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Total Synthesis and Structural Elucidation of Azaspiracid-1. Synthesis-Based Analysis of Originally Proposed Structures and Indication of Their Non-Identity to the Natural Product

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Abstract: The key building blocks (**6**, **7**, and **8**) for the intended construction of the originally proposed structures of azaspiracid-1, a potent marine-derived neurotoxin, were coupled and the products elaborated to the targeted compounds (**1a**,**b**) and their C-20 epimers (**2** and **3**). The assembly of the three intermediates was accomplished by a dithiane-based coupling reaction that united the C_1-C_{20} (**7**) and $C_{21}-C_{27}$ (**8**) fragments, followed by a Stille-type coupling which allowed the incorporation of the $C_{28}-C_{40}$ fragment (**6**) into the growing substrate. Neither of the final products (**1a**,**b**) matched the natural substance by TLC or ^{1}H NMR spectroscopic analysis, suggesting one or more errors in the originally proposed structure for this notorious biotoxin.

Introduction

In the preceding paper,[1] we described the stereoselective construction of the three key building blocks **6**, **7**, and **8** (Figure 3) required for our projected total synthesis of the proposed structure of azaspiracid-1 (**1a**, Figure 1).[2] These sequences delivered all three fragments in both enantiomeric forms as needed to ensure the eventual assignment of the relative and absolute stereochemistry of the natural product. In this article we describe the coupling of these key building blocks and the elaboration of the resulting products to the targeted molecules, the two diastereomeric azaspiracid-1 structures **1a** and **1b** (Figure 1). This accomplishment, however, only proved that the originally proposed structure (**1a** or **1b**) was in error. This finding prompted the synthesis of the two C-20-*epi* diastereomers **2** and **3** (Figure 1), neither of which matched the natural product, thereby proving their non-identity to the true structure of azaspiracid-1. This, in turn, left the project open for new speculations and initiatives for the deconvolution of the still remaining puzzle of this intriguing natural product. The demystification of the true structure of azaspiracid-1 as that depicted by **1** (Figure 2) will be described in a subsequent publication.[3]

Results and Discussion

1. Coupling of the C_1-C_{20} and $C_{21}-C_{27}$ Fragments and Synthesis of the ABCDE Domain. Of the two available choices to assemble the C_1-C_{20} (**7**), $C_{21}-C_{27}$ (**8**), and $C_{28}-C_{40}$ (**6**) fragments into the targeted azaspiracid-1 architecture, we opted for that involving initial union of **7** and **8** through a dithiane coupling,[4] followed by subsequent incorporation of **6** into the growing molecule (i.e., **5**) through a Stille reaction,[5] as outlined retrosynthetically in Figure 3. As a prelude to the dithiane-based coupling of **7** and **8**, we carried out a preliminary investigation involving the simpler and, therefore, more plentiful partners dithiane **9** and carbonyl compounds **10**, **11**, **11a**, and **11b** (Table 1) in order to develop the necessary technology for what was expected to be a challenging task. Table 1 summarizes the results of this study. Thus, employing at first the aldehyde substrate corresponding to structure **10** and *t*-BuLi or *n*-BuLi as the base to form the lithio derivative of dithiane **9** in various solvents and different temperatures led to no product containing both fragments. Monitoring the formation of the expected lithiated compound from **9** by D_2O quenching/^{1}H NMR spectroscopic analysis led to the conclusion that the desired intermediate, although initially formed, was incapable of being properly trapped with the aldehyde partner (**10**, entries 1−6, Table 1), being too short-lived for a useful reaction.

Faced with this predicament, we then employed the *n*-BuLi−*n*-Bu_2Mg reagent, which is known to provide a longer-lived

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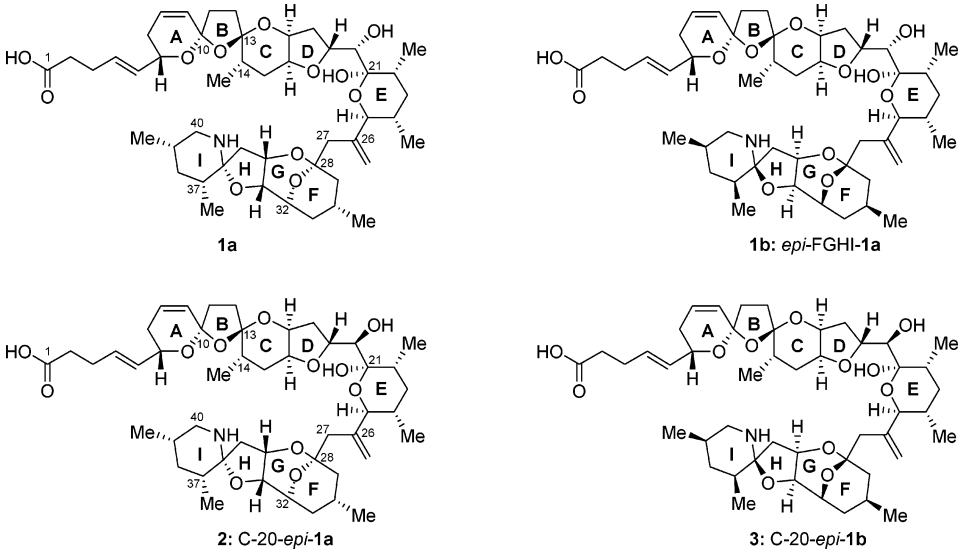

Figure 1. Two of the originally proposed structures of azaspiracid-1, **1a** and **1b**, and synthesized C-20 epimers **2** and **3**.

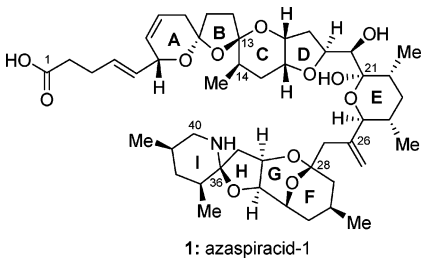

Figure 2. Revised structure of azaspiracid-1 (**1**).

organometallic species from dithianes,[6] expecting a positive outcome in the coupling reaction. Indeed, an encouraging improvement was observed, with yields up to 42% under certain conditions (entry 7, Table 1), but the coupling product consisted of a random (ca. 1:1) mixture of the two diastereomeric alcohols. Having exhausted all hope for further improvement with aldehyde **10** as the electrophile partner in this reaction, we adopted a series of alternative electrophilic equivalents, most notably activated esters. Such partners, we reasoned, could not only offer advantages such as a more reliable coupling substrate in this carbon−carbon bond-forming process but also, most importantly, provide us with an opportunity to control the C-20 stereochemistry through the subsequent reduction of the expected ketone (in contrast to the aldehyde coupling that proceeded in a nonstereoselective manner, as mentioned above). Among the activated esters utilized (e.g., 2-pyridinethiol **11a**,[7] benzotriazole **11b**,[8] and pentafluorophenol **11**,[9] entries 8−10, Table 1), the pentafluorophenol ester proved the best, leading to a 63% yield of the desired ketone (entry 10, Table 1). The required pentafluoro ester **11** was prepared by a four-step sequence from TBDPS derivative **13** [(i) TBAF-induced desilylation, 88% yield; (ii) Swern oxidation,[10] 94% yield; (iii) $NaClO_2$-based oxidation, 82% yield; and (iv) DCC coupling with pentafluorophenol, 85% yield], as shown in Scheme 1.

With the coupling reaction of fragments **9** and **11** at a satisfactory level of efficiency, we proceeded to explore the sequence to the complete model ABCDE ring framework as shown in Scheme 1. For this investigation, we utilized the C_5-C_{27} ketone **16** obtained by coupling of the ABCD segment **11** with dithiane **8** according to the *n*-BuLi−*n*-Bu_2Mg-based procedure (63% yield). From the many reducing reagents tested, only DIBAL-H performed well in this reaction, yielding, at −90 °C in CH_2Cl_2, the desired and anticipated alcohol as a single stereoisomer in a reaction that also cleaved the pivaloate ester, leading to compound **17** (55% yield). The lost ester group was easily re-introduced (PivCl, py, 75% yield) to furnish the next intermediate, **18**, from which the dithiane moiety was removed by the action of $PhI(OCOCF_3)_2$ (78% yield).[11] The so generated keto silyl bis-ether (**19**) was treated with TBAF, which allowed the liberated trihydroxy ketone to spontaneously fold on itself, affording the hydroxy hemiketal **20** in 85% yield. For the purposes of assigning the stereochemistry of the reduction, this 1,2-diol system was treated with triphosgene and pyridine, giving the cyclic carbonate **21**, whose structural rigidity allowed its ^1H NMR spectra and NOE studies to be particularly revealing.[12] Indeed, the observed NOEs (see Scheme 1) confirmed the shown (and desired) stereochemistry for the C-20 hydroxyl group which was generated a few steps back by the DIBAL-H reduction of the carbonyl group at that position.

2. Synthesis of Two of the Diastereomers of the Originally Proposed Structures of Azaspiracid-1. With the development of the synthetic technology for the establishment of the ABCDE domain of azaspiracid-1, the road was now open for advancement of the C_1-C_{20} fragment **22**[1] to the C_1-C_{27} allylic acetate **29** (Scheme 2), as needed for the planned Stille reaction with stannane **6**. To this end, the lithium anion derived from **8** and *n*-BuLi−*n*-Bu_2Mg in THF at 25 °C as described above was

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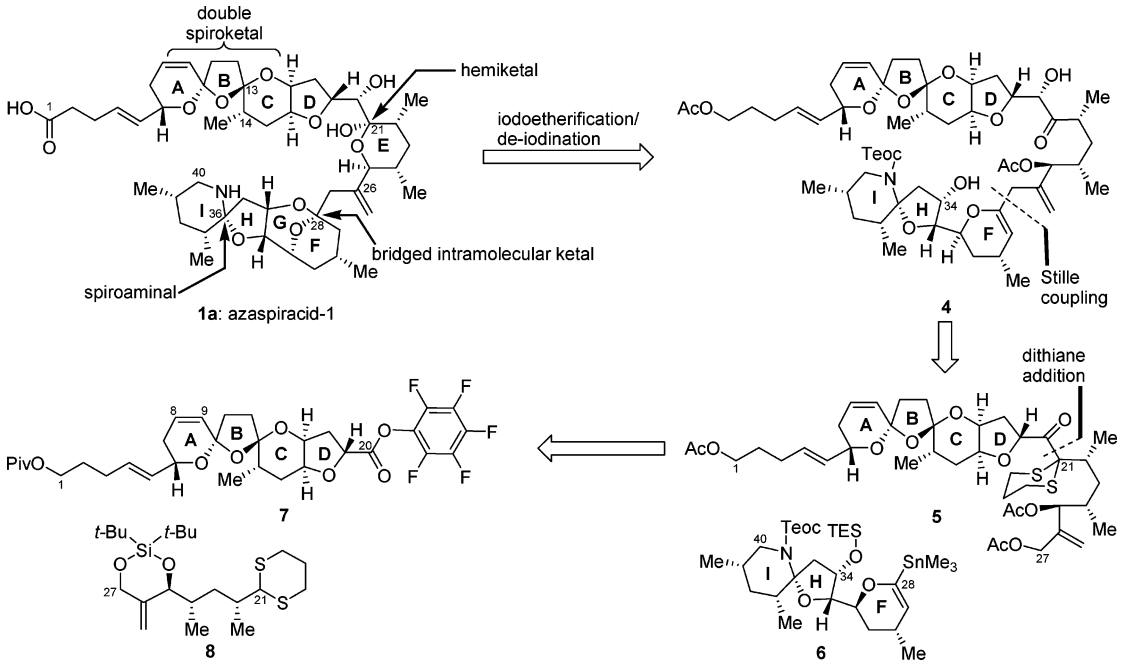
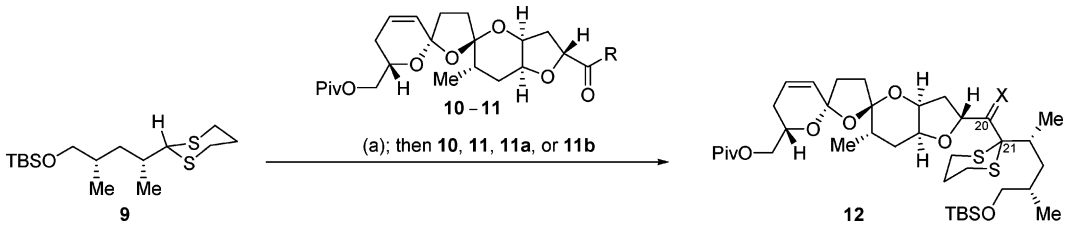
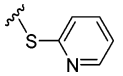
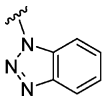
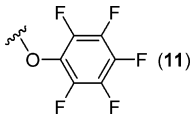


Figure 3. Retrosynthetic analysis of the originally proposed structure of azaspiracid-1 (**1a**).

Table 1. Optimization of Dithiane Coupling for the Formation of the $C_{20}-C_{21}$ Bond of Azaspiracid-1

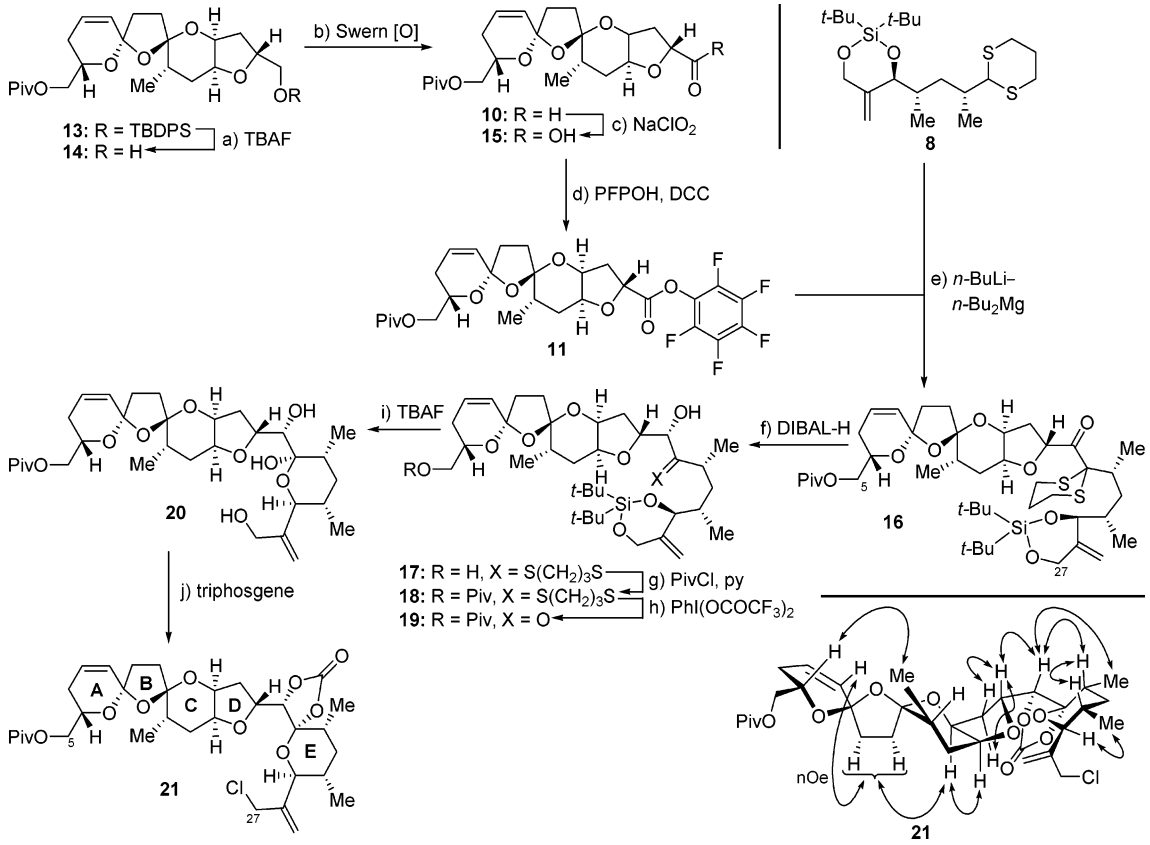


entry	(a) reagents and conditions	R	% deuterium exchange[a]	yield (%)[b]	X
1	*t*-BuLi, HMPA, THF, −78 °C, 1 h	H (**10**)	92	10	H, OH
2	*t*-BuLi, HMPA, THF, 0 °C, 5 min	H (**10**)	30	0	H, OH
3	*t*-BuLi, THF, 25 °C, 1 h	H (**10**)	77	0	H, OH
4	*t*-BuLi, Et_2O, 25 °C, 1 h; $MgBr_2$	H (**10**)	85	0	H, OH
5	*n*-BuLi, NaO-*t*-Bu, THF, −78 °C, 1 h	H (**10**)	17	0	H, OH
6	*n*-BuLi, THF, 0 °C, 30 min	H (**10**)	50	0	H, OH
7	*n*-BuLi−*n*-Bu_2Mg, THF, −90 °C, 1 h	H (**10**)	80	10−42[c]	H, OH
8	*n*-BuLi−*n*-Bu_2Mg, THF, −90 °C, 1 h	 (**11a**) (**11a**)		0	O
9	*n*-BuLi−*n*-Bu_2Mg, THF, −90 °C, 1 h	 (**11b**) (**11b**)		0	O
10	*n*-BuLi−*n*-Bu_2Mg, THF, −90 °C, 1 h	 (**11**) (**11**)		63	O

[a] D_2O addition after the indicated reaction time. [b] Addition of **10**, **11**, **11a**, and **11b** at −90 °C. [c] Combined yield of a 1:1 mixture of diastereoisomers.

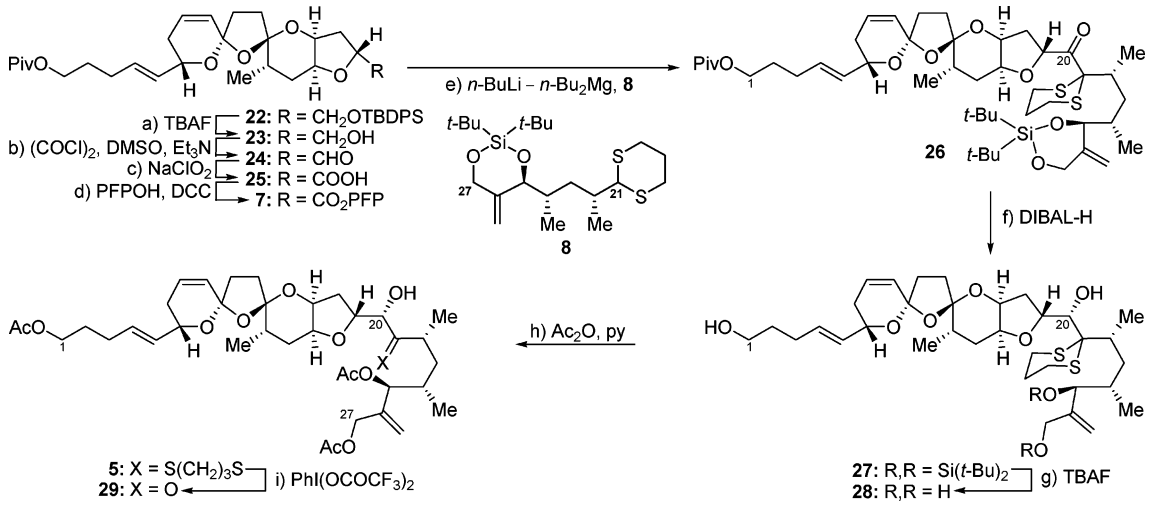
reacted at −90 °C with pentafluoro ester **7** [prepared from silyl ether **22** by desilylation with TBAF, Swern oxidation of the resulting alcohol **23** to the corresponding aldehyde (**24**), and further oxidation to the carboxylic acid (**25**) followed by DCC-mediated coupling with pentafluorophenol (64% yield over the four steps)] to afford dithiane ketone **26** in 63% yield, as shown in Scheme 2. The DIBAL-H reduction of **26** proceeded, as expected, stereoselectively and in 55% yield, furnishing a single

Scheme 1. Model Dithiane Coupling and Structural Confirmation of All Stereocenters in the ABCDE Ring System (C_5-C_{27} Domain)[a]



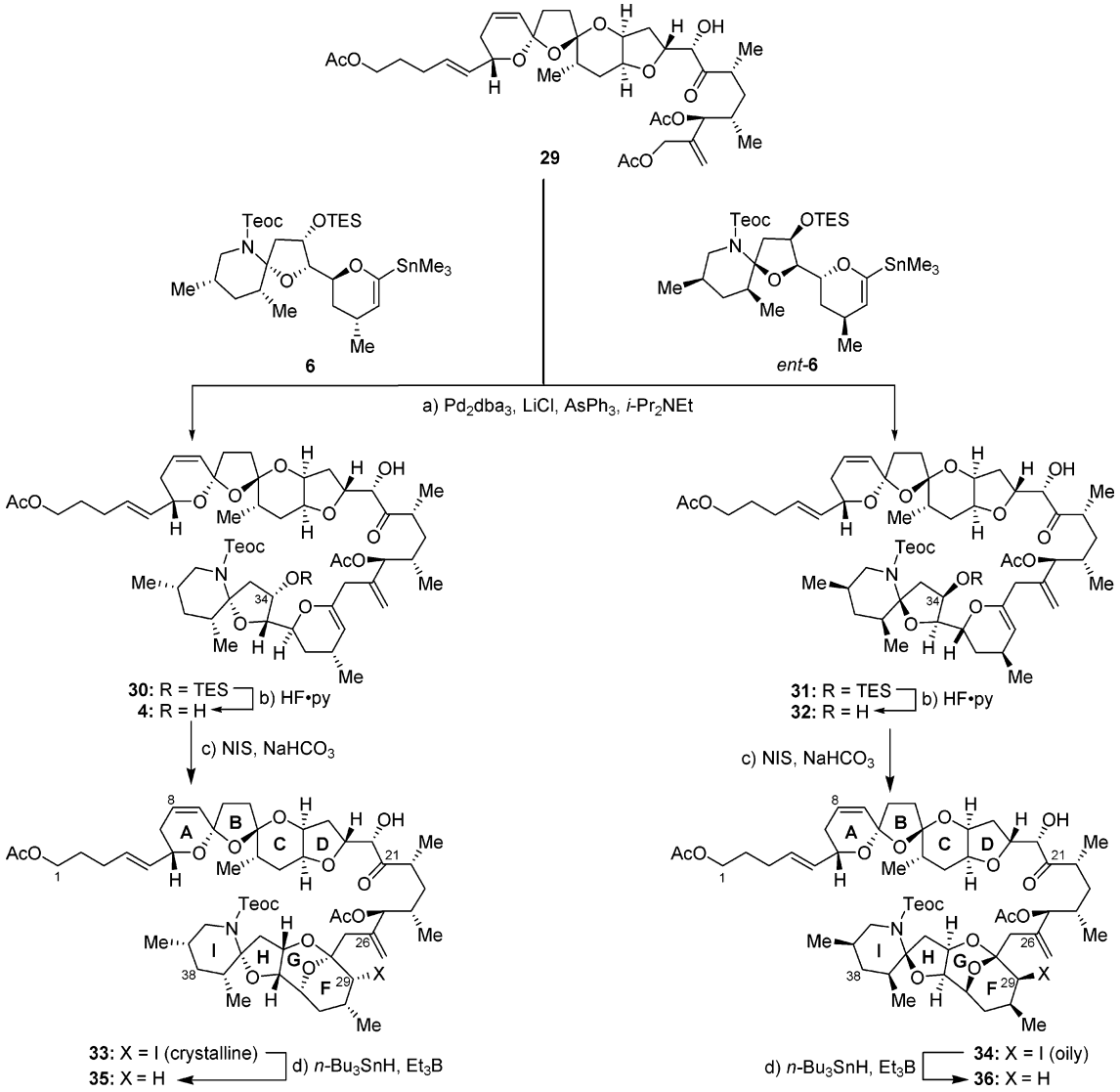
[a] Reagents and conditions: (a) TBAF (1.0 M in THF, 5.0 equiv), THF, 25 °C, 2 h, 88%; (b) $(COCl)_2$ (5.0 equiv), DMSO (11 equiv), CH_2Cl_2, −78 °C, 30 min; then Et_3N (22 equiv), −78 → 0 °C, 94%; (c) $NaClO_2$ (6.0 equiv), NaH_2PO_4 (6.0 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 1.5 h; (d) pentafluorophenol (1.5 equiv), DCC (2.0 equiv), CH_2Cl_2, 25 °C, 2.5 h, 78% over two steps; (e) **8** (9.0 equiv), *n*-BuLi−*n*-Bu_2Mg (1.1 M in hexanes, 6.0 equiv), THF, 0 → 25 °C, 1.5 h; then **35**, −90 °C, 15 min, 63%; (f) DIBAL-H (1.0 M in CH_2Cl_2, 10 equiv), CH_2Cl_2, −90 °C, 1.5 h, 55%; (g) PivCl (3.0 equiv), py (10 equiv), 0 → 25 °C, 12 h, 75%; (h) $PhI(OCOCF_3)_2$ (2.2 equiv), MeCN:pH 7 buffer (4:1), 0 °C, 78%; (i) TBAF (1.0 M in THF, 5.0 equiv), THF, 25 °C, 16 h, 85%; (j) triphosgene (2.0 equiv), py (15 equiv), CH_2Cl_2, −78 → 25 °C, 1 h, 54%. Abbreviations: Piv, trimethylacetyl; TBDPS, *tert*-butyldiphenylsilyl; TBAF, tetra-*n*-butylammonium fluoride; DMSO, dimethyl sulfoxide; PFP, pentafluorophenyl; DCC, dicyclohexylcarbodiimide; DIBAL-H, diisobutylaluminum hydride.

Scheme 2. Synthesis of Stille Coupling Partner **29**[a]



[a] Reagents and conditions: (a) TBAF (1.0 M in THF, 2.0 equiv), THF, 0 → 25 °C, 3 h, 93%; (b) $(COCl)_2$ (5.0 equiv), DMSO (11 equiv), CH_2Cl_2, −78 → −60 °C, 2 h; then Et_3N (22 equiv), −78 → −30 °C, 1 h, 89%; (c) $NaClO_2$ (4.0 equiv), NaH_2PO_4 (4.0 equiv), 2-methyl-2-butene (5.0 equiv), *t*-BuOH:H_2O (5:1), 25 °C, 2 h, 95%; (d) pentafluorophenol (1.2 equiv), DCC (1.5 equiv), CH_2Cl_2, 25 °C, 2 h, 82%; (e) **8** (9.0 equiv), *n*-BuLi−*n*-Bu_2Mg (1.1 M in hexanes, 6.0 equiv), THF, 0 → 25 °C, 1.5 h; then **7**, −90 °C, 15 min, 63%; (f) DIBAL-H (1.0 M in CH_2Cl_2, 10 equiv), CH_2Cl_2, −90 °C, 1.5 h, 55%; (g) TBAF (1.0 M in THF, 5.0 equiv), THF, 25 °C, 16 h, 78%; (h) Ac_2O (50 equiv), pyridine:CH_2Cl_2 (1:1), 0 → 25 °C, 16 h, 84%; (i) $PhI(OCOCF_3)_2$ (2.2 equiv), MeCN:pH 7 buffer (4:1), 0 °C, 78%.

Scheme 3. Stille Coupling of Stannanes **6** and *ent*-**6** with Allylic Acetate **29**, and Arrival at Octacyclic Ring Systems **35** and **36**[a]



[a] Reagents and conditions: (a) Pd_2dba_3 (0.3 equiv), $AsPh_3$ (0.3 equiv), LiCl (6.0 equiv), *i*-Pr_2NEt (12 equiv); then **6** or *ent*-**6** (0.03 M in THF, syringe pump addition), NMP, 45 °C, 4 h, 66% for **30**, 56% for **31**; (b) HF·py (excess), THF:pyridine (1:1), 0 → 25 °C, 2.5 h; (c) NIS (10 equiv), $NaHCO_3$ (30 equiv), THF, 0 °C, 16 h, 67% for **33**, 63% for **34** over two steps; (d) Et_3B (1.0 M in hexanes, 3.0 equiv), *n*-Bu_3SnH:toluene (1:2), 0 °C, 5 min, 92% for **35**, 94% for **36**. Abbreviations: NIS, *N*-iodosuccinimide; TES, triethylsilyl; Teoc, 2-(trimethylsilyl)ethoxycarbonyl; dba, dibenzylideneacetone; NMP, *N*-methylpyrrolidone.

diol with the C-20 hydroxyl group, assumed at this point to be of the same configuration as that obtained before with the reduction of **16** (Scheme 1). This assignment was to be confirmed later on in the synthesis by X-ray crystallographic analysis of a descendent compound (vide infra). Exposure of the latter compound (**27**) to TBAF resulted in the cleavage of the cyclic silyl bis-ether, generating tetraol **28** (78% yield), which was selectively converted to triacetate **5** with Ac_2O in the presence of pyridine in 84% yield. Being shielded more by its surroundings than the other three, the C-20 hydroxyl group remained inert under these conditions. The dithiane group was then removed from compound **5** by the action of $PhI(OCOCF_3)_2$[11] to afford, in 78% yield, triacetoxy ketone **29**, representing the required C_1-C_{27} Stille coupling partner.

The next task toward the total synthesis of the originally proposed structure of azaspiracid-1 called for the union of allylic acetate **29** with stannane **6** by a Stille coupling reaction. Due to the complexity and sensitivity of the substrates involved, success in this process required considerable experimentation. As shown in Table 2, employment of $Pd(PPh_3)_4$ as a catalyst in conjunction with LiCl[13] and *i*-Pr_2NEt in THF at 45 °C led to no coupling product formation, with only decomposition observed (entry 1). Switching to $Pd_2(dba)_3$ as the catalyst under the same conditions resulted in coupling (60% combined yield); however, the product was found to be a mixture of the desired compound (**30**) and its C-25 acetoxy epimer (C-25-*epi*-**30**), with **30** predominating in ca. 2:1 ratio. Faced with this epimerization problem, which we attributed to the high reactivity of the catalyst, we then proceeded to tame its behavior by employing $AsPh_3$ as an additional ligand in the reaction mixture. It was reasoned that the bulkiness of this ligand combined with its

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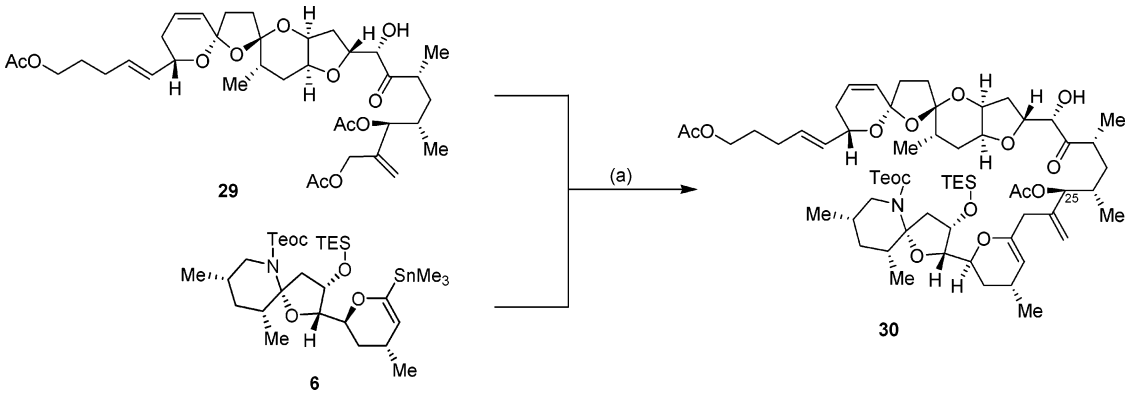
Table 2. Optimization of the Stille Coupling of **29** with **6**



entry	reagents and conditions (a)[a]	temp (°C)	time (h)	yield (%) **30**:C_{25}-*epi*-**30**
1	10 mol % $Pd(PPh_3)_4$, LiCl, *i*-Pr_2NEt	45	15	0:0
2	10 mol % $Pd(dba)_3$, LiCl, *i*-Pr_2NEt	45	12	40:20
3	10 mol % $Pd_2(dba)_3$, 80 mol % $AsPh_3$, LiCl, *i*-Pr_2NEt	45	16	10:0
4	10 mol % $Pd_2(dba)_3$, 40 mol % $AsPh_3$, LiCl, *i*-Pr_2NEt	45	8	50:0
5	10 mol % $Pd_2(dba)_3$, 20 mol % $AsPh_3$, LiCl, *i*-Pr_2NEt	45	5	60:0
6	10 mol % $Pd_2(dba)_3$, 10 mol % $AsPh_3$, LiCl, *i*-Pr_2NEt	45	4	66:0

[a] Compound **29** was dissolved in NMP and LiCl and *i*-Pr_2NEt were added. To this mixture was added palladium catalyst and in entries 2, 3, 4, and 5, ligand additive $AsPh_3$. Compound **6** was added as a solution in THF at the indicated temperature and over the time period listed.

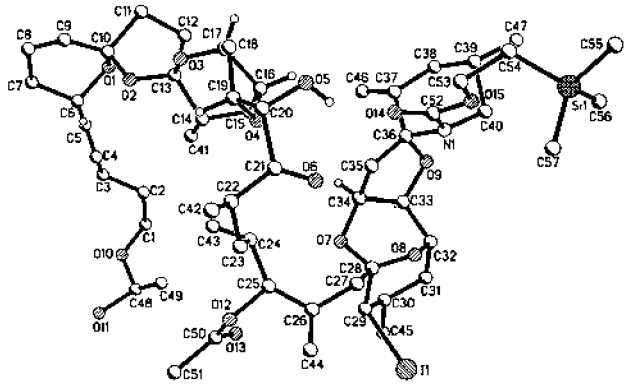


Figure 4. ORTEP drawing of iodide **33**.

accelerating effect would result in the desired compromise between reaction rate acceleration and stereochemical integrity preservation at C-25.[14] Indeed, with some fine-tuning of stoichiometry, we were able to achieve a 66% yield of coupling product **30** without any observable epimerization. In this respect, it was interesting to note both the acceleration of the reaction and the increase in yield as the amount of $AsPh_3$ was decreased from 80 to 10 mol %, matching the mol % of the palladium catalyst [$Pd(dba)_3$] (entries 3−6, Table 2).

Boasting all carbons needed for the azaspiracid-1 molecule, intermediate **30** was then ready for further advancement, as shown in Scheme 3. Because the relative stereochemistry between the ABCDE and FGHI domains was not known at the time, we went through the sequence with both isomers of the FGHI domain (**6** and *ent*-**6**). The next task was to forge ring G and thus complete the FGHI domain of the molecule. Removal of the TES group from the C-34 oxygen generated hydroxy enol ether **4**, whose iodoetherification reaction (NIS, $NaHCO_3$)[15] furnished, in 67% overall yield over the two steps, iodoether **33** as a single diastereomer. Iodide **33** pleasantly surprised us with its nice crystals suitable for X-ray crystallographic analysis, which confirmed its structure (and those of its predecessor compounds) as shown (see ORTEP drawing, Figure 4). It should be noted that the $Hg(OAc)_2$ technology[16] that was successfully employed to forge this key ring (G) in a model system[1] led to decomposition when applied to substrate **4**.

Having accomplished its mission of forging ring G and proving beyond doubt the configuration of the growing molecule, the iodide residue was then ejected reductively from intermediate **33** by exposure to excess *n*-Bu_3SnH and Et_3B[17] as a radical initiator in toluene at 0 °C, to afford the octacyclic system **35** in 92% yield. In an analogous manner and in similar yields, the diastereomeric compound **36** was synthesized from intermediate **29** and the enantiomer of **6** (*ent*-**6**), as depicted in Scheme 3. All that now separated these two compounds, **35** and **36**, from the targeted diastereomeric structures of azaspiracid-1, **1a**,**b**, was the casting of the final ring (E) and a few functional group adjustments.

The final stages of the drive toward the targeted azaspiracid-1 structures, **1a**,**b**, are shown in Scheme 4. The secondary alcohol in **35** was protected as a TES ether (TESOTf, 2,6-lutidine, 89% yield) to allow selective oxidation at C-1. Thus, cleavage of the C-1 acetate (K_2CO_3, MeOH, 85% yield), followed first by a Swern oxidation [$(COCl)_2$, DMSO; Et_3N] and then by a $NaClO_2$ oxidation (81% overall yield for the two steps), led to carboxylic acid **43** through intermediates **37**, **39**, and **41**. The TES group was then removed from **43** by treatment with TBAF to afford acetoxy compound **45** (87% yield), a substrate thought to be separated from the final target (**1a**) by only one step, deacetylation. Indeed, on exposure of this compound (**45**) to LiOH, the acetate was cleaved and an inseparable mixture[18] of

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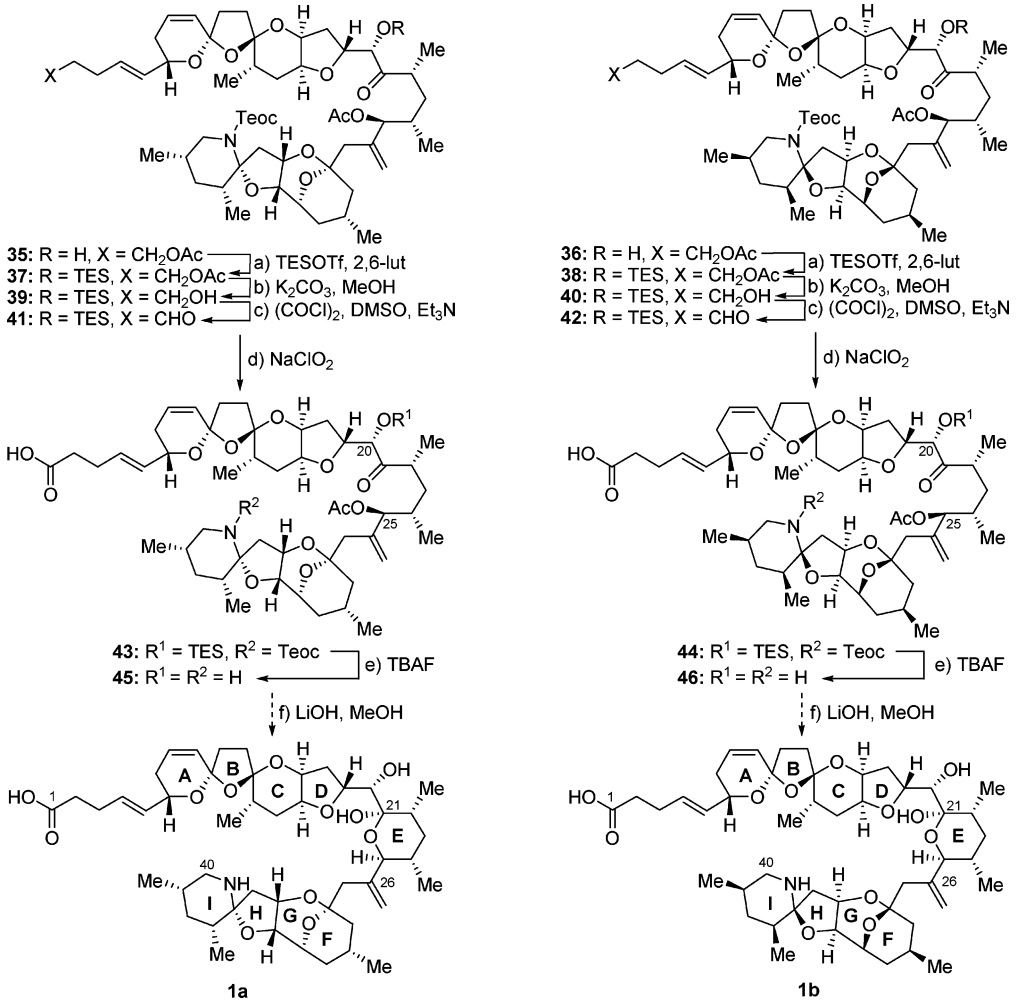
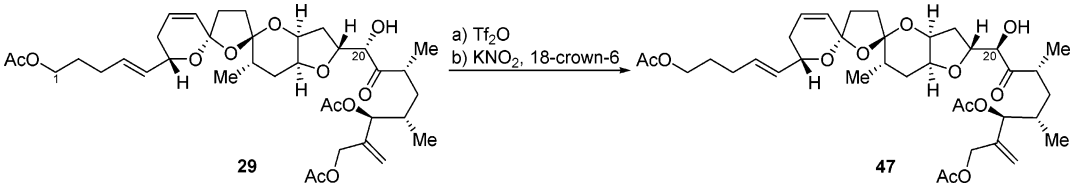
(15) (a) Adinolfi, M.; Parrilli, M.; Barone, G.; Laonigro, G.; Mangoni, L. *Tetrahedron Lett.* **1976**, 3661. (b) Haaima, G.; Weavers, R. T. *Tetrahedron Lett.* **1988**, *29*, 1085.
(16) Carnevale, G.; Davini, E.; Iavarone, C.; Trogolo, C. *J. Chem. Soc., Perkin. Trans. 1: Org. Bioorg. Chem.* **1990**, 989.
(17) Miura, K.; Ichinose, Y.; Nozaki, K.; Fugami, K.; Oshima, K.; Utimoto, K. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 143.

Scheme 4. Final Stages and Completion of the Total Synthesis of the Proposed Azaspiracid-1 Structures **1a** and **1b**[a]



[a] Reagents and conditions: (a) TESOTf (10 equiv), 2,6-lutidine (20 equiv), CH_2Cl_2, −78 → 0 °C, 10 min, 89% for **37** and 81% for **38**; (b) K_2CO_3 (10 equiv), MeOH, 25 °C, 2 h, 85% for **39** and 87% for **40**; (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, 81% for **41** and 79% for **42** over two steps; (e) TBAF (5.0 equiv), THF, 25 °C, 2 h, 87% for **45**, 92% for **46**; (f) LiOH (10 equiv), MeOH:H_2O (5:1), 25 °C, 16 h, 45% (for the total inseparable mixture of at least two compounds represented by **1a**) and 37% (for the total inseparable mixture of at least two compounds represented by **1b**).

Scheme 5. Inversion of C-20 Stereocenter[a]

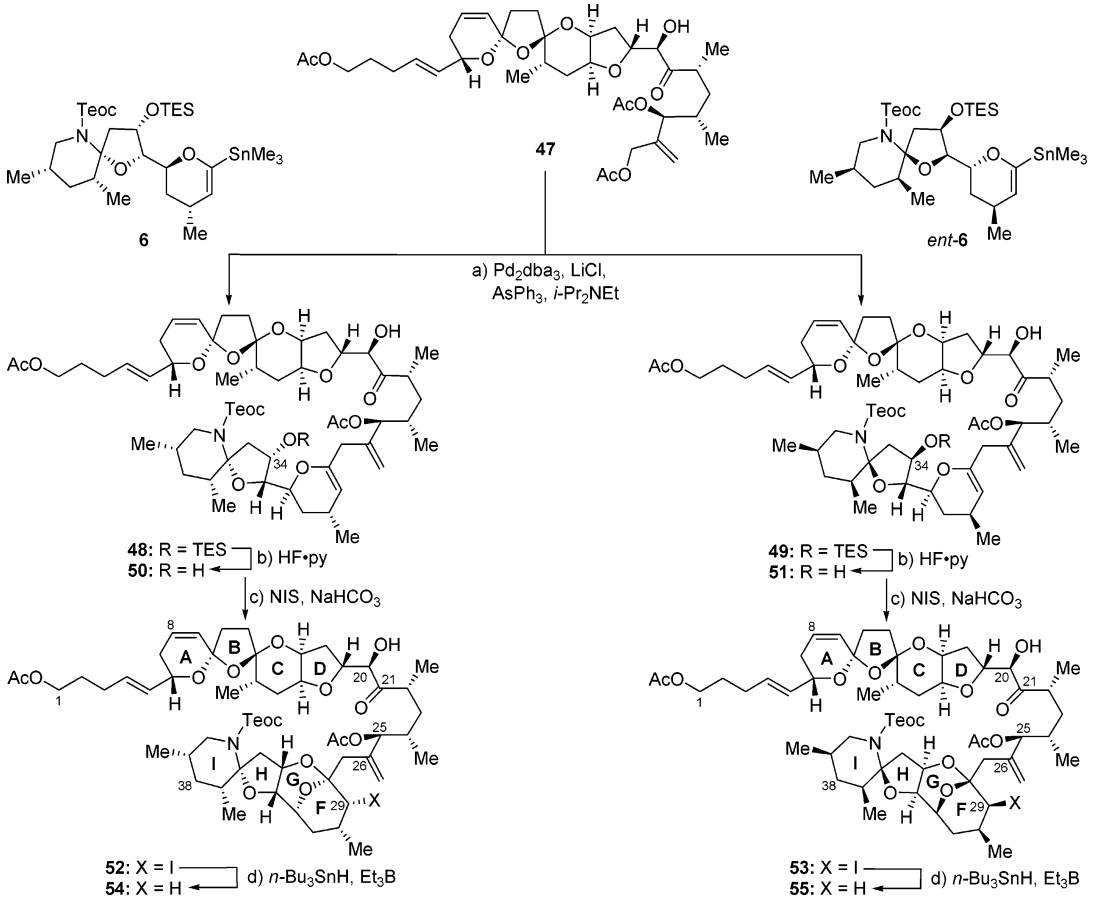


[a] Reagents and conditions: (a) Tf_2O (10 equiv), py (20 equiv), 4-DMAP (0.2 equiv), CH_2Cl_2, 0 °C, 1.5 h, 68%; (b) KNO_2 (5.0 equiv), 18-crown-6 (5.0 equiv), DMF, 25 °C, 24 h, 45%. Abbreviations: Tf, trifluoromethanesulfonyl; 4-DMAP, 4-(dimethylamino)pyridine; DMF, dimethylformamide.

products (at least two) was obtained, none of which matched the natural product by TLC or ^{1}H NMR spectroscopy. The same sequence was then employed to advance the second diastereomer **36** to its final destination, **1b**, through intermediates **38**, **40**, **42**, **44**, and **46**, but again the obtained inseparable mixture (at least two components) did not contain any compound matching the natural substance by TLC or ^{1}H NMR spectroscopy. Although we had no definitive evidence to support a claim that precursors **45** and **46** led to structures **1a** and **1b** either as transient species or as components of the obtained mixtures upon treatment with LiOH, the fact that the natural substance was not present in the mixture was clear, for neither its ^{1}H NMR signals were observed in the spectrum of the isolated mixture of compounds nor did it show up in the TLC experiments that included an authentic sample. The conclusion that the originally proposed structures (i.e., **1a**,**b**) were wrong was also supported by the ^{1}H NMR spectra of compounds **45** and **46** and the

(18) Separation could not be obtained either by preparative TLC (silica gel, CH_3Cl:CH_3OH:H_2O 20:3:1) or by HPLC according to the conditions of Satake et al.[2]

Scheme 6. Synthesis of the C-20 Epimeric Octacyclic Systems **54** and **55**[a]



[a] Reagents and conditions: (a) Pd_2dba_3 (0.3 equiv), $AsPh_3$ (0.3 equiv), LiCl (6.0 equiv), *i*-Pr_2NEt (12.0 equiv); then **6** or *ent*-**6** (0.03 M in THF, syringe pump addition), NMP, 45 °C, 4 h, 58% for **48**, 61% for **49**; (b) HF•py (excess), THF:pyridine (1:1), 0 → 25 °C, 2.5 h; (c) NIS (10 equiv), $NaHCO_3$ (30 equiv), THF, 0 °C, 16 h, 68% for **52**, 60% for **53** over two steps; (d) Et_3B (1.0 M in hexanes, 3.0 equiv), *n*-Bu_3SnH:toluene (1:2), 0 °C, 5 min, 79% for **54**, 85% for **55**.

intermediates leading up to them, which consistently exhibited significant differences in the proton chemical shifts from the ones reported for the natural substance, especially for H-6, H-7, H-8, and H-9.

3. Synthesis of Two of the C-20 Epimers of the Originally Proposed Structures and a Second-Generation Late-Stage Sequence. At this stage we decided to pursue the synthesis of the C-20 epimers of structures **1a**,**b**, despite our suspicions that there was more to the structural discrepancy between these structures and that of the natural product (based on ^{1}H NMR spectroscopic differences). We did this because it was the most immediate option available to us at the time, and also in the hope that, even if it did not tell the entire story, this expedition may guide us, at least to some extent, in the right direction. The inversion of the C-20 hydroxyl group proved nontrivial, as numerous attempts to bring this about with Mitsunobu inversion,[19] stereoselective reduction[20] of the C-20 carbonyl compound, or S_N2-type displacement employing substrates with several leaving groups were met with failure. It was finally found that triflate formation (Tf_2O, py, 4-DMAP, 68% yield from **29**), followed by reaction of the intermediate triflate with KNO_2 in the presence of 18-crown-6, resulted in complete inversion of configuration at C-20,[21] leading to **47** in 45% yield (Scheme 5).

Schemes 6 and 7 summarize the chemistry used to arrive at the azaspiracid C-20 epimers (**2** and **3**) following the previously described chemistry (see Scheme 3). Unfortunately, as with **1a**,**b**, the last step (LiOH-induced cleavage of the C-25 acetate) proved problematic in terms of yielding a single product.[18] As before, and as we expected, no component of the resulting mixtures corresponded to natural azaspiracid-1 by TLC or ^{1}H NMR spectroscopic analysis. Furthermore, and as we brought it to an end, this excursion, like the ones before it, left us with no clue as to the true structure of the natural product.

Convinced of the fact that the originally proposed structural assignments for azaspiracid-1 were in error, but troubled by our failure to produce clean samples of these structures in the final operation, we set out to modify the final stages of the synthesis so as to avoid exposure of the precursors to LiOH in the final step. Since these advanced intermediates proved robust to TBAF conditions, we targeted the C-25 TBS ether as the penultimate precursor to the targeted structures, and thus followed the sequence shown in Scheme 8. The diacetate **66** was selectively prepared from tetraol **28** by treatment with AcCl in the presence

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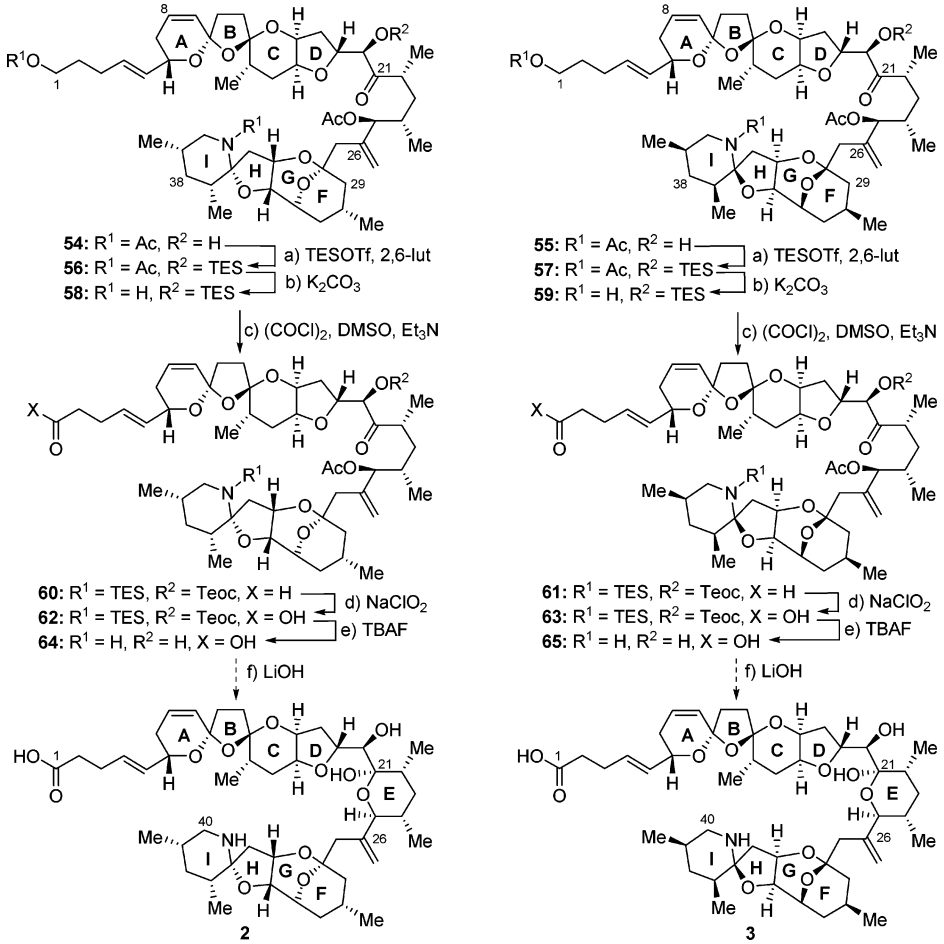
(21) Jain, R.; Kamau, M.; Wang, C.; Ippolito, R.; Wang, H.; Dulina, R.; Anderson, J.; Gange, D.; Sofia, M. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2185.

Scheme 7. Final Sequences of the Total Syntheses of C-20-*epi*-**1a** (**2**) and C-20-*epi*-**1b** (**3**)[a]



[a] Reagents and conditions: (a) TESOTf (10 equiv), 2,6-lutidine (20 equiv), CH_2Cl_2, −78 → 0 °C, 10 min; (b) K_2CO_3 (10 equiv), MeOH, 25 °C, 2 h; (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, 42% for **62** and 51% for **63** over four steps; (e) TBAF (5.0 equiv), THF, 25 °C, 2 h, 77% for **64**, 80% for **65**; (f) LiOH (10 equiv), MeOH:H_2O (5:1), 25 °C, 16 h, 45% (for the total inseparable mixture of at least two compounds represented by **2**) and 38% (for the total inseparable mixture of at least two compounds represented by **3**).

of 2,4,6-collidine in CH_2Cl_2 at −78 °C (89% yield) and converted to the C-25 TBS ether **67** with TBSOTf and 2,6-lutidine (82% yield). Oxidative cleavage of the dithiane moiety from the latter compound [$PhI(OCOCF_3)_2$, 61% yield] then allowed the generation of hydroxy ketone-allylic acetate **68**, which underwent smooth Stille coupling with stannane **6** as before (Pd_2dba_3, LiCl, $AsPh_3$, *i*-Pr_2NEt, 63% yield) to afford coupled product **69**. The TES group was then selectively removed from **69** by the action of TBAF (88% yield), and the resulting dihydroxy compound was subjected to the iodoetherification conditions developed previously (NIS, $NaHCO_3$), affording iodoether **71** in 60% yield. Reductive removal of the iodide residue from the latter comound (**71**) then led to octacyclic intermediate **72** (55% yield), whose advancement to carboxylic acid **76** proceeded smoothly as with the previous series, and through intermediates **73** (TESOTf, 2,6-lutidine, 90% yield), **74** (K_2CO_3, MeOH, 79% yield), and **76** [$(COCl)_2$, DMSO; Et_3N, followed by $NaClO_2$ oxidation, 80% yield over the two steps]. The three silicon-based protecting groups guarding the originally proposed structure **1a** were then removed by exposure to TBAF, but the product was, once again, an inseparable mixture of at least two compounds,[18] none of which matched natural azaspiracid-1 by TLC or ^1H NMR spectroscopy. Changing the fluoride source for the final step (e.g., TASF, HF·py, HF·Et_3N) did not improve the outcome.

Conclusion

Having carried out the above-described investigations, we had proven the mistaken structural identity originally ascribed to azaspiracid-1, but we were nowhere near knowing the real structure of this fascinating substance. Where was, or were, the mistake(s)? That was the lingering question, one that could not be answered through the information available to us at the time. At this juncture, we concluded that wherever the error(s), a drastically new approach had to be injected into our synthetic program if we were to make headway toward elucidating the azaspiracid-1 riddle. The new inspiration and assistance would come from the natural substance itself, despite its extreme scarcity, as we will describe in a subsequent publication.[3]

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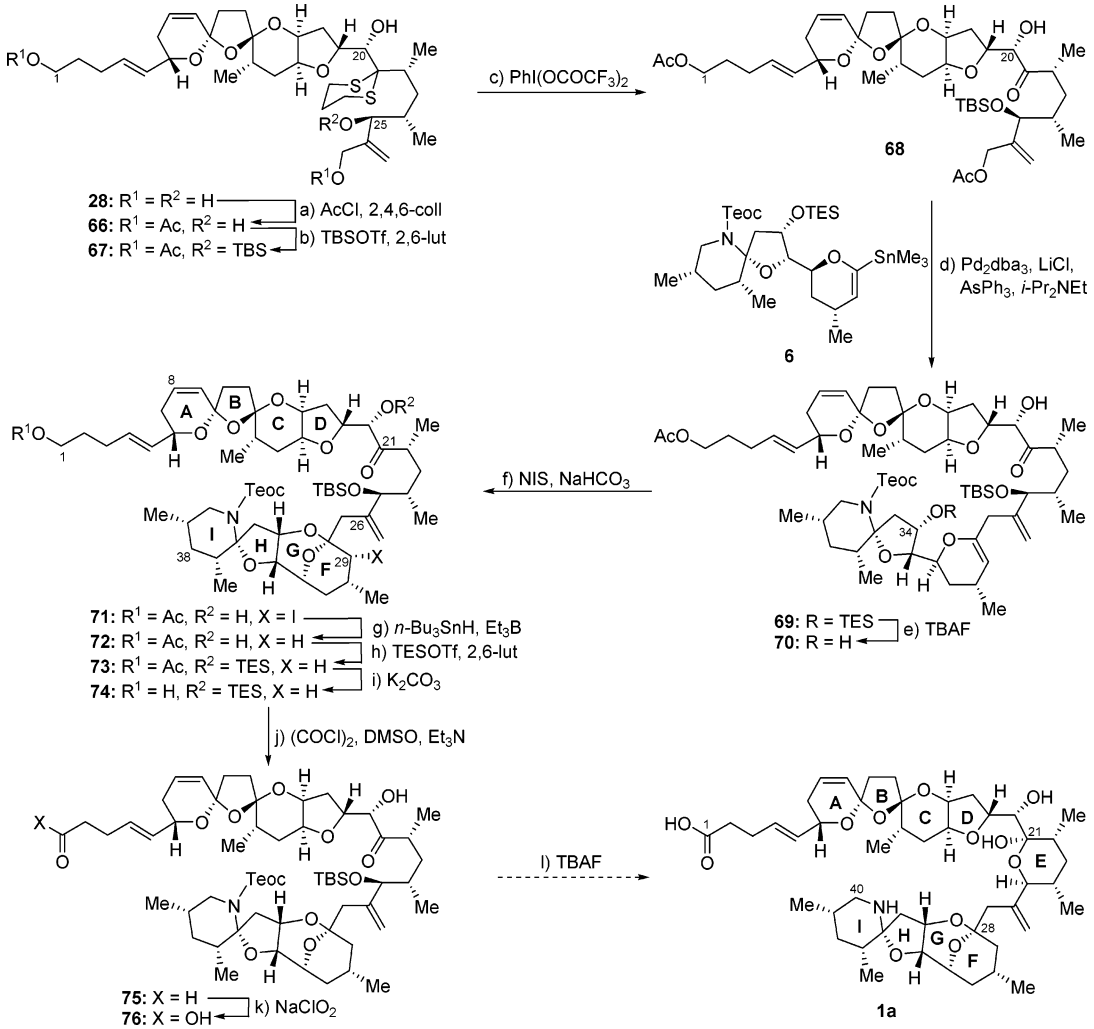
Scheme 8. Second Generation Approach toward the Originally Proposed Structure **1a**[a]



[a] Reagents and conditions: (a) AcCl (20 equiv), 2,4,6-collidine (40 equiv), CH_2Cl_2, −78 °C, 6 h, 89%; (b) TBSOTf (10 equiv), 2,6-lutidine (20 equiv), CH_2Cl_2, −78 → 0 °C, 30 min, 82%; (c) $PhI(OCOCF_3)_2$ (2.0 equiv), MeCN:pH 7 buffer (4:1), 0 °C, 61%; (d) **68**, Pd_2dba_3 (0.3 equiv), $AsPh_3$ (0.3 equiv), LiCl (6.0 equiv), *i*-Pr_2NEt (12 equiv); then **4** (3.0 equiv, 0.03 M in THF, syringe pump addition), NMP, 40 °C, 1 h, 63%; (e) TBAF (1.0 M in THF, 1.2 equiv), THF, 0 °C, 20 min, 88%; (f) NIS (2.0 equiv), $NaHCO_3$ (10 equiv), THF, 0 °C, 12 h, 60%; (g) Et_3B (1.0 M in hexanes, 0.2 equiv), *n*-Bu_3SnH:toluene (1:2), 0 °C, 5 min, 55%; (h) TESOTf (10 equiv), 2,6-lutidine (20 equiv), CH_2Cl_2, −78 → 0 °C, 10 min, 90%; (i) K_2CO_3 (10 equiv), MeOH, 25 °C, 2 h, 79%; (j) $(COCl)_2$ (10 equiv), DMSO (20 equiv), CH_2Cl_2, −78 °C, 1 h; then Et_3N (50 equiv), −78 → −20 °C, 30 min; (k) $NaClO_2$ (10 equiv), NaH_2PO_4 (10 equiv), 2-methyl-2-butene (excess), *t*-BuOH:H_2O (4:1), 25 °C, 30 min, 80% over two steps; (l) TBAF (5.0 equiv), THF, 25 °C, 15 h, 45% (for the total inseparable mixture of at least two compounds represented by **1a**). Abbreviation: TBS, *tert*-butyldimethylsilyl.

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

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