1H NMR (600 MHz, CD_3OD/0.5% CD_3COOD): $\delta =$ 5.35 (s, 1H), 5.31 (s, 1H), 4.52 (d, $J = 10.1$ Hz, 1H), 4.26 (br s, 1H), 3.81 (d, $J = 1.8$ Hz, 1H), 2.85–2.70 (m, 2H), 2.64–2.56 (m, 1H), 2.49 (dd, $J = 14.5$, 4.8 Hz, 1H), 2.44–2.38 (m, 1H), 2.40 (d, $J = 14.5$ Hz, 1H), 2.32 (d, $J = 14.5$ Hz, 1H), 2.30–2.22 (m, 2H), 2.10–1.95 (m, 3H), 1.85–1.71 (m, 2H), 1.60 (d, $J = 12.7$ Hz, 1H), 1.56–1.49 (m, 1H), 1.46–1.39 (m, 2H), 1.27–1.23 (m, 2H), 1.23 (d, $J = 7.0$ Hz, 3H), 1.17–1.04 (m, 1H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.93 (d, $J = 6.1$ Hz, 3H), 0.91 (d, $J =$ 7.0 Hz, 3H), 0.89 ppm (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 177.2, 143.1, 120.4, 97.9, 95.9, 92.0, 79.6, 75.8, 72.6, 45.5, 43.4, 43.1, 42.4, 40.0, 38.0, 37.6, 37.0, 35.4, 33.1, 31.8, 26.4, 23.8, 19.8, 18.0, 17.5, 16.2$ ppm; HRMS (MALDI): calcd for $C_{26}H_{41}NO_5H^+$ [M+H^+]: 448.3057; found: 448.3051.

FGHI-*epi*-**4**: $R_f = 0.29$ (silica gel, EtOAc/MeOH 9:1); $[\alpha]_D^{25} = -18.0$ (MeOH, $c = 0.30$); IR (film): $\tilde{\nu}_{max} = 3374, 2962, 2921, 2854, 1729, 1452, 1411, 1261, 1090, 1021, 865, 800, 701, 666\ cm^{-1}$; ^1H NMR (600 MHz, CD_3OD/0.5% CD_3COOD): $\delta = 5.40$ (s, 1H), 5.37 (s, 1H), 4.44 (d, $J = 9.6$ Hz, 1H), 4.37 (br s, 1H), 4.03 (d, $J = 2.6$ Hz, 1H), 3.25 (d, $J = 12.3$ Hz, 1H), 3.05 (t, $J = 12.3$ Hz, 1H), 2.72 (dd, $J = 14.9$, 4.4 Hz, 1H), 2.66–2.60 (m, 1H), 2.48 (d, $J = 14.5$ Hz, 1H), 2.40–2.30 (m, 1H), 2.32 (d, $J = 14.5$ Hz, 1H), 2.30–2.20 (m, 1H), 2.15–2.08 (m, 2H), 2.04–2.00 (m, 1H), 1.83 (dd, $J = 14.0$, 5.7 Hz, 1H), 1.73 (d, $J =$ 14.0 Hz, 1H), 1.58–1.51 (m, 1H), 1.46–1.39 (m, 2H), 1.35–1.25 (m, 3H), 1.25 (d, $J = 7.0$ Hz, 3H), 1.18–1.09 (m, 2H), 1.00 (d, $J = 6.6$ Hz, 3H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.97 (d, $J = 6.2$ Hz, 3H), 0.95 ppm (d, $J = 6.1$ Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD): $\delta = 177.1, 142.5, 121.0, 98.1, 95.9, 92.4, 79.5, 75.8, 72.7, 45.7, 43.3, 43.2, 41.8, 40.0, 37.9, 37.7, 37.4, 35.6, 32.6, 31.8, 26.3, 23.9, 19.8, 17.9, 17.4, 16.2$ ppm; HRMS (MALDI): calcd for $C_{26}H_{41}NO_5H^+$ [M+H^+]: 448.3057; found: 448.3057.

8: $R_f = 0.19$ (silica gel, $CHCl_3$/MeOH/H_2O 20:3:1); $[\alpha]_D^{25} = +49.6$ (MeOH, $c = 0.4$); IR (film): $\tilde{\nu}_{max} = 3359, 2922, 2948, 1700, 1575, 1455, 1393, 1257, 1116, 1064, 1043, 1012, 793, 621\ cm^{-1}$; ^1H NMR (600 MHz, C_5D_5N): $\delta = 4.71$ (br s, 1H), 4.46 (br s, 1H), 3.59 (br s, 1H), 2.95 (s, 2H), 2.92 (t, $J = 12.3$ Hz, 1H), 2.83–2.75 (m, 1H), 2.34–2.27 (m, 1H), 2.20–2.13 (m, 2H), 2.10–2.04 (m, 1H), 1.95–1.88 (m, 1H), 1.80–1.71 (m, 2H), 1.57–1.50 (m, 1H), 1.49–1.42 (m, 2H), 1.31–1.24 (m, 1H), 0.91 (d, $J = 5.7$ Hz, 3H), 0.78 (d, $J = 4.8$ Hz, 3H), 0.72 ppm (d, $J =$ 5.3 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD): $\delta = 177.4, 97.2, 95.7, 80.2, 75.3, 72.2, 52.0, 48.2, 43.1, 39.6, 37.7, 35.4, 31.2, 30.8, 26.5, 23.9, 19.6, 16.1$ ppm; HRMS (MALDI): calcd for $C_{18}H_{29}NO_5H^+$ [M+H^+]: 340.2118; found: 340.2122.

21: $R_f = 0.22$ (silica gel, EtOAc/hexanes 1:2); $[\alpha]_D^{25} = +45.0$ ($CHCl_3$, $c = 1.1$); IR (film): $\tilde{\nu}_{max} = 3439, 2924, 2360, 2320, 1739, 1450, 1378, 1320, 1235, 1155, 1091, 1049, 1020, 978, 855, 800, 667\ cm^{-1}$; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 6.07$ (ddd, $J = 9.9$, 5.1, 2.2 Hz, 1H), 5.80–5.74 (m, 2H), 5.62 (dd, $J = 15.7$, 6.1 Hz, 1H), 4.60–4.55 (m, 1H), 4.52–4.48 (m, 1H), 4.28–4.26 (m, 1H), 3.94–3.93 (m, 1H), 3.83–3.81 (m, 1H), 3.75 (s, 3H), 3.58–3.53 (m, 1H), 2.49–2.42 (m, 4H), 2.31–2.25 (m, 1H), 2.21–1.97 (m, 9H), 1.89–1.87 (m, 1H), 1.58–1.51 (m, 1H), 0.98 ppm (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 173.4, 131.1, 130.1, 129.1, 128.5, 111.4, 104.2, 78.9, 76.3, 75.9, 68.6, 64.8, 51.5, 35.7, 33.9, 33.6, 31.0, 29.9, 29.7, 27.6, 23.4, 15.6$ ppm; HRMS (MALDI): calcd for $C_{22}H_{32}O_7Na^+$ [M+Na^+]: 431.2040; found: 431.2060.

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