Total Synthesis of the Novel Immunosuppressant Sanglifehrin A

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Abstract: The total synthesis of the novel immunosuppressant sanglifehrin A (SFA, **1**) is described. The approach is flexible, convergent, and stereoselective. The use of Paterson's aldol methodology was pivotal for the preparation of the novel, highly substituted spirolactam fragment of SFA. The 22-membered macrocyclic core of the molecule and the coupling of this fragment to the spirolactam moiety were successfully achieved using selective intra- and intermolecular Stille reactions, respectively. Carbodiimide-based protocols were employed for the synthesis of the tripeptide backbone.

Introduction

The discovery of immunosuppressive agents such as cyclosporin A (CsA) and FK506 has led to a significant increase in the success of organ and bone marrow transplantation and to a greater understanding of the molecular basis of signal transduction pathways.[1] These structurally distinct natural products form two different drug−protein complexes that inhibit the phosphatase activity of the intracellular signaling molecule calcineurin.[2] Such inhibition blocks T-cell activation and prevents host rejection of transplants. Recently, an exciting new immunosuppressive compound, sanglifehrin A (SFA, **1**), was discovered by scientists at Novartis[3] during their screening for compounds that would interfere with signaling molecules other than calcineurin. Produced by *Streptomyces* sp A92-309110 found in a soil sample in Dembo-Bridge in Malawi, SFA possesses impressive biological properties.[4] These include strong binding to cyclophilin, immunosuppressive activity, and inhibition of both T-cell and B-cell proliferation.[3,4] Studies concerning the mode of action of SFA and analogues thereof should advance our understanding of the immune response at the molecular level and thereby facilitate the design of immunosuppressants in the future.

The structure of **1** has been fully elucidated by spectroscopic and X-ray crystallographic techniques[5] and is shown in Figure 1. Key features include a novel, highly substituted [5,5] spirolactam moiety and a 22-membered macrocycle possessing a peptidic backbone characterized by unusual β-substituted piperazic acid and *m*-hydroxyphenylalanine units. The macrocycle also contains four contiguous chiral centers at positions 14−17 as well as endo- and exocyclic *E*,*E* cumulated diene units. Such challenging structural novelty combined with exciting biological activity led us to tackle the total synthesis of **1**. In planning our approach, we hoped to develop a convergent, flexible, and stereocontrolled route that would minimize protecting group manipulations and provide a platform from which entry to analogues of biological interest could ultimately be realized. A detailed account of our successful endeavors toward the first total synthesis of **1** is presented in this paper.[6]

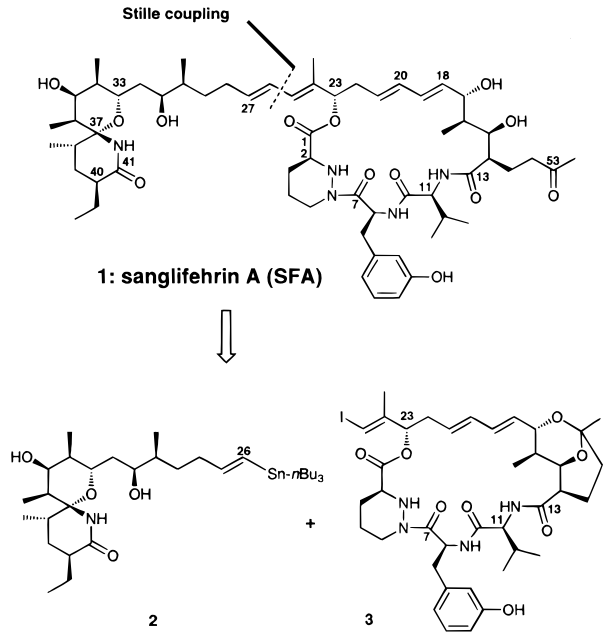

Figure 1. Structure and retrosynthetic analysis of sanglifehrin A.

Results and Discussion

Retrosynthetic Analysis. Recognizing the importance of developing a convergent synthesis of **1,** we divided the molecule

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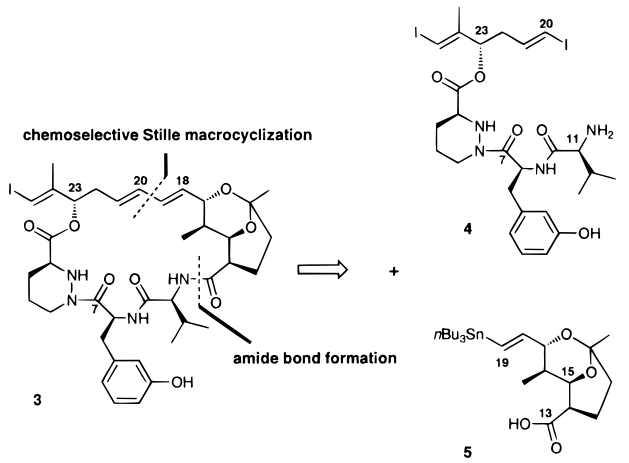

Figure 2. Retrosynthetic analysis of the macrocyclic core **3** of sanglifehrin A.

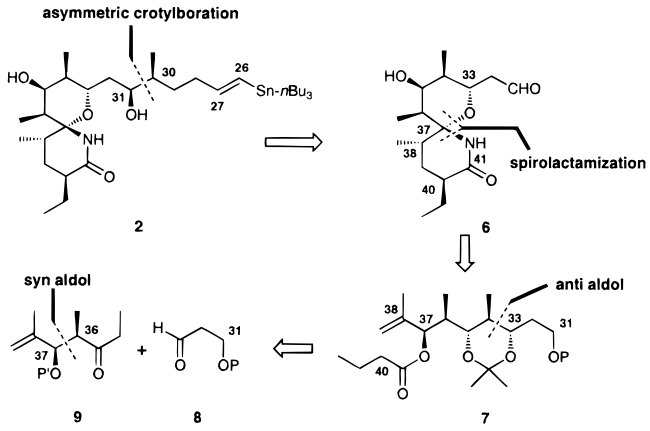

Figure 3. Retrosynthetic analysis of spirolactam fragment **2** of sanglifehrin A.

into two principal fragments resulting from the cleavage of the C25−C26 σ bond of the exocyclic diene unit (Figure 1). This disconnection revealed the *E*-vinylstannane **2** and the *E*-trisubstituted alkenyl iodide **3**, union of which was envisaged via a palladium-mediated Stille coupling.[7] Notably, intramolecular protection of the C15,C17 diol unit and the C53 methyl ketone in **1** via ketalization was proposed given their convenient proximity.

Our approach to the macrocyclic core of **1** involves a novel Stille macrocyclization[8] and a projected racemization-free amide bond formation (Figure 2). Thus, sequential disconnection of the C19−C20 and N12−C13 σ bonds revealed the bis(vinyl)-iodoamine **4** and the vinylstannane ketal carboxylic acid **5** as potential key intermediates. This approach was expected to be challenging principally because formation of the desired 22-membered macrocycle would require the chemoselective participation of the C20 vinyl iodide. To our knowledge, when we began this project there were no reports in the literature of such an intramolecular chemoselective Stille cyclization. Key projected steps for the synthesis of **4** and **5** include stereoselective epoxidation and regioselective epoxide opening for the introduction of the C15 and C14 stereocenters, respectively, asymmetric allylboration for the incorporation of the C23 stereogenic center, and carbodiimide-based coupling protocols for the generation of the tripeptide backbone.

Figure 3 outlines the retrosynthetic analysis of the spirolactam fragment of **1**. We envisioned the introduction of the desired C30 and C31 stereogenic centers via asymmetric crotylboration of the spirolactam aldehyde **6**. Key steps proposed for the conversion of stereopentad **7** to **6** include a stereoselective Ireland−Claisen rearrangement,[9] a substrate-controlled hydroboration, and a thermodynamically controlled spirolactamization for the incorporation of the C40, C38, and C37 stereocenters. We planned to synthesize the C31−C39 fragment **7** using methodology developed by Paterson and co-workers.[10] This would require an antialdol coupling reaction between the achiral aldehyde **8** and ethyl ketone **9**, followed by in situ carbonyl reduction.

Synthesis of the C13−C19 Vinylstannane Ketal Carboxylic Acid 5. The sequence leading to the required **5** is shown in Scheme 1. The known α,β-unsaturated ester **10**[11] was initially converted to the triisopropylsilyloxy (TIPS) ether **11** (TIPSCl, imidazole, 95%) which was then reduced with DIBAL (87%) to the corresponding allylic alcohol **12**. *m*CPBA-mediated epoxidation of allylic alcohol **12** provided epoxide **13** in quantitative yield and in good selectivity (β:α epoxide ratio ~6:1). Regiospecific ring opening[12] of epoxide **13** with 3-butenylmagnesium bromide[13] in the presence of CuI led to olefinic diol **14** (79% yield), which was chemoselectively converted to the primary pivaloate ester **15** (95%). After removal of the TIPS protecting group with tetra-*n*-butylammonium fluoride (TBAF, 81%), Wacker oxidation[14] of diol pivaloate ester **16** was followed by the proposed acid-induced ketalization (vide supra) of the intermediate methyl ketone providing ketal **17** in excellent yield (88% for two steps). Unmasking of the C18 primary hydroxyl function was achieved by debenzylation of ketal **17** (H_2, 10% Pd/C) providing hydroxy ketal **18** which was oxidized to aldehyde ketal **19** (83% for two steps) using TPAP/NMO.[15] A one-carbon homologation of **19** to acetylenic ketal **20** was next achieved in 98% yield using the Ohira−Bestmann reagent[16] in the presence of K_2CO_3. Conveniently, the pivaloate group was also cleanly removed during this operation once an excess of K_2CO_3 was added. A key requirement for the success of this reaction was that an equimolar amount of the diazophosphonate and base be premixed prior to the addition of aldehyde ketal **19**; otherwise the reaction is particularly sensitive to epimerization at C17. Confirmation of the expected stereochemical outcome of the epoxidation and the ring-opening reactions was provided by X-ray crystallographic analysis of acetylenic ketal **20** (see Figure 4).[17] A highly regio- and stereoselective

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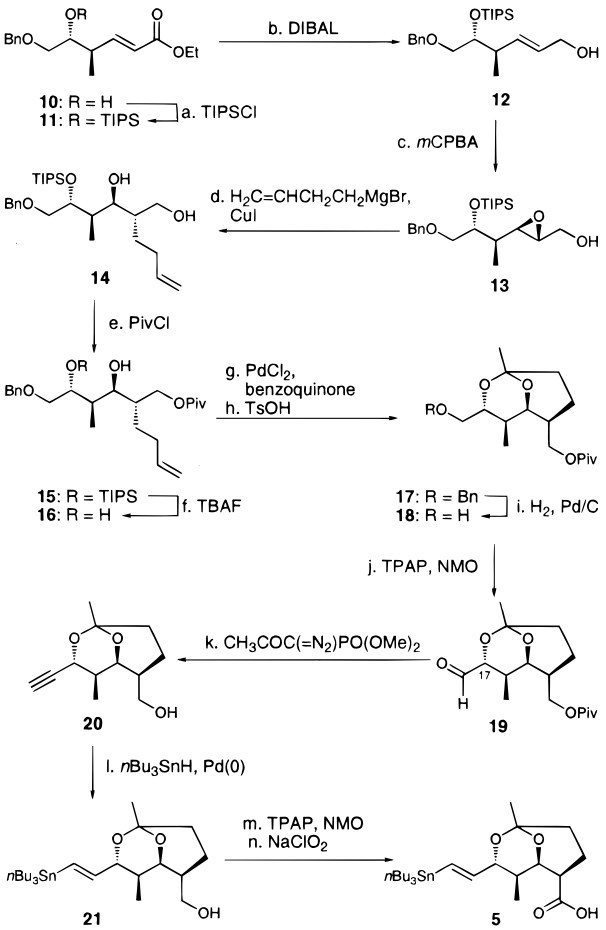
(17) The crystal data, atomic coordinates, etc., have been deposited with the Cambridge Crystallographic Data Centre.

Scheme 1. Synthesis of Carboxylic Acid **5**[a]



[a] Reagents and conditions: (a) 2.0 equiv of TIPSCl, 3.0 equiv of imidazole, DMF, 60 °C, 24 h, 95%; (b) 3.0 equiv of DIBAL, CH_2Cl_2, −78 °C, 2 h, 87%; (c) 1.5 equiv of *m*CPBA, CH_2Cl_2, −25 °C, 100%, β:α epoxide ratio ~6:1; (d) 5.0 equiv of $H_2C{=}CHCH_2CH_2MgBr$, 1.0 equiv of CuI, Et_2O/THF (1:1), −40 → −20 °C, 18 h, 79%; (e) 25 equiv of PivCl, 50 equiv of pyridine, 25 °C, 24 h, 95%; (f) 2.0 equiv of TBAF, THF, 25 °C, 1 h, 81%; (g) 0.1 equiv of $PdCl_2$, 1.5 equiv of benzoquinone, DMF/H_2O (7:1), 25 °C, 3 h; (h) 0.05 equiv of TsOH·H_2O, benzene, reflux, 88% for two steps; (i) H_2, 0.1 equiv of 10% Pd/C, EtOH, 25 °C, 1 h, 100%; (j) 0.05 equiv of TPAP, 3.0 equiv of NMO, 4 Å MS, CH_2Cl_2, 25 °C, 20 min; (k) 5.0 equiv of $MeC(O)C(=N_2)PO(OMe)_2$, 5.0 equiv of K_2CO_3, MeOH, 0 → 25 °C, 13 h, then 5.0 equiv of K_2CO_3, 25 °C, 24 h, 73% for two steps; (l) 4.0 equiv of nBu_3SnH, 0.3 equiv of $PdCl_2(PhCN)_2$, 0.6 equiv of P(*o*-tol)$_3$, 4.0 equiv of iPr_2NEt, CH_2Cl_2, −20 °C, 1 h, 80%; (m) 0.05 equiv of TPAP, 3.0 equiv of NMO, 4 Å MS, CH_2Cl_2, 25 °C, 15 min; (n) 6.0 equiv of $NaClO_2$, 2.0 equiv of NaH_2PO_4, 10 equiv of 2-methyl-2-butene (2 M in THF), *t*BuOH:H_2O (5:1), 25 °C, 15 min (good yield, see Scheme 6). *m*CPBA *m*-chloroperbenzoic acid, TBAF = tetra-*n*-butylammonium fluoride, TsOH = *p*-toluensulfonic acid, TPAP = tetra-*n*-propylammonium perruthenate, and NMO = *N*-methylmorpholine *N*-oxide.

palladium(0)-mediated hydrostannylation[18] of **20** provided vinylstannane ketal **21** (79%) which was oxidized in two steps to the desired **5** in good overall yield.

An alternative approach to **20** that offers the advantage of both a shorter synthetic sequence and higher overall yield is presented in Scheme 2. This sequence is primed by the asymmetric crotylboration (67%) of propargylic aldehyde **22**[19] with Brown's *trans*-crotyl (+) diisopinocampheylborane.[20]

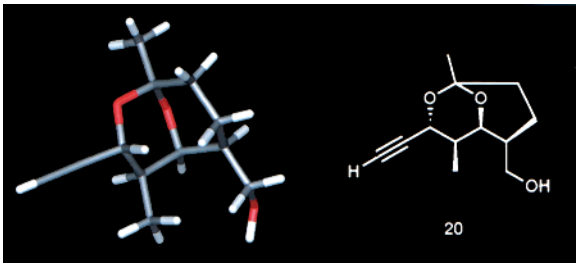
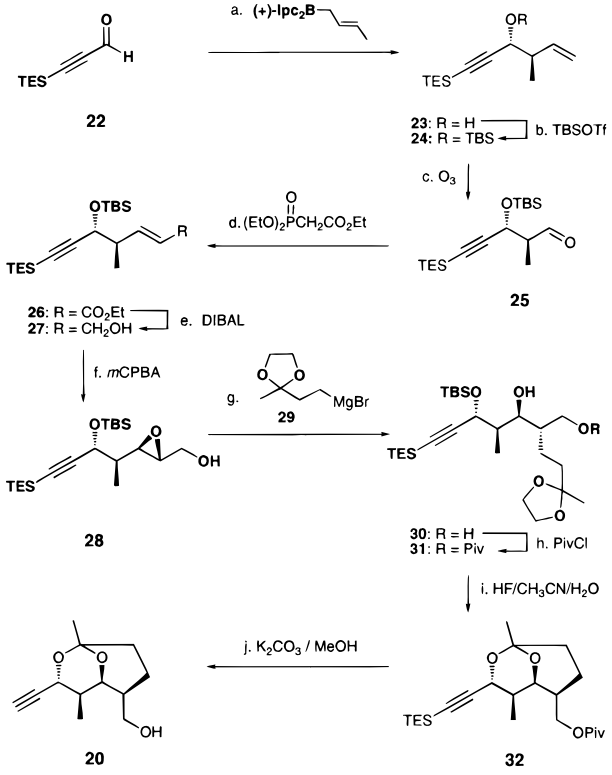


Figure 4. X-ray crystal structure of hydroxy alkyne **20**.

Scheme 2. Alternative Synthesis of Hydroxy Alkyne **20**[a]



[a] Reagents and conditions: (a) 2.5 equiv of (*E*)-crotyldiisopinocampheylborane, THF, −78 °C; then 15 equiv of $NaBO_3{\cdot}4H_2O$, THF/H_2O (1:1), 25 °C, 12 h, 67%; (b) 1.5 equiv of TBSOTf, 2.0 equiv of 2,6 lutidine, CH_2Cl_2, 0 → 25 °C, 12 h, 97%; (c) O_3, 0.2 equiv of Sudan 7B, CH_2Cl_2, −78 °C; then 1.5 equiv of PPh_3, −78 → 25 °C, 12 h; (d) 3.0 equiv of $(EtO)_2P(=O)CH_2CO_2Et$, 3.0 equiv of NaH, THF, −78 → 25 °C, 2 h, 82% for two steps; (e) 2.5 equiv of DIBAL, −78 °C, 0.5 h, 92%; f. 2.0 equiv of *m*CPBA, −30 °C, 17 h, 92%, β:α epoxide ratio ~79:21; (g) 6.0 equiv of **29**, −40 °C, 3 h; (h) 1.3 equiv of PivCl, pyridine, 0 → 25 °C, 78% for two steps; (i) HF:CH_3CN:H_2O, 1:10:1, 25 °C, 2 h, 76%; (j) 5.0 equiv of K_2CO_3, MeOH, 25 °C, 48 h, 92%. *m*CPBA = *m*-chloroperbenzoic acid.

Silylation of the resulting acetylenic alcohol **23** with TBSOTf provided acetylenic silyl ether **24** (97%), which was transformed in a two-step sequence (ozonolysis, followed by phosphonate anion olefination) to the acetylenic α,β-unsaturated ester **26** via acetylenic aldehyde **25** (82% for two steps). Reduction of **26** with DIBAL then furnished acetylenic allylic alcohol **27** in 92% yield. The remaining two stereocenters at C14 and C15 were introduced by a similar sequence of epoxide formation (**27** → **28**, 92% yield, β:α epoxide ratio ~79:21) and ring-opening reactions as described for allylic alcohol **12** (Scheme 1). This alternative route, however, results in a more expedient arrival at acetylenic ketal **20** by directly incorporating oxygenation at C53 through the use of a nucleophilic 3-ketobutyl equivalent

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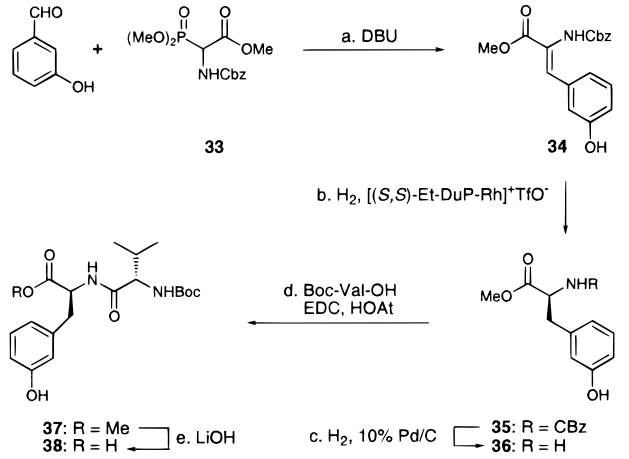
Scheme 3. Synthesis of Fragment **38**[a]

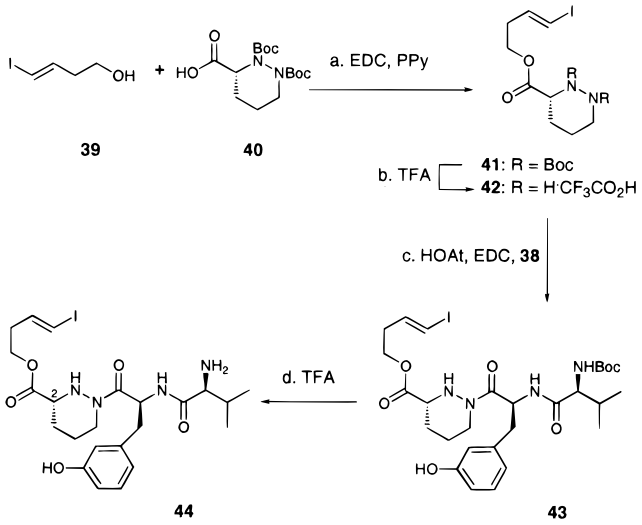


[a] Reagents and conditions: (a) 2.0 equiv of DBU, CH_2Cl_2, 25 °C, 2 h, 90%; (b) 0.7 mol % of [(*S*,*S*)-Et-DuP−Rh)$^+$TfO$^-$, 60 psi, 96 h, 98% ee, 90%; (c) H_2, 10% Pd/C, MeOH, 25 °C, 12 h, 96%; (d) 3.0 equiv of EDC, 3.0 equiv of HOAt, CH_2Cl_2, 0 → 25 °C, 3 h, 78%; (e) 2.0 equiv of LiOH, THF/H_2O (3:1), 0 → 25 °C, 1.5 h, 89%. DBU = 1,8-diazobicyclo[5.4.0]undec-7-ene, [(*S*,*S*)-Et-DuP−Rh]$^+$TfO$^-$ = (+)-1,2-bis[(2*S*,5*S*)-2,5-diethylphospholano]benzene (1,5-cyclooctadiene)rhodium(I) trifluoromethanesulfonate, Boc-Val-OH = *N*-(*tert*-butoxycarbonyl)-L-valine), HOAt = 1-hydroxy-7-azabenzotriazole, and EDC = 1-(3-(dimethylamino)propyl)-3-ethylcarbodimide hydrochloride.

for the ring-opening reaction and by simplifying the deprotecting strategy. Thus, regioselective ring opening of acetylenic epoxide **28** with ketal−magnesium bromide **29**[21] followed by chemoselective pivaloate formation provided acetylenic hydroxy ketal pivaloate **31** via acetylenic hydroxy ketal **30** (66% for two steps). Removal of the *tert*-butylsilyloxy group (HF/CH_3CN/H_2O, 1:10:1, 76% yield) was accompanied by transketalization furnishing acetylenic internal ketal pivaloate **32**. Concomitant cleavage of the pivaloate and triethylsilyl groups in acetylenic internal ketal pivaloate **32** in the presence of K_2CO_3 then completed the sequence leading to acetylenic internal ketal alcohol **20** in 92% yield.

Synthesis of the Tripeptide Fragment of SFA. The synthesis of the macrocyclic core of **1**, requiring an efficient preparation of the tripeptide fragment **4** (Figure 2), began with the stereoselective assembly of the intermediate dipeptide carboxylic acid derivative **38** as outlined in Scheme 3. The key step involved the enantioselective hydrogenation of the α,*β*-didehydro amino acid derivative **34**. Thus, the condensation of *N*-benzyloxycarbonylglycine phosphonate **33** with *m*-hydroxybenzaldehyde in the presence of DBU furnished the diastereomerically pure (*E*)-**34** in 90% yield.[22] Asymmetric hydrogenation[23] of **34** using catalytic [(*S*,*S*)-Et-DuP−Rh]$^+$TfO$^-$ provided the amino acid derivative **35** in excellent *ee* (98%) and yield (90%). Hydrogenolysis of the Cbz group from **35** (96%) followed by carbodiimide-mediated coupling of the resulting amino acid methyl ester **36** in the presence of HOAt[24] with Boc-protected valine then led to dipeptide derivative **37** (78%), which underwent smooth hydrolysis upon treatment with LiOH providing the dipeptide carboxylic acid derivative **38** (89%). With the requisite C7−N12 fragment in hand, the tripeptide fragment was ready to be assembled.

Scheme 4. Synthesis of Model Fragment 44 (C_2-epi as Compared to Sanglifehrin A)[a]



[a] Reagents and conditions: (a) 2.0 equiv of EDC, 0.1 equiv of PPy, 1.0 equiv of *i*Pr_2NEt, CH_2Cl_2, 0 → 25 °C, 80%; (b) TFA:CH_2Cl_2 (1:1), 0 → 25 °C, 2 h; (c) 1.0 equiv of HOAt, 3.0 equiv of *i*Pr_2NEt, 1.2 equiv of EDC, CH_2Cl_2, 0 → 25 °C, 3.5 h, 66% for two steps; (d) TFA:CH_2Cl_2 (1:10), 0 → 25 °C, 2 h (good yield). PPy = 4-pyrrolidinopyridine, TFA = trifluoroacetic acid.

At this stage, we decided to initiate a model study to test the efficacy of the proposed Stille coupling for the preparation of the 22-membered macrocycle.[25] To this end, we chose to replace the trisubstituted vinyl iodide at C23 in tripeptide derivative **3** (Figure 2) with a hydrogen atom. As shown in Scheme 4, the synthesis of model tripeptide ester derivative **44** (note the C_2-epi stereochemistry of this model system as compared to sanglifehrin A) was achieved by a short four-step sequence. Thus, coupling of alcohol **39**[26] with the Boc-protected piperazic acid derivative **40**[27] (C_2-epi as compared to sanglifehrin A) in the presence of EDC provided 2(*R*)-di-Boc-piperazic acid ester **41** in good yield (80%). Treatment of **41** with trifluoroacetic acid (TFA) removed the Boc protecting groups from the nitrogen atoms providing piperazic acid ester **42** which was regioselectively acylated with dipeptide carboxylic acid derivative **38** at the less hindered *β*-position in the presence of EDC/HOAt (66% for two steps) providing tripeptide ester *N*-Boc derivative **43**. Removal of the Boc protecting group from **43** with TFA then furnished tripeptide ester derivative **44** in good yield.

Given the success of this approach, we next set about preparing the fully functionalized tripeptide fragment of **1** in an identical manner (Scheme 5). The requisite hydroxybis(vinyl iodide) **47** was readily prepared from the known iodo aldehyde **45**[28] in two steps. Thus, the chromium(II)-mediated Takai reaction[29] was used for the stereoselective introduction of the C19−C20 (*E*)-vinyl iodide (57% yield), and the C17 hydroxyl group of the resulting product was unmasked by desilylation with TBAF (88% yield) to afford **47** via bis(vinyl iodide) silyl

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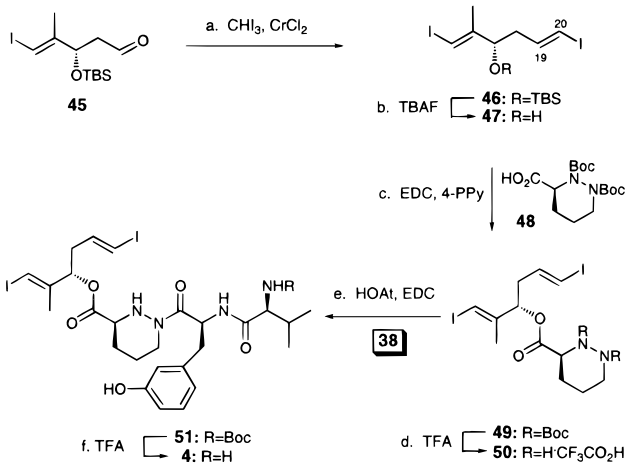
Scheme 5. Synthesis of Fragment **4**[a]



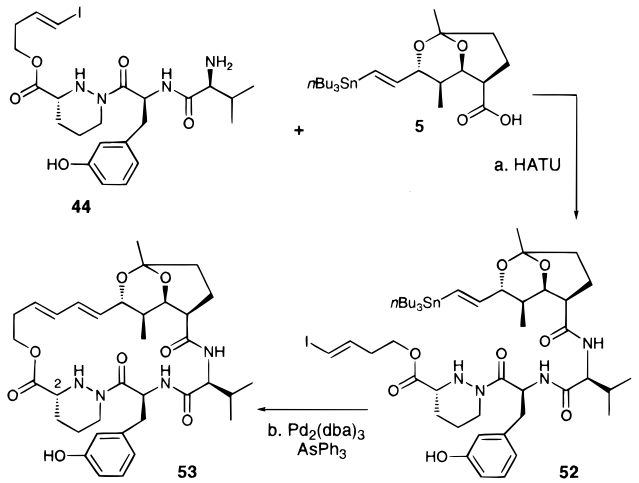
[a] Reagents and conditions: (a) 6.0 equiv of $CrCl_2$, 2.0 equiv of CHI_3, 0 → 25 °C, dioxane/THF (9:1), 12 h, 57%; (b) 1.2 equiv of TBAF, THF, 0 → 25 °C, 15 min, 88%; (c) 2.0 equiv of EDC, 0.1 equiv of PPy, 1.0 equiv of *i*Pr_2NEt, 2.0 equiv of **48**, CH_2Cl_2, 0 → 25 °C, 64%; (d) TFA/CH_2Cl_2 (1:1), 0 → 25 °C, 2 h; (e) 1.0 equiv of HOAt, 3.0 equiv of *i*Pr_2NEt, 1.2 equiv of EDC, CH_2Cl_2, 0 → 25 °C, 3.5 h, 66% for two steps; (f) TFA:CH_2Cl_2 (1:10), 0 → 25 °C, 2 h.

ether **46**. Coupling of **47** with Boc-protected amino acid **48**[27] in the presence of EDC/PPy provided ester 2(*S*)-*N*-Boc-piperazic ester **49** (64% yield) from which both Boc groups were removed upon treatment with TFA to afford 2(*S*)-piperazic ester **50**. Regioselective acylation of **50** with dipeptide carboxylic acid derivative **38** (Scheme 3) (EDC/HOAt) then furnished fragment *N*-Boc-tripeptide ester **51** (66% yield for two steps), which was deprotected with TFA providing tripeptide ester **4** in good yield (Scheme 5). Interestingly, attempts to directly couple the preformed tripeptide unit with **47** failed to provide **51** in good yield (<30%).

Synthesis of the Macrocyclic Core of SFA. The synthesis of the 2(*R*)-sanglifehrin A model system **53** (note that this model has the C_2-epi stereochemistry of sanglifehrin A) is outlined in Scheme 6. Thus union of tripeptide ester derivative **44** with vinylstannane ketal carboxylic acid **5** in the presence of EDC/HOAt resulted in a disappointingly low yield of tripeptide ester **52**. Fortunately, the coupling was more efficiently effected using HATU,[24] which furnished **52** in 51% overall yield from vinylstannane ketal **21** (see Scheme 1) with no detectable epimerization. With the cyclization precursor in hand, the crucial macrocyclization was tried next. In light of the reported superiority of $AsPh_3$ over PPh_3 ligands in the palladium(0)-mediated Stille coupling reaction.[30] we decided to attempt the cyclization in the presence of $Pd_2(dba)_3$·$CHCl_3$/$AsPh_3$ and *i*Pr_2NEt. To our delight, the ring closure of **52** proceeded under these conditions, albeit in 40% yield, at room temperature and in the presence of the free phenol and amine functionalities (Scheme 6).

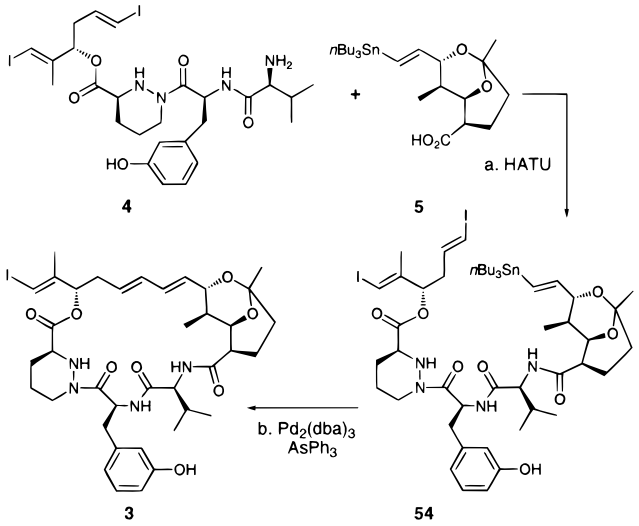
In a parallel study, attempts were made to access **53** via a ring-closing metathesis reaction; in contrast to the Stille reaction, however, none of the desired macrocycle **53** was detected in the complex mixture of products formed upon exposure to (cy_3P)$RuCl_2CHPh$ catalyst.[31] Interestingly, Wagner and co-workers[32] recently reported the successful use of a ring-closing metathesis reaction to access a more simplified model macrocycle of **1** possessing the C18−C21 diene unit. Encouraged by the successful construction of the C_2-epi sanglifehrin A model system via the intramolecular Stille coupling reaction, we proceeded to build sanglifehrin A itself following the chartered strategy.

Scheme 6. Synthesis of Sanglifehrin A Model System **53** (C_2-epi)[a]



[a] Reagents and conditions: (a) 1.0 equiv of HATU, 4.0 equiv of *i*Pr_2NEt, DMF, 0 → 25 °C, 10 h, 51% for three steps from **21** (Scheme 1); (b) 0.15 equiv of $Pd_2(dba)_3$·$CHCl_3$, 0.6 equiv of $AsPh_3$, 10 equiv of *i*Pr_2NEt, DMF, 25 °C, 3 h, 40%. HATU, *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; $Pd_2(dba)_3$·$CHCl_3$, tris(dibenzylideneacetone)dipalladium (0)−chloroform adduct.

Scheme 7. Synthesis of Sanglifehrin Macrocycle **3**[a]



[a] Reagents and conditions: (a) 1.0 equiv of HATU, 4.0 equiv of *i*Pr_2NEt, DMF, 0 → 25 °C, 10 h, 50% for three steps from **21** (Scheme 1); (b) 0.15 equiv of $Pd_2(dba)_3$·$CHCl_3$, 0.6 equiv of $AsPh_3$, 10 equiv of *i*Pr_2NEt, DMF, 25 °C, 36 h, 62%.

The synthesis of the fully functionalized macrocyclic core of **1** is presented in Scheme 7. Thus, in a manner identical to that described for the model system (Scheme 6), the tripeptide **4** was coupled with vinylstannane ketal carboxylic acid **5** providing the final precursor before the key macrocyclization, tripeptide ester **54** (HATU, *i*Pr_2NEt, 50% yield from vinylstannane ketal **21**, Scheme 1). Pleasantly, treatment of **54** with $Pd_2(dba)_3$·$CHCl_3$/$AsPh_3$ in DMF (1.0 mM) for 36 h led to the exclusive formation of the desired sanglifehrin A cyclic intermediate **3** in an isolated yield of 62%. More prolonged reaction times led to significantly reduced yields of sanglifehrin

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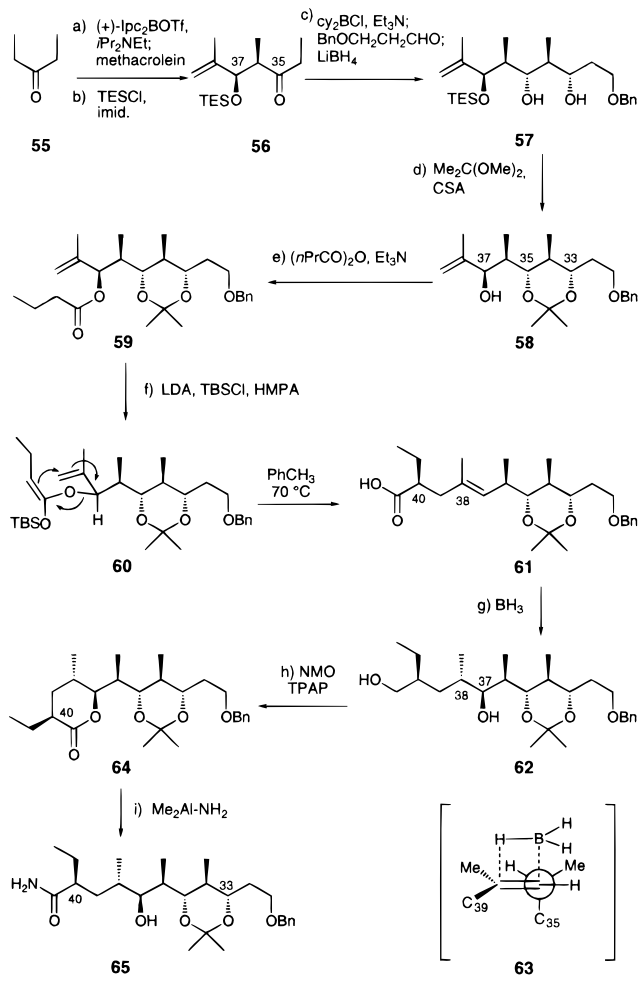
Scheme 8. Synthesis of Fragment **65**[a]



[a] Reagents and conditions: (a) 1.3 equiv of (+)-Ipc_2BOTf, 4.0 equiv of iPr_2NEt, THF, −78 °C, 2 h; then 5.0 equiv of methacrolein, −78 → −10 °C, 10 h; H_2O_2 (30% aq)/CH_3OH/H_2O pH 7 buffer (1.3:5:1), 0 °C, 3 h; (b) 1.2 equiv of TESCl, 1.8 equiv of imidazole, CH_2Cl_2, 0 °C, 2 h, 74% for two steps; (c) 1.5 equiv of cy_2BCl, 1.5 equiv of Et_3N, Et_2O, 0 °C, 1.5 h; then 2.0 equiv of $BnO(CH_2)_2CHO$, −78 → −10 °C, 4 h; then 10 equiv of $LiBH_4$, −78 → 25 °C, 12 h; 15 equiv of $NaBO_3 \cdot 4H_2O$, THF/H_2O (3:2), 15 °C, 12 h, 72%; (d) 0.1 equiv of CSA, 30 equiv of $Me_2C(OMe)_2$, acetone, 72 h, 95%; (e) 2.0 equiv of $(nPrCO)_2O$, 6.0 equiv of Et_3N, CH_2Cl_2, 25 °C, 24 h, 98%; (f) 1.4 equiv of LDA, 6.0 equiv of TBSCl, THF, −78 °C; then HMPA/THF (1:5), −78 → 0 °C, 1 h; toluene, 70 °C, 2 h, 84%; (g) 5.0 equiv of $BH_3 \cdot$THF, THF, −20 °C, 17 h; 15 equiv of $NaBO_3 \cdot 4H_2O$, THF/H_2O (3:2), 15 °C, 12 h; **62**/diastereoisomer ∼5:1 ratio; (h) 0.01 equiv of TPAP, 6.0 equiv of NMO, CH_2Cl_2, 25 °C, 8 h, 51% for two steps; (i) 20 equiv of Me_2Al-NH_2, CH_2Cl_2, 25 °C, 2 h, 90%; TES = triethylsilyl, cy_2BCl = dicyclohexylboron chloride, CSA = camphorsulfonic acid, LDA = lithium diisopropylamide, HMPA = hexamethylphosphoramide, TPAP = tetra-*n*-propylammonium perruthenate, NMO = *N*-methylmorpholine-*N*-oxide, and DMP = Dess−Martin periodinane.

A cyclic intermediate **3**. Having successfully addressed the synthesis of the macrocycle, the next task at hand was to develop an approach to the spirolactam fragment of **1**.

Synthesis of the Spirolactam Fragment of SFA. The enantioselective synthesis of the spirolactam residue of **1** began with the assembly of the key C31−N42 fragment acetonide amide **65** (Scheme 8), which possesses six of the seven requisite stereocenters. The short, nine-step sequence leading to **65** is shown in Scheme 8. Asymmetric aldol methodology[10] and substrate-controlled hydroboration were used to install the array of alternating oxygenated and methyl functionalities on the C33−C38 backbone, while the Ireland−Claisen rearrangement[9] was elected to introduce the C40 stereocenter. Thus, formation of the (*Z*)-boron enolate from diethyl ketone **55** and (+)-diisopinocamphenyl boron triflate in the presence of iPr_2NEt[33] followed by the addition of methacrolein[10a] provided, after silylation with TESCl, the syn aldol derivative TES-protected aldol **56** in 74% overall yield. The absolute stereochemistry of **56** was tentatively assigned as 3*R*, 4*R* based on analogy with examples reported by Paterson[10a,34] for which reaction stereocontrol was rationalized in terms of a chairlike transition state in which steric interactions between the Ipc ligands and the substituents in the transition-state core are minimized. Coupling of the (*E*)-dicyclohexylboron enolate derived from ethyl ketone TES-protected aldol **56** and cy_2BCl-iPr_2NEt with 3-benzyloxypropanal followed by in situ reduction with lithium borohydride[10c] provided stereopentad diol **57** in 72% yield (Scheme 8). The rationale for the sense of stereochemical control of such one-pot aldol−reduction reactions has been discussed by Paterson and co-workers.[35] Proceeding with the synthesis, the requisite Ireland−Claisen precursor acetonide ester **59** was readily prepared in two steps from diol **57**. Thus, treatment of diol **57** with 2,2-dimethoxypropane in the presence of camphorsulfonic acid (CSA) led to the concomitant formation of the C33−C35 syn acetonide and the unmasking of the C37 hydroxyl group furnishing hydroxyacetonide **58** in 95% yield. Analysis of the ^1H and ^{13}C NMR spectra[36] of the resulting **58** was in complete agreement with the expected stereochemical outcome of the anti aldol bond construction and the in situ reduction (vide supra).

Acylation of **58** with butyric anhydride in the presence of Et_3N provided acetonide ester **59** (98%), which underwent enolization−silylation (LDA, TBSCl) to generate the required acetonide keteneacetal **60**. Thermolysis of **60** in toluene followed by hydrolysis of the resulting silyl ester then provided acetonide carboxylic acid **61** in 85% yield and as a single diastereoisomer. The *S* stereochemistry of the ethyl-substituted chiral center and the *E* geometry about the trisubstituted double bond were tentatively assigned on the assumption that the Ireland−Claisen rearrangement proceeded via the well-precedented chairlike transition state[9] (see structure **60**, Scheme 8). This assumption was subsequently confirmed unambiguously by X-ray analysis of a more advanced intermediate (vide infra).

At this stage, the final relevant stereogenic center at C38 was introduced by a substrate-controlled regio- and stereoselective hydroboration of trisubstituted alkene acetonide carboxylic acid **61**. Thus, treatment of **61** with borane at −25 °C followed by oxidative workup provided a ∼5:1 mixture of diastereomeric diols in favor of the desired acetonide diol **62**. The stereochemistry of the newly formed stereocenters at C37 and C38 were predicted by invoking Houk's transition-state model[37] in which the largest group on the chiral center flanking the double bond in **61** is anti to the attacking borane and allylic 1,3-strain is minimized as illustrated in **63** (see Scheme 8). Attempts to improve the selectivity were unsuccessful since the alkene was unreactive in the presence of more bulky hydroborating reagents (thexylborane and 9-BBN). Moreover, the employment of low temperature (−25 °C) was critical to the success of the

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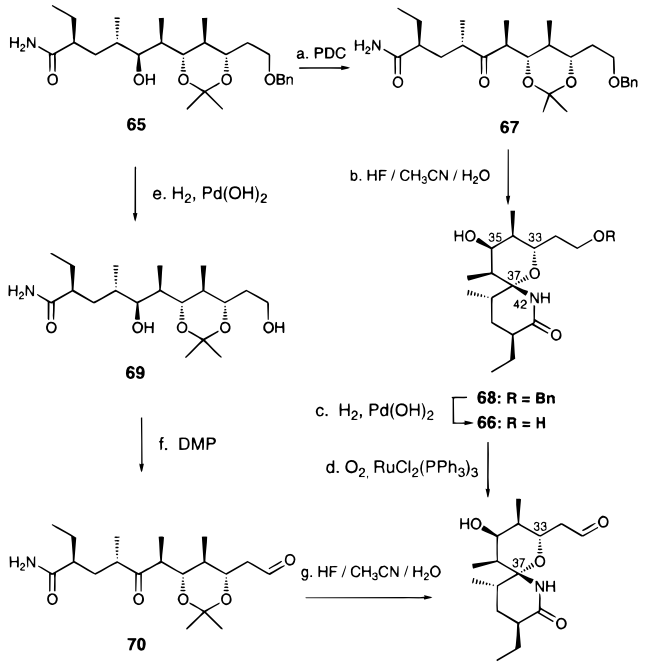
Scheme 9. Synthesis of Spirolactam **6**[a]



[a] Reagents and conditions: (a) PDC, CH_2Cl_2, 25 °C, 12 h, 76%; (b) $CH_3CN/HF/H_2O$ (20:1:1), 25 °C, 36 h, 95%; (c) H_2, 20% $Pd(OH)_2/C$ (cat.), EtOH, 25 °C, 12 h, 99%; (d) 0.08 equiv of $RuCl_2(PPh_3)_3$, air, C_6H_6, 25 °C, 3 h, 83%; (e) H_2, 20% $Pd(OH)_2/C$ (cat.), EtOH, 25 °C, 12 h, 100% (f) 2.7 equiv of DMP, 4.0 equiv of pyridine, CH_2Cl_2, 25 °C, 30 min, 55%; (g) $CH_3CN/HF/H_2O$ (20:1:1), 25 °C, 36 h, 95%. DMP = Dess−Martin periodinane.

hydroboration reaction since higher temperatures favored formation of the C33 isopropyl ether via intramolecular reduction of the acetonide function.[38] Oxidation of acetonide diol **62** in the presence of its C37−C38 diastereoisomer with TPAP/NMO[15] led to the direct formation of a mixture of diastereoisomeric lactones from which the desired compound acetonide lactone **64** was isolated in 51% yield from **61** after chromatography. The final step in the sequence leading to the C31−N42 fragment required the conversion of **64** to acetonide amide **65**. To this end, treatment of **64** with dimethylaluminum amide[39] furnished **65** in 90% yield. The next task at hand was the elaboration of **65** to the spirolactam core of **1**.

Prior to presenting our synthesis of the spirolactam hydroxyaldehyde **6** (Scheme 9) it is relevant at this stage to comment on computer modeling that was carried out on the natural and unnatural spirolactams diol **66** and C37-epi-**66**, respectively, shown in Figure 5. Molecular dynamics followed by minimization with the CFF91 force field[40] showed that the natural spirolactam is more stable than the unnatural isomer by ∼5 kcal/mol. Thus, under thermodymanic control, stabilizing anomeric effects and destabilizing steric factors, considered together, are expected to direct spirocyclization in favor of the natural spirolactam. Moreover, the natural compound is probably further stabilized by an intramolecular H-bond between *H*N-42 and H*O*-C35. The confirmation of this assumption was provided by X-ray crystallographic analysis of a key cyclized intermediate (vide infra).

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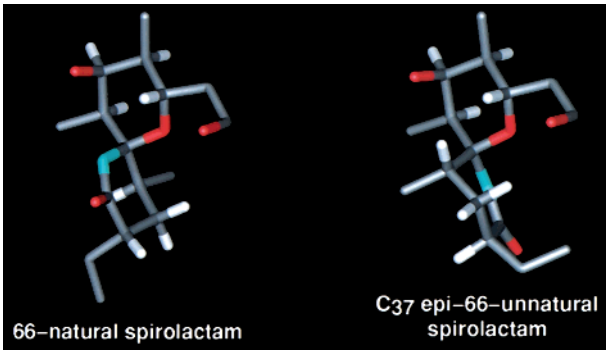
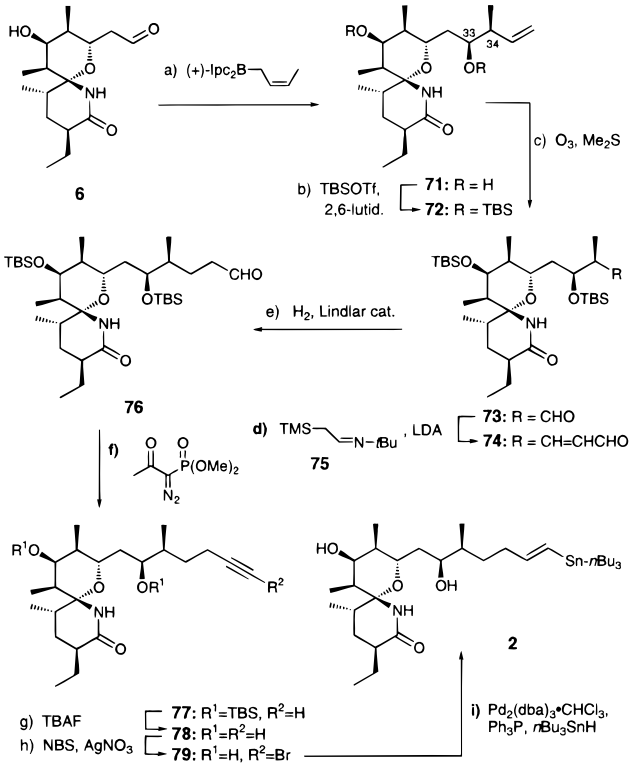


Figure 5. Computer modeling of natural and unnatural spirolactam diols.

Scheme 10. Synthesis of Sanglifehrin A Spirolactam **2**[a]



[a] Reagents and conditions: (a) 5.0 equiv of (*Z*)-crotyldiisopinocampheylborane, THF, −78 °C, 2 h, −78 → 25 °C, 1 h; $NaBO_3 \cdot 4H_2O$, THF/H_2O (3:2), 25 °C, 12 h, 67%, **71**:*β*-isomer ∼7:3 ratio; (b) 2.4 equiv of TBSOTf, 3.6 equiv of 2,6-lutidine, CH_2Cl_2, −10 → 25 °C, 4 h, 92%; (c) O_3, 100 equiv of Me_2S, CH_2Cl_2, −78 → 25 °C, 45 h, 61%; (d) 3.0 equiv of LDA, 3.0 equiv of $TMSCH_2CH{=}N\text{-}t$Bu, THF, −78 → 0 °C, 2.5 h, 68%; (e) H_2, Lindlar catalyst, MeOH, 25 °C, 14 h, 92%; (f) 2.0 equiv of $MeC(=O)C(=N_2)P(=O)(OMe)_2$, 2.5 equiv of K_2CO_3, CH_3OH, 0 → 25 °C, 10 h, 98%; (g) 8.0 equiv of TBAF, THF, 45 °C, 48 h, 87%; (h) 1.2 equiv of NBS, 0.3 equiv of $AgNO_3$, acetone, 25 °C, 30 min, 69%; (i) 0.1 equiv of $Pd_2(dba)_3 \cdot CHCl_3$, 0.8 equiv of Ph_3P, 2.2 equiv of nBu$_3$SnH, 25 °C, 30 min, 70%.

Two alternative routes to **6** are presented in Scheme 9. In the first approach, **65** was transformed into acetonide ketoamide **67** on treatment with PDC. The crucial spirocyclization of **67** was then carried out in the presence of hydrofluoric acid in aqueous acetonitrile. Under these conditions, acetonide hydrolysis and cyclization ensued and the spirolactam hydroxy benzyl ether **68** was isolated as a single diastereoisomer in 95% yield. Hydrogenolysis of benzyl ether **68** (H_2, $Pd(OH)_2$ catalyst 20 wt % on carbon) led to spirolactam diol **66**, X-ray crystallographic analysis of which was in complete accord with the predicted stereochemistry at each of the seven stereogenic

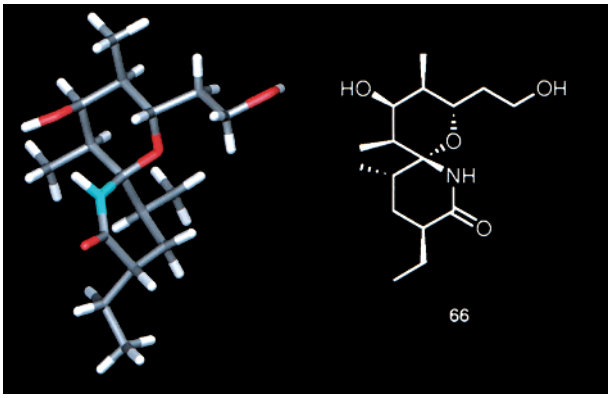


Figure 6. X-ray crystal structure of spirolactam diol **66**.

centers (see Figures 5 and 6). Chemoselective oxidation of the primary hydroxyl function in **66** with oxygen in the presence of tris(triphenylphosphine)ruthenium(II) dichloride catalyst[41] then provided spirolactam hydroxyaldehyde **6** in 83% yield. In a shorter sequence leading to **6**, the primary hydroxyl group was unmasked prior to the spirolactamization step. Thus, debenzylation of **65** (H_2, $Pd(OH)_2$ catalyst 20 wt % on carbon) was followed by bis-oxidation of the resulting acetonide hydroxyamide **69** with Dess−Martin periodinane[42] furnishing keto aldehyde **70** in 55% yield. Complete dehydration of the amide function in **69** resulted unless the oxidation was carried out in the presence of excess pyridine. The use of PDC to effect this oxidation led to significantly reduced yields of **70** (∼30%) due to difficulties encountered during isolation. Acid-induced spirocyclization of **70** then provided **6** stereoselectively and in 95% yield.

The completion of the synthesis of the fully functionalized left-hand fragment of **1** is shown in Scheme 10. Crotylboration of spirolactam hydroxyaldehyde **6** with Brown's *cis*-crotylborane [(+)-Ipc_2B(*cis*-crotyl)] provided a 7:3 mixture of diastereomeric homoallylic alcohols (67% combined yield) that was readily separated by chromatography. Literature precedents using B-crotyl$(Ipc)_2$ suggested that the major diastereoisomer was that corresponding to the indicated stereochemistry at C33−C34 for olefinic spirolactam diol **71**. Unambiguous confirmation of this assumption was established at a later stage in the sequence (vide infra). Treatment of diol **71** with TBSOTf in the presence of 2,6-lutidine (92%) followed by ozonolysis and Me_2S workup provided spirolactam aldehyde **73** (61%) via olefinic spirolactam diol bis(silyloxy ether) **72**. A two-carbon homologation of spirolactam aldehyde **73** to spirolactam aldehyde **76** was achieved via the corresponding spirolactam α,β-unsaturated aldehyde **74**. Thus, reaction of **73** with the lithioderivative of silyl aldimine **75**[43] followed by hydrogenation of the double bond using Lindlar's catalyst[44] directly provided **76** in 64% yield for two steps. Attempts to convert **71** directly to **74** via cross-metathesis with acrolein[45] failed. Next, **76** was transformed into acetylenic spirolactam **77** in excellent yield with $MeC(=O)C(=N_2)P(=O)(OMe)_2$. X-ray crystallographic analysis of **77** confirmed its structure (see Figure 7), including the anticipated stereochemical outcome of the crotylboration (**6** → **71**). At this point the *tert*-butyldimethylsilyloxy protecting groups were removed (**77** → **78**, TBAF, 87% yield) since the free hydroxyl

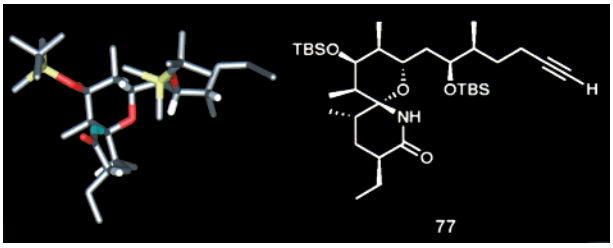
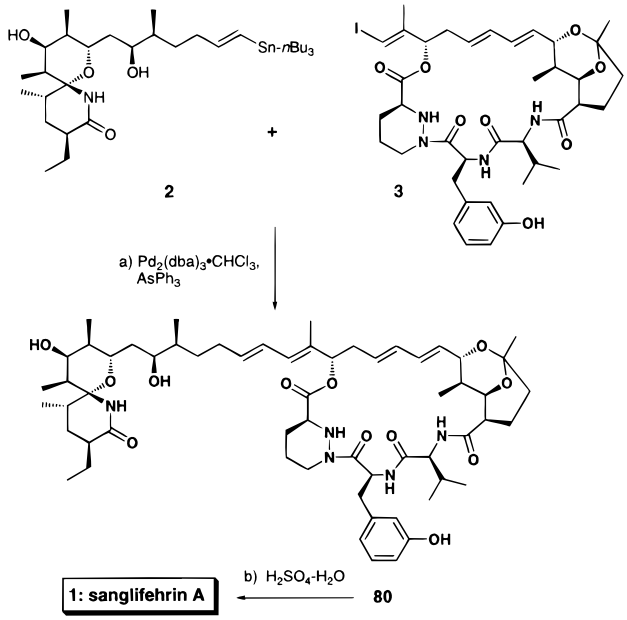


Figure 7. X-ray crystal structure of alkyne **77**.

Scheme 11. Total Synthesis of Sanglifehrin A (**1**)[a]



[a] Reagents and conditions: (a) 0.1 equiv of $Pd_2(dba)_3$•$CHCl_3$, 0.2 equiv of $AsPh_3$, 10 equiv of iPr_2NEt, DMF, 40 °C, 5 h, 45%; (b) 2.0 equiv of 2 N H_2SO_4, THF/H_2O (4:1), 25 °C, 7 h, 50% conversion (by HPLC), 33%.

groups were not expected to be detrimental to subsequent transformations (Scheme 10). In light of the excellent regiospecificity and stereoselectivity observed during the palladium-catalyzed hydrostannylation of bromoalkynes,[46] we chose to access the targeted stannane **2** from acetylenic spirolactam diol **78** via the intermediacy of bromoacetylenic spirolactam **79**. Thus, bromination of **78** with *N*-bromosuccinimide in the presence of a catalytic quantity of silver nitrate[47] provided **79** (69%). This was converted to vinylstannane spirolactam diol **2** on treatment with tri-*n*-butyltin hydride and in situ-generated catalytic $Pd(PPh_3)_4$. With both key coupling partners, **2** and sanglifehrin A cyclic intermediate **3** in hand, the stage was set for the final coupling.

Final Stages of the Total Synthesis of SFA. The completion of the total synthesis of **1** is presented in Scheme 11. Treatment of a mixture of vinylstannane spirolactam **2** and sanglifehrin A cyclic intermediate **3** with a catalytic amount of in situ-generated palladium(0) tetrakistriphenylarsine[30] in DMF at 40 °C led to sanglifehrin A internal ketal **80** in 45% isolated yield. Finally, **1** was generated by unravelling ketal **80** through exposure to aqueous sulfuric acid as described by the Novartis group.[3] Synthetic **1** had physical properties identical to those of an

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authentic sample kindly provided by Dr R. Metternich of Novartis.

Conclusion

In conclusion, a convergent, highly stereocontrolled total synthesis of SFA has been achieved. The palladium-mediated Stille coupling[7] is the keystone of the strategy for the stereospecific construction of the macrocycle and for the union of the left and right-hand fragments of **1**. Highlights of the synthesis include the use of Paterson's aldol methodology[11] for the synthesis of the spirolactam fragment and carbodiimide-based protocols for the construction of the tripeptide backbone. The flexibility of the described strategy allows it to be adapted for the generation of a wide variety of sanglifehrin analogues. Moreover, based on the developed chemistry, a solid-phase version amenable to combinatorial synthesis is imaginable.[48] The described research could ultimately facilitate chemical biology studies in the field of immunosuppression in general and possibly in the elucidation of the, as yet, unknown mechanism of action of this exciting new immunosuppressant, in particular.

(48) During the preparation of this paper, researchers from Novartis reported a convenient procedure for the degradation of sanglifehrin A thereby making fragments of this molecule readily available to be incorporated into combinatorial libraries: Metternich, R.; Denni, D. Thai, B.; Sedrani, R. *J. Org. Chem.* **1999**, *64*, 9632−9639.

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Supporting Information Available: Experimental methods and characterization for **2**, **3**, **6**, **11**, **12**, **14**−**18**, **20**, **21**, **23**−**28**, **30**−**32**, **34**−**38**, **41**−**43**, **46**, **47**, **49**−**54**, **56**−**59**, **61**, **64**−**74**, **76**−**80**, and **sanglifehrin A (1)**. This material is available free of charge via Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

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