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Rapid, High-Yield, Solid-Phase Synthesis of the Antitumor Antibiotic Sansalvamide A Using a Side-Chain-Tethered Phenylalanine Building Block

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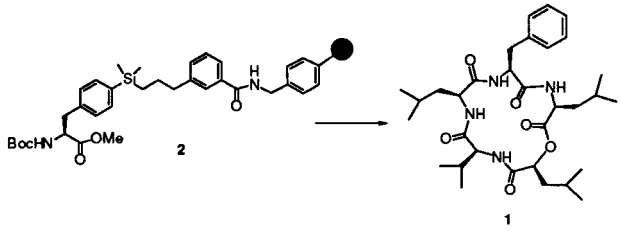
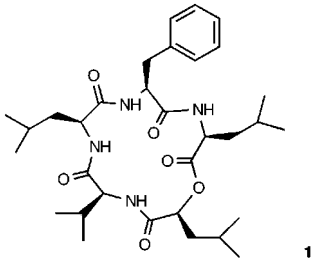
ABSTRACT



A 10-step solid-phase synthesis of the cytotoxic depsipeptide sansalvamide A (1) has been accomplished in an overall yield of 67% with >95% purity employing polymer-bound phenylalanine building block 2. Both the *N*- and *C*-termini of 2 are extended followed by on-resin head-to-tail macrocyclization of the linear peptide in a high yield. This should be a general stategy for the synthesis of diverse libraries of cyclic peptides and depsipeptides that contain exclusively phenylalanine and other hydrophobic side chains.

Sansalvamide A (**1**) is a cyclic depsipeptide produced by a marine fungus of the genus *Fusarium* found on Little San Salvador Island, Bahamas.[1] Structurally, sansalvamide A is



composed of four hydrophobic amino acids (Phe, 2 Leu, Val) and one hydrophobic hydroxy acid ((*S*)-2-hydroxy-4-methylpentanoic acid; *O*-Leu), with five stereogenic centers all having *S*-stereochemistry, as determined by an extensive NMR analysis and chiral capillary GC of the ester derivatives of the amino acids after acid hydrolysis. This highly lipophilic natural product was found to have significant cancer cell cytotoxicity with a mean IC_{50} value of 27.4 μg/mL against the National Cancer Institute's 60 cell-line panel. Therefore, an efficient synthetic methodology to sansalvamide A, which would allow extensive diversity for biological screening purposes, is highly desirable.

Many of the naturally occurring pseudopeptides, such as Dolastin 10 and 15,[2] and cyclic peptides[3] are hydrophobic in nature and pharmacologically active. Although the precise correlation between biological activities and hydrophobicity is unknown, at least one phenylalanine or modified phenyl-

(1) Belofsky, G. N.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1999**, *40*, 2913−2916.

(2) (a) Poncet, J.; Hortala, L.; Busquet, M.; Gueritt-Voegelein, F.; Thoret, S.; Pierre, A.; Atassi, G.; Jouin, P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2855−2858. (b) Akaji, K.; Hayashi, Y.; Kiso, Y.; Kuriyama, N. *J. Org. Chem.* **1999**, *64*, 405−411. (c) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J. C. *Tetrahedron* **1993**, *49*, 9150−9170.

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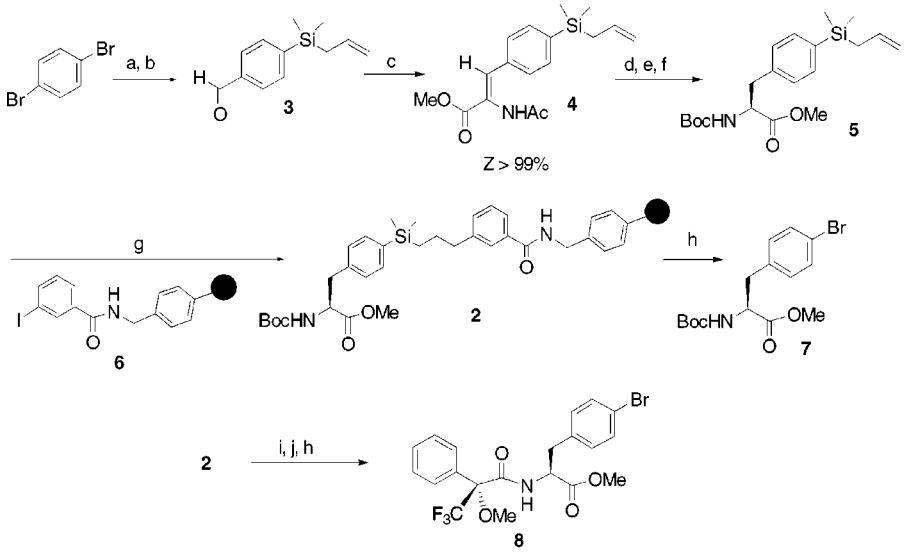
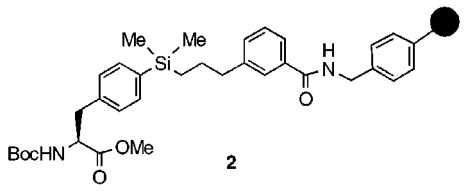
Scheme 1. Synthesis of Phenylalanine Building Block **2**[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, then allylchlorodimethylsilane, 82%; (b) *t*-BuLi, THF, −78 °C, then DMF, 82%; (c) methyl 2-acetamido-2-(dimethoxyphosphinyl)acetate, tetramethylguanidine, THF, −78 °C to room temperature, 86%; (d) [((*S*,*S*)-Et-DuPHOS)-Rh]$^+$ (1 mol %), H_2 (1 atm), CH_2Cl_2, 23 h, 100%; (e) $(Boc)_2O$, DMAP (cat.), THF, reflux, 1 h; (f) hydrazine, MeOH, 4 h, 93% for two steps; (g) 9-BBN, THF, room temperature, 5 h, then **6**, DMF, $Pd(PPh_3)_4$, 2 *N* Na_2CO_3, 75 °C, 3 days; (h) Br_2 in CH_2Cl_2, 20 min; (i) 2% thioanisole and 50% TFA in CH_2Cl_2, 15 min; (j) (*R*)-MTPACl (3 equiv), DIPEA (4 equiv), CH_2Cl_2, 4 h.

alanine residue is commonly found in these compounds. Given the role of phenylalanine as an important pharmacophore in many biologically active agents, solid-phase protocols to phenylalanine-containing compounds are highly desirable.

Recently, we reported arylsilane-based "traceless linker" strategies[4] to attach the aromatic side chain of phenylalanine[5] and β-homophenylalanine[6] to a polystyrene resin, based on the earlier work of Plunkett and Ellman.[4a] Our work accommodates the elaboration of solid-phase bound aromatic amino acid residues in both the *N*- and *C*-terminal directions, which serves as an efficient synthetic tool for constructing libraries of peptides and peptidomimetics containing exclusively phenylalanine and other hydrophobic side chains. The synthesis of cyclic peptides and depsipeptides is generally hampered by low yields in the cyclization step, which requires high dilution conditions and is accompanied by dimerization and oligomerization side reactions; in this case, not only are yields low but purification becomes tedious. Here we report an improved synthetic procedure for the polymer-bound phenylalanine building block **2** and its use



in the first synthesis of the hydrophobic cyclic depsipeptide antitumor antibiotic sansalvamide A. The methodology reported here should prove to be general for the rapid, high-yield synthesis of libraries of cyclic depsipeptides and cyclic peptides containing hydrophobic side chains exclusively.

Our original synthetic method for the polymer-bound phenylalanine building block **2** involves a C−C bond-forming reaction of lithiated Schollkopf's bislactim ether with 4-allyldimethylsilylbenzyl bromide as a key step which results in the formation of two diastereomers in a ratio of 94 to 6.[5] The tedious column chromatography procedure required for the separation of the major isomer led us to investigate an alternative synthetic route for the silylated phenylalanine building block **2** (Scheme 1).

Lithiation of 1,4-dibromobenzene with *n*-butyllithium followed by treatment with allylchlorodimethylsilane gave 1-allyldimethylsilyl-4-bromobenzene; further lithiation with *tert*-butyllithium and reaction with DMF produced 4-allyldimethylsilylbenzaldehyde (**3**). Tetramethylguanidine-mediated condensation of **3** with Horner−Emmons reagent afforded a (*Z*)-enamide ester (**4**) exclusively.[7] Subsequent

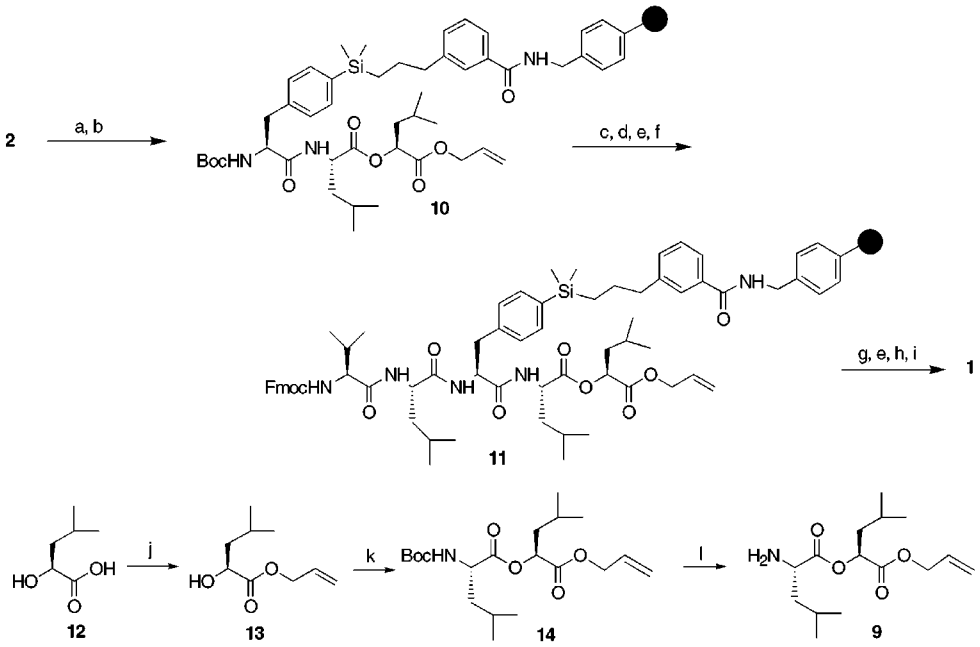
(3) (a) Hawkins, G. J.; Lavin, M. F.; Marshall, K. A.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1990**, *33*, 1634−1638. (b) Degnan, B. M.; Hawkins, C. J.; Lavin, M. F.; McCaffrey, E. J.; Parry, D. L.; van den Brenk, A. L.; Watters, D. J. *J. Med. Chem.* **1989**, *32*, 1349−1354. (c) Norley, M. C.; Pattenden, G. *Tetrahedron Lett.* **1998**, *39*, 3087−3090. (d) Freeman, D. J.; Pattenden, G. *Tetrahedron Lett.* **1998**, *39*, 3251−3254. (e) McKeever, B.; Pattenden, G. *Tetrahedron Lett.* **1999**, *40*, 9317.

(4) (a) Plunkett, M. J.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 6006−6007. (b) Plunkett, M. J.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 2885−2893. (c) Chenera, B.; Finkelstein, J. A.; Veber, D. F. *J. Am. Chem. Soc.* **1995**, *117*, 11999−12000. (d) Boehm, T. L.; Showalter, H. D. H. *J. Org. Chem.* **1996**, *61*, 6498−6499. (e) Woolard, F. X.; Paetsch, J.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 6102−6103. (f) Hu, Y.; Porco, J. A. Jr.; Labadie, J. W.; Gooding, O. W.; Trost, B. M. *J. Org. Chem.* **1998**, *63*, 4518−4521. (g) Newlander, K. A.; Chenera, B.; Veber, D. F.; Yim, N. C. F.; Moore, M. L. *J. Org. Chem.* **1997**, *62*, 6726−6732. (h) Han, Y.; Walker, S. D.; Young, R. N. *Tetrahedron Lett.* **1996**, *37*, 2703−2706.

(5) Lee, Y.; Silverman, R. B. *J. Am. Chem. Soc.* **1999**, *121*, 8407−8408.

(6) Lee, Y.; Silverman, R. B. *Org. Lett.* **2000**, *2*, 303−306.

Scheme 2. Synthesis of Sansalvamide A[a]



[a] Reagents and conditions: (a) LiOH (5 equiv), THF/H_2O (7:1), room temperature, 16 h; (b) HBTU (4 equiv), DIPEA (4 equiv), **9** (4 equiv), NMP, 16 h; (c) 2% thioanisole and 50% TFA in CH_2Cl_2, 15 min; (d) Fmoc-Leu-OH (4 equiv), HBTU (4 equiv), DIPEA (4 equiv), NMP, 6 h; (e) 20% piperidine in DMF, 40 min; (f) Fmoc-Val-OH (4 equiv), HBTU (4 equiv), DIPEA (4 equiv), NMP, 6 h; (g) $CHCl_3$/AcOH/NMM (37:2:1), $Pd(PPh_3)_4$ (4 equiv), 3 h; (h) HBTU (4 equiv), DIPEA (4 equiv), NMP, 16 h; (i) 2% thioanisole and 50% TFA in CH_2Cl_2, 36 h; (j) allyl bromide, K_2CO_3, acetone, 48 h, 95%; (k) Boc-Leu-OH, DMAP (cat), DCC, CH_2Cl_2, 48 h, 63%; (l) 50% TFA in CH_2Cl_2, 5 min.

asymmetric catalytic hydrogenation of **4** with the commercially available [((*S*,*S*)-Et-DuPHOS)-Rh]$^+$ catalyst system in CH_2Cl_2 under H_2 (1 atm) created the desired chiral α-carbon in quantitative yield without affecting the terminal olefin of the allyl group.[8] Interchange of the *N*-acetyl group for *N*-Boc was carried out in one pot by treatment of the chiral intermediate with Boc_2O in the presence of a catalytic amount of DMAP in refluxing THF followed by hydrolysis of the resulting mixed imide with excess hydrazine in MeOH for 4 h. This provided **5** in 93% for two steps.[9] Hydroboration of the terminal olefin of **5**, followed by Suzuki coupling of the generated borane complex with 3-iodobenzamidomethylpolystyrene (**6**),[10] resulted in the production of resin-bound phenylalanine building block **2**. The loading level (0.12 mmol/g) of **2** was determined by the mass balance of **7**, which was obtained by cleaving **2** from the resin with bromine in CH_2Cl_2.[11] Treatment of **2** with 50% TFA in CH_2Cl_2 for 15 min, followed by reaction with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) in the presence of base and subsequent cleavage of the product with bromine in CH_2Cl_2, produced Mosher amide **8**.[12] Analysis of both ^1H and ^{19}F NMR spectra of **8** indicated high enantiopurity (>98% ee) of polymer-bound building block **2**.

As illustrated in Scheme 2, solid-phase synthesis of sansalvamide A was initiated by deprotection of the ester group of **2**, followed by reaction of the resulting acid with *O*-allyl Leu-*O*-Leu (**9**) under standard amide coupling conditions (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), diisopropylethylamine (DIPEA), and *N*-methylpyrrolidinone (NMP)), to give **10**. The depsipeptide ester **9** required for the synthesis of **10** was prepared in three steps: (1) allylation of hydroxy acid **12** with allyl bromide and K_2CO_3 in acetone; (2) condensation of **13** with Boc-Leu-OH in the presence of catalytic (dimethylamino)pyridine (DMAP) and dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 to give **14**; (3) cleavage of the *N*-Boc group with TFA. After deprotection of the *N*-Boc protecting group and extension of the *N*-terminus using Fmoc strategy in a three-step sequence (coupling to Fmoc-Leu-OH/deprotection of Fmoc/coupling to Fmoc-Val-OH), protected linear intermediate **11** was produced. The allyl ester was removed with Pd(0) in $CHCl_3$/AcOH/*N*-methylmorpholine (NMM) (37:2:1),[13] which was followed by cleavage of the Fmoc

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(10) Prepared by amide coupling of 3-iodobenzoic acid with aminomethylated polystyrene (1.02 mmol/g, 100−200 mesh, purchased from Novabiochem Corp.) employing diisopropylcarbodiimide and HOBT in DMF for 2 days at room temperature.

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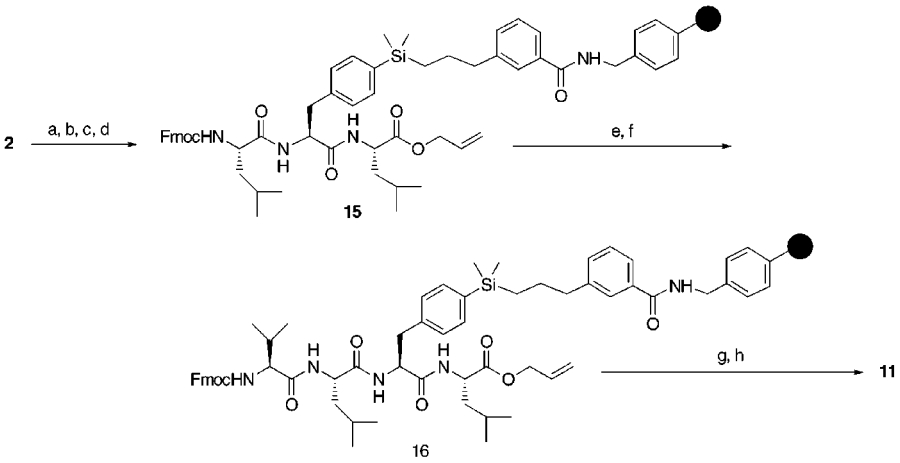
(12) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

Scheme 3. Alternative Synthesis of Intermediate **11** in the Synthesis of Sansalvamide A[a]



[a] Reagents and conditions: (a) LiOH (5 equiv), THF/H_2O (7:1), room temperature, 16 h; (b) *O*-allylLeu (4 equiv), HBTU (4 equiv), DIPEA (4 equiv), NMP, 16 h; (c) 2% thioanisole and 50% TFA in CH_2Cl_2, 15 min; (d) Fmoc-Leu-OH (4 equiv), HBTU (4 equiv), DIPEA (4 equiv), NMP, 6 h; (e) 20% piperidine in DMF, 40 min; (f) Fmoc-Val-OH (4 equiv), HBTU (4 equiv), DIPEA (4 equiv), NMP, 6 h; (g) $CHCl_3$/AcOH/NMM (37:2:1), $Pd(PPh_3)_4$ (4 equiv), 3 h; (h) **13**, MSNT, NMI, CH_2Cl_2, 16 h.

group. Cyclization of the depsipeptide on the polymer support was readily achieved with HBTU and DIPEA in NMP for 16 h. One important advantage of using a solid-phase strategy is that cyclization of the linear (depsi)peptide can occur without the need for dilute conditions and without linear polymer byproduct formation. Finally, cleavage of the cyclized product from the support was performed with 50% TFA/CH_2Cl_2 for 36 h to release sansalvamide A, identical by NMR spectral comparison to natural sansalvamide A,[14] in a 67% yield based on the initial loading level of **2**. The high purity (>95%) of the crude product determined by ^{1}H NMR spectral analysis indicated that the solid-phase methodology proceeded efficiently.

Except for the solution synthesis of **9**, the synthesis of sansalvamide A shown in Scheme 2 is resin bound. To devise a more efficient and general methodology which can be utilized for automated solid-phase synthesis of sansalvamide A analogues, on-resin ester bond formation was carried out (Scheme 3). The resin-bound phenylalanine building block **2** was elaborated in both the *C*- and *N*-directions to give resin-bound diprotected tetrapeptide **16**. Deprotection of the allyl group in **16** followed by coupling of the resulting carboxylic acid with **13** in the presence of 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) and *N*-methylimidazole (NMI) in CH_2Cl_2[15] afforded **11**, which again led to the synthesis of sansalvamide A in comparable yield and purity as described in Scheme 2.

In conclusion, a 10-step total synthesis of the antitumor antibiotic sansalvamide A was carried out from the phenylalanine building block **2** in an overall yield of 67% with >95% purity. Although side-chain tethering of an amino acid has been previously utilized for solid-phase cyclic peptide synthesis, this method has been limited to amino acids having a polar (heteroatomic) side chain which is attached to the polymer support.[13,16] The advantages of the solid-phase approach, therefore, have been lost for those cyclic (depsi)-peptides that contain only hydrophobic side chains. The polymer-bound phenylalanine building block developed in this study, however, can provide rapid access to cyclic peptides and depsipeptides that consist exclusively of hydrophobic side chains. An added advantage of the silicon-based traceless linker strategy[4a] is that the arylsilyl linkage used is compatible with a variety of reaction conditions, as described in the present synthesis, and can be cleaved under several different conditions, which is desirable for linkers used in the synthesis of complex molecules. This methodology should prove to be a general rapid and high-yield approach to the synthesis of libraries of cyclic peptides and depsipeptides containing exclusively phenylalanine and other hydrophobic amino acids.

Supporting Information Available: Complete experimental details and product characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) A sample of sansalvamide A was kindly donated by Professor William Fenical of Scripps Institution of Oceanography (University of California, San Diego).

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(16) For example see (a) Sabatino, G.; Chelli, M.; Mazzucco, S.; Ginanneschi, M.; Papini, A. M. *Tetrahedron Lett.* **1999**, *40*, 809−812. (b) Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639−2642. (c) Kates, S. A.; Sole, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 1549−1552. (d) Sugiura, T.; Hamada, Y.; Shioiri, T. *Tetrahedron Lett.* **1987**, *28*, 2251−2254. (e) Zhong, H. M.; Greco, M. N.; Maryanoff, B. E. *J. Org. Chem.* **1997**, *62*, 9326−9330. (f) Cabrele, C.; Langer, M.; Beck-Sickinger, A. G. *J. Org. Chem.* **1997**, *64*, 44353-4361.