Antineoplastic Agents. 400. Synthesis of the Indian Ocean Marine Sponge Cyclic Heptapeptide Phakellistatin 2[†,1a]

George R. Pettit,* Monte R. Rhodes, and Rui Tan

Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287-2404

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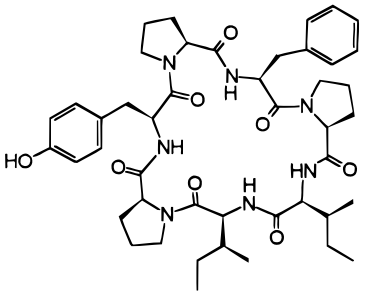
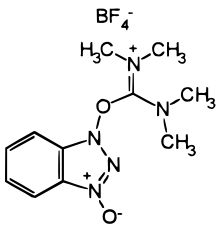
Solution-phase synthesis of the marine sponge constituent phakellistatin 2 (**1**), cyclo(Tyr-Pro-Phe-Pro-Ile-Ile-Pro), was completed using a combination of stepwise coupling and (4 + 3) segment condensation. Use of diethyl phosphorocyanidate for the peptide bond formations gave the linear heptapeptide in 54% yield. Cyclization was achieved in high yields utilizing TBTU (**2**), BOP-C1 (**3**), PyBroP (**4**), and HOAt (**5**), resulting in 50−65% yields of phakellistatin 2 (**1**) depending on the method employed. The synthetic cyclic peptide was chemically but not biologically identical with the natural product.

The isolation of new cyclic peptides from marine sponges has been increasingly productive. Illustrative are the Pohnpei *Cribrochalina olemda* kapakahines[2a] (related to phakellistatin 3[2b]), the Philippine *Aciculites orientalis* aciculitins,[2c] the Federated State of Micronesia *Phakellia* sp. phakellistatins,[2d] and the Indonesia *Ircinia dendroides* waiakeamide,[2e] of which the first three groups contain cancer cell growth inhibitory members. An early advance with such potentially useful Porifera cyclic peptide constituents[2b,3] was our isolation [9.6 × 10^{-5}% yield and structural elucidation (by high-field 2D-NMR and HRFABMS techniques)] of phakellistatin 2 (**1**) from the Republic of Comoros *Phakellia carteri*.[4] This cyclic heptapeptide (**1**) exhibited cell growth inhibitory properties against the murine P388 lymphocytic leukemia (ED_{50} 0.34 μg/mL) and a selection of human cancer cell lines. A total synthesis of phakellistatin 2 (**1**) was undertaken to increase the supply for further biological studies and to ascertain whether the natural product might contain an exceptionally potent antineoplastic substance[5] in a trace amount too small to detect[6] by the chemical and physical techniques employed for isolation and structure determination.

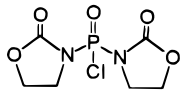
For the synthesis of the cyclic heptapeptide **1**, a solution-phase synthetic strategy[7] was employed involving Fmoc N-terminal protection[8,9] and *tert*-butyl ester C-terminal protection, similar to that which we recently used to obtain axinastatins 2 and 3[6a] and stylopeptide 1.[6b] The *tert*-butyl ester for C-terminal protection was utilized owing to its ability to resist nucleophilic attack and diketopiperazine formation.[10] Peptide bond formation was accomplished with DEPC,[1b,11] starting from *tert*-butyl proline (**7**).[6a,12] When DEA[9a] was used as the base for Fmoc cleavage, a large amount of dibenzofulvene (DBF) was formed, accompanied by significant (5−10%) epimerization and overall lowered yields. Therefore, TAEA[1b,13] with a phosphate buffer (pH 5.5) was used to assist in removing DBF, reduce epimerization, and result in 70% or higher yields on the average. Peptide bond formation was conducted with DIEA as the base in dichloromethane (DCM shown to give less epimerization[14] than DMF[15]). The use of this base was further supported by previous studies[6,8] and the stability of the Fmoc group toward DIEA.[10c] These improvements were employed to synthesize the requisite tri- and tetrapeptide segments shown in Schemes 1 and 2, respectively. The two



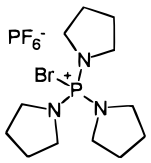
1, Phakellistatin 2



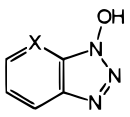
2, TBTU



3, BOP-Cl



4, PyBroP



5, HOAt, X = N
6, HOBt, X = CH

units were then coupled to form the linear heptapeptide (Scheme 3). Since cyclization would be based on a proline unit, which is turn inducing[16a] and minimizes racemization,[16b,c] the formation of phakellistatin 2 was expected to be favored by this approach.

Cyclization of heptapeptide **13** to provide phakellistatin 2 was readily accomplished utilizing the following techniques: TBTU[17] (**2**)/DIEA in DCM, BOP-C1[18] (**3**)/DIEA in DCM, PyBroP[19] (**4**)/DIEA in DCM, and TBTU with HOAt (**5**),[20] the pyridyl variant of the well-known[21] HOBt (**6**). All of these methods afforded yields of 45% or better. The use of TBTU (**2**) gave the highest consistent yields (55%). The synthetic phakellistatin 2 (**1**) was found to be chemically

[†] Dedicated to the memory of Dr. C. Gordon Zubrod (1914−1999), a great pioneer of cancer chemotherapy and friend.

* To whom correspondence should be addressed. Phone: (602) 965-0186. Fax: (602) 965-8558. E-mail: bpettit@asu.edu.

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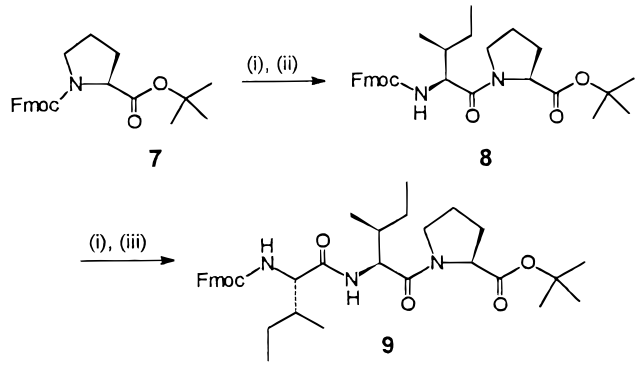
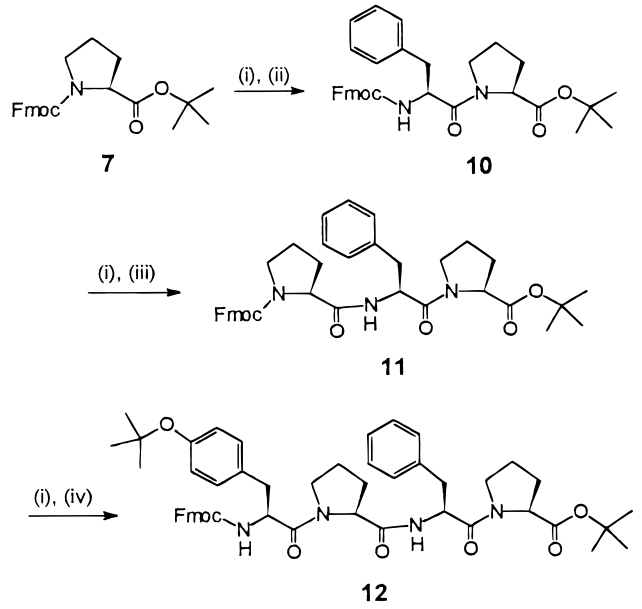
Scheme 1[a]



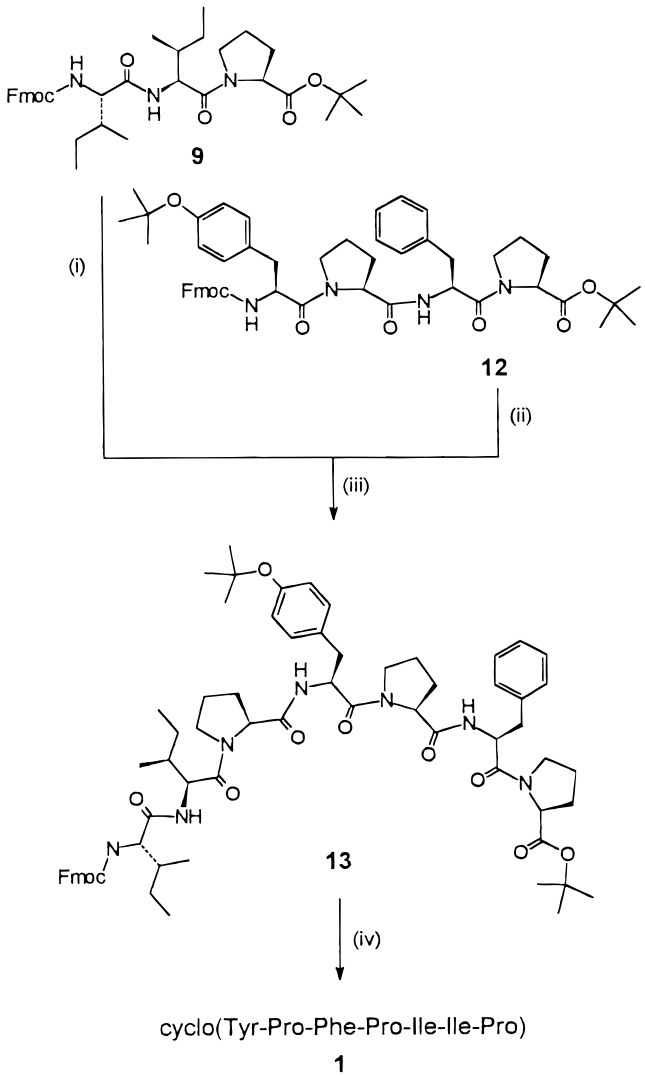
[a] Key: (i) TAEA, DCM; (ii) Fmoc-L-Ile, DEPC, DIEA, DCM, 72%; (iii) Fmoc-L-Ile, DEPC, DIEA, DCM, 84%.

Scheme 2[a]



[a] Key: (i) TAEA, DCM; (ii) Fmoc-L-Phe, DEPC, DIEA, DCM, 71%; (iii) Fmoc-L-Pro, DEPC, DIEA, DCM, 81%; (iv) Fmoc-L-Tyr-OtBu, DEPC, DIEA, DCM, 75%.

Scheme 3[a]



[a] Key: (i) TFA, DCM; (ii) TAEA, DCM; (iii) DEPC, DIEA, DCM, 54%; (iv) method A, B, C, or D.

identical with the natural product by comparison of high-field (500 MHz) ^{1}H and ^{13}C NMR data (see Tables 1 and 2), IR, TLC, mixture mp, and $[\alpha]_D$, but not biologically identical as noted below.

Biological evaluation of the synthetic phakellistatin 2 and the natural sample against the P388 lymphocytic leukemia cell line showed cell growth inhibitory activity of ED_{50} 24 μg/mL as compared with the natural peptide's activity of ED_{50}, 0.34 μg/mL. Comparison of the synthetic cyclic peptide with a minipanel of six cell lines from the NCI's primary screening panel of human cancer cell lines[22] showed no activity compared with the natural peptide. The results of these biological experiments again suggest that cyclic peptides of this nature may be capable of capturing/complexing strongly active antineoplastic agents in amounts undetectable by current high-field NMR, high-resolution MS, and chromatographic techniques, as we have recently discovered.[23,6] Alternatively, a reviewer has thoughtfully suggested that these differences might result from conformational changes arising from the proline units.

After this study was completed, Kessler[24] summarized in preliminary form a solid-phase peptide synthesis based on the structure assigned phakellistatin 2. In the final step, they chose cyclization between Ile and Ile under the assumption that Pro would induce a β-turn in the $i + 1$ position. That strategy might explain their observation that the product did not coincide exactly with the NMR data we reported for natural phakellistatin 2. Furthermore, their product was not compared with an authentic specimen of natural phakellistatin 2. To remove any conceivable doubt about the correctness of the structure we originally assigned to natural phakellistatin 2 and confirmed in the present study, we again meticulously purified specimens of the natural and synthetic phakellistatin 2 and repeated the comparison investigation. Both specimens were purified by the same HPLC techniques and individually restudied by high-field NMR (500 MHz). In addition, the high-field ^{1}H NMR and ^{13}C NMR spectra were repeated using an equiweight mixture (cf. Table 1) of the natural and synthetic phakellistatin 2 (**1**). All of the chromatographic and spectral comparison procedures using the natural and synthetic specimens gave identical results.

Experimental Section

General Procedure. All solvents except for acetonitrile (HPLC grade, EM Science) and DMF (anhyd, Aldrich) were redistilled. Dichloromethane was distilled from calcium hydride and stored over 4-Å molecular sieves. The Fmoc L-amino acids (NovaBiochem and Sigma−Aldrich) were used as re-

Table 1. Phakellistatin 2[a] High-Field (500 MHz) NMR Assignments Recorded in CD_3OD

carbon	^{13}C NMR	^{1}H NMR
Pro*		
CO	172.74p	
a-CH	59.89n	4.44 (1H, d, 8.0)
b-CH_2	31.78p	1.96 (1H)
		2.12 (1H)
c-CH_2	22.56p	1.89 (1H)
		2.12 (1H)
d-CH_2	48.24p	3.41 (1H, m)
		3.59 (1H, m)
Pro**		
CO	172.99p	
a-CH	63.26n	4.54 (1H, brd, 8.5)
b-CH_2	32.78p	2.16 (1H)
		2.27 (1H, m)
c-CH_2	23.01p	1.67 (1H)
		1.91 (1H)
d-CH_2	47.67p	3.45 (1H, m)
		3.60 (1H, m)
Pro***		
CO	173.23p	
a-CH	62.34n	3.21 (1H, d, 8.0)
b-CH_2	31.70p	0.96 (1H, m)
		1.91 (1H)
c-CH_2	22.85p	1.40 (1H, m)
		1.69 (1H)
d-CH_2	47.67p	3.34 (1H)
		3.36 (1H)
Ile*		
CO	172.99p	
a-CH	59.94n	3.77 (1H, d, 10.5)
b-CH	35.71n	1.90 (1H, m)
b′-CH_3	16.28n	0.73 (3H, d, 7.0)
c-CH_2	27.12p	1.13 (1H, m)
		1.36 (1H, m)
d-CH_3	10.64	0.79 (3H, t, 7.0)
Ile**		
CO	174.51p	
a-CH	55.80n	4.33 (1H, d, 9.5)
b-CH	38.35n	1.58 (1H, m)
b′-CH_3	15.16n	0.89 (3H, d, 7.2)
c-CH_2	26.08p	1.18 (1H, m)
		1.67 (1H)
d-CH_3	11.01n	0.89 (1H, t, 7.2)
Phe		
CO	172.79p	
a-CH	55.63n	4.41 (1H, dd, 12/5.0)
b-CH_2	38.41p	2.93 (1H, t, 12.5)
		3.18 (1H)
Ar-C1	136.88p	
Ar-C2, 6	130.63n	7.22 (2H, d, 7.5)
Ar-C3, 5	130.05n	7.32 (2H, t, 7.5)
Ar-C4	128.68n	7.29 (1H, t, 7.3)
Tyr		
CO	170.48p	
a-CH	54.34n	4.66 (1H, dd, 7.0/3.5)
b-CH_2	37.66p	3.08 (1H)
		3.14 (1H)
Ar-C1	127.37p	
Ar-C2, 6	131.98n	6.87 (2H, d, 8.3)
Ar-C3, 5	116.14n	6.68 (2H, d, 8.5)
Ar-C4	157.77p	

*,**,***Denotes individual amino acid units of the same type without any implied sequence information. [a] Data resulting from an equiweight (2.5 mg each) mixture of natural and synthetic.[b] The coupling constants refer to Hz. The n and p notations correspond to APT results in which n indicates one or three protons and p refers to none or two protons attached.

Table 2. ^{13}C NMR Spectral Data Comparison of Natural and Synthetic Phakellistatin 2

carbon assignment	natural phakellistatin 2	synthetic phakellistatin 2
Ile-CO	174.5	174.45
Pro-CO	173.22	173.17
Pro-CO/Ile-CO	172.99	172.94
Phe-CO	172.78	172.76
Pro-CO	172.74	172.70
Tyr-CO	170.46	170.42
Tyr-Ar-C_4	157.77	157.74
Phe-Ar-C_1	136.87	136.85
Tyr-Ar-$C_{2,6}$	131.98	131.96
Phe-Ar-$C_{2,6}$	130.64	130.60
Phe-Ar-$C_{3.5}$	130.06	130.03
Phe-Ar-C_4	128.68	128.64
Tyr-Ar-C_1	127.35	127.33
Tyr-Ar-$C_{3,5}$	116.13	116.11
Pro-αCH	63.25	63.22
Pro-αCH	62.34	62.31
Ile-αCH	59.93	59.91
Pro-αCH	59.89	59.86
Ile-αCH	55.80	55.77
Phe-αCH	55.62	55.60
Tyr-αCH	54.33	54.30
Pro-αCH_2	48.24	48.22
(Pro-δCH_2) × 2	47.68	47.67
Phe-β-CH_2	38.41	38.39
Ile-βCH_2	38.35	38.33
Tyr-βCH_2	37.63	37.63
Ile-βCH_2	35.71	35.69
Pro-βCH_2	32.77	32.77
Pro-βCH_2	31.78	31.77
Pro-βCH_2	31.69	31.68
Ile-γCH_2	27.12	27.10
Ile-γCH_2	26.08	26.06
Pro-γCH_2	23.01	23.00
Pro-γCH_2	22.85	22.84
Pro-γCH_2	22.56	22.54
Ile-β′-CH_3	16.28	16.27
Ile-β′-CH_3	15.16	15.17
Ile-δCH_3	11.03	11.03
Ile-δCH_3	10.65	10.65

ceived. BOP-Cl was supplied by TCI America, and PyBroP was supplied by Advanced ChemTech. Diethyl phosphorocyanidate (93%), diisopropylethylamine, diethylamine, TBTU, TAEA, and trifluoroacetic acid were used as received from Sigma−Aldrich Co. The HOAt synthesis was performed by nitration of commercially available 3-hydroxypyridine,[25] methylation of the resulting 2-nitro-3-pyridinol,[26] and treatment of the resulting methyl ether with excess hydrazine.[27] Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate unless noted otherwise. Thin-layer chromatography was performed using silica gel GHLF Uniplates (Analtech), and the plates were visualized by UV light (254 nm) and/or 2% Ninhydrin−ethanol (by heating for 2−3 min). Chromatographic purification of products was accomplished by flash chromatography[28] using Merck silica gel 60 (230−400 mesh). For a summary of the instrumental methods and equipment refer to ref 6.

General Deprotection Method.[13] A solution of the *N*-Fmoc *tert*-butyl ester (5.00 g) in dichloromethane (50 mL) was stirred (under argon) at room temperature, and TAEA (20 equiv) was added. After addition of the yellow-green base, the solution became yellow and cloudy. Stirring was continued for 1 h, and deprotection was observed (by TLC) to be complete. The mixture was washed with brine (3 × 25 mL), followed by a phosphate buffer solution (25.3 g K_2HPO_4/12.3 g KH_2PO_4 in 250 mL of water, 2 × 25 mL). The total aqueous phase was extracted with dichloromethane (25 mL), and the combined organic solution was dried ($MgSO_4$), filtered, and reduced (~20 mL) in volume. The amino acid *tert*-butyl ester was used directly without further purification.

General Peptide Bond-Forming Method. The *N*-Fmoc-amino acid (1 equiv) was added to a flame-dried flask under argon and dissolved in anhydrous dichloromethane (~0.3 M) containing several drops of DMF to assist dissolution (if

necessary). The solution was cooled (−10 °C) and stirred, and DEPC (calculated volume of 93% pure DEPC was added to give 1.1 equiv) was added via syringe. After being stirred for 15 min, a solution of the amino acid *tert*-butyl ester in dichloromethane was added. DIEA (1.1 equiv) was added via syringe and the solution allowed to warm to rt over the course of 2 h. The reaction mixture was stirred at rt for 6 h, whereupon TLC monitoring indicated the desired *N*-Fmoc-peptide-OBu^t and a minor amount of dibenzofulvene (DBF). The reaction mixture was washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, water, and brine. Drying, filtration, and solvent removal gave the crude product.

***N*-Fmoc-Ile-Pro-OBu^t (8).** Using the above general procedures, *N*-Fmoc-Pro-OBu^t (**7**, 10.0 g; 25.4 mmol) was N-deprotected and coupled with *N*-Fmoc-Ile. The resulting crude product was purified chromatographically (3:1 hexane−EtOAc as eluent) to give 9.3 g (72%) of a foamy solid that crystallized slowly from dichloromethane: mp 47−49 °C; R_f 0.24 (3:1 hexanes−EtOAc); $[\alpha]^{24}_D$ −37° (*c* 0.31, $CHCl_3$); EIMS *m*/*z* 506 (15), 433 (12), 394 (5), 336 (7), 308 (35), 284 (75), 264 (13), 211 (100), 178 (100); *anal.* C 70.82%, H 7.42%, N 5.81%, calcd for $C_{30}H_{38}N_2O_5$, C 71.12%, H 7.56%, N 5.53%.

***N*-Fmoc-^{3}Ile-^{2}Ile-^{1}Pro-OBu^t (9).** As summarized above, *N*-Fmoc-Ile-Pro-OBu^t (**8**, 8.00 g; 15.8 mmol) was N-deprotected and condensed with *N*-Fmoc-Ile. The clear oily product was chromatographed (2:1 hexanes−EtOAc) to give 8.65 g (88.3%) of a colorless foam: mp 96−98 °C; R_f 0.23 (2:1 hexane−EtOAc); $[\alpha]^{24}_D$ −58° (*c* 0.20, $CHCl_3$); λ_{max} ($CHCl_3$)/nm (log ϵ) 267 (4.35), 289 (3.87), 301 (3.91); IR ν_{max} (Nujol)/cm^{-1} 3290, 3067, 2966, 2877, 1724, 1641, 1537, 1448, 1367, 1242, 1155, 1035, 910, 758; EIMS *m*/*z* 619 (10), 546 (5), 449 (10), 367 (2), 311 (4), 255 (14), 225 (12), 178 (100); ^{1}H NMR δ 0.86 (t, J = 7.5 Hz, 3H, ^{2}Ile γ-CH_3), 0.90 (t, J = 7.5 Hz, 3H, ^{3}Ile γ-CH_3), 1.01 (d, J = 7.0 Hz, 6H, ^{2,3}Ile δ-CH_3), 1.12 (m, 4H, ^{2,3}Ile γ-CH_2), 1.45 (s, 9H, C(CH_3)$_3$), 1.57−1.85 (m, 2H, ^{2,3}Ile β-CH), 1.94 (m, 2H, Pro γ-CH_2), 2.04 (t, J = 8.5 Hz, 1H, Pro β-CH_2), 2.17 (m, 1H, Pro β-CH_2), 3.65 (dd, J = 13, 7 Hz, 1H, Pro δ-CH_2), 3.81 (dd, J = 13, 7 Hz, 1H, Pro δ-CH_2), 4.06 (t, J = 7 Hz, 1H, ^{2}Ile α-CH), 4.22 (t, J = 7 Hz, 1H, ^{3}Ile α-CH), 4.38 (m, 3H, Pro α-CH, Fmoc β-CH_2), 4.63 (t, J = 8 Hz, 1H, Ile α-CH), 5.38 (d, J = 8.5 Hz, 1H, ^{2}Ile-N*H*), 6.50 (d, J = 9.0 Hz, 1H, ^{3}Ile-N*H*), 7.31 (t, J = 7.5 Hz, 2H, Fmoc Ar-H), 7.40 (t, J = 7.5 Hz, 2H, Fmoc Ar-H), 7.59 (t, J = 7.5 Hz, 2H, Fmoc Ar-H), 7.76 (t, J = 7.5 Hz, 2H, Fmoc Ar-H); ^{13}C NMR (100 MHz) δ 10.82 (^{2}Ile δ-CH_3), 11.18 (^{3}Ile δ-CH_3), 15.05 (^{2}Ile γ-CH_3), 15.33 (^{3}Ile γ-CH_3), 24.28 (Pro γ-CH_2), 24.60 (^{2}Ile γ-CH_2), 24.65 (^{3}Ile γ-CH_2), 27.74 (Pro C(*C*H_3)$_3$), 28.96 (Pro β-CH_2), 37.19 (^{2}Ile β-CH), 37.65 (^{3}Ile β-CH), 46.97 (Fmoc α-CH), 47.25 (Pro δ-CH_2), 54.74 (^{2}Ile α-CH), 59.38 (^{3}Ile α-CH), 59.54 (Pro α-CH), 66.80 (Fmoc −CH_2), 81.00 (Pro *C*(CH_3)$_3$), 119.68, 124.93, 124.99, 126.83, 127.40 (Fmoc Ar-*C*H), 141.02 (Fmoc Ar-*C*q), 143.67 (Fmoc Ar-*C*q), 156.02 (urethane CO), 170.23 (^{2}Ile CO), 170.88 (^{3}Ile CO), 171.33 (Pro CO); *anal.* C 69.78%, H 8.16%, N 6.72%, calcd for $C_{36}H_{49}N_3O_6$, C 69.76%, H 7.97%, N 6.78%.

***N*-Fmoc-Phe-Pro-OBu^t (10).** For preparation of this dipeptide *N*-Fmoc-Pro-OBu^t (**7**, 12.0 g; 30.5 mmol) was N-deprotected and combined with *N*-Fmoc-Phe. The pale yellow oily product was purified by chromatography in 5:2 hexane−EtOAc to afford a solid that crystallized from EtOAc−hexane to give colorless prisms (13.1 g; 80%): mp 145.1−145.4 °C; R_f 0.48 (2:1 hexanes−EtOAc); $[\alpha]^{25}_D$ −78° (*c* 0.54, $CHCl_3$); EIMS *m*/*z* 540 (20), 467 (10), 393 (20), 342 (15), 227 (10), 178 (100); *anal.* C 73.6%, H 6.89%, N 5.09%, calcd for $C_{33}H_{46}N_2O_5$, C 73.3%, H 6.71%, N 5.18%.

***N*-Fmoc-^{3}Pro-^{2}Phe-^{1}Pro-OBu^t (11).** The preceding *N*-Fmoc-Phe-Pro-OBu^t (**10**, 10.0 g; 18.5 mmol) was N-deprotected and coupled with *N*-Fmoc-Pro. The resulting clear oil was separated chromatographically employing 3:1 EtOAc−hexane as eluent to give a solid that crystallized from ether−hexane (9.44 g, 80%) as a colorless solid: mp 110−111 °C; R_f 0.27 (3:1 EtOAc−hexane); $[\alpha]^{25}_D$ −86° (*c* 1.0, $CHCl_3$); EIMS *m*/*z* 637 (1), 564 (1), 467 (1), 415 (4), 346 (6), 303 (20), 247 (50), 178 (100); *anal.* C 71.66%, H 7.02%, N 6.49%, calcd for $C_{38}H_{43}N_3O_6$, C 71.6%, H 6.8%, N 6.58%.

***N*-Fmoc-^{4}Tyr(OBu^t)-^{3}Pro-^{2}Phe-^{1}Pro-OBu^t (12).** *N*-Fmoc-Pro-Phe-Pro-OBu^t (**11**, 5.1 g, 8 mmol) was N-deprotected and coupled (cf. the general methods) with *N*-Fmoc-OBu^t-Tyr. The oily product was subjected to chromatographic separation using 3:1 EtOAc−hexane to provide the tetrapeptide as a colorless foam that precipitated from ether−hexane (5.14 g, 75%) to give a colorless solid: mp 90−92 °C; R_f 0.45 (3:1 EtOAc−hexane); $[\alpha]^{24}_D$ −47° (*c* 0.32, $CHCl_3$); λ_{max} ($CHCl_3$/nm (log ϵ) 228 (4.1), 267 (4.3), 289 (3.8), 300 (3.8); IR ν_{max} (Nujol)/cm^{-1} 3408, 3292, 3057, 2978, 2879, 1722, 1641, 1506, 1477, 1367, 1255, 1161, 1037, 898, 738; ^{1}H NMR δ 1.26 (d, J = 10.8 Hz, 2H, Pro γ-CH_2), 1.27 (s, 9H, Pro C(CH_3)$_3$), 1.33 (dd, J = 10.8, 1.2 Hz, 2H, Pro γ-CH_2), 1.37 (d, J = 2.1 Hz, 1H, Pro γ-CH_2), 1.47 (s, 9H, Tyr C(CH_3)$_3$), 1.65 (m, 1H, Pro CH_2), 1.80 (m, 1H, Pro CH_2), 1.95 (m, 2H, Pro β-CH_2), 2.14 (m, 2H, Pro β-CH_2), 2.86 (d, J = 6.6 Hz, 2H, Tyr β-CH_2), 2.97 (dd, J = 15, 6.6 Hz, 2H, Phe β-CH_2), 3.10 (m, 2H), 3.20 (dd, Pro δ-CH_2), 3.31 (dd, Pro δ-CH_2), 3.37 (m, 1H, Pro δ-CH_2), 3.65 (m, 1H, Pro δ-CH_2), 4.11 (dd, Fmoc β-CH_2), 4.25 (m, 2H, Fmoc β-CH_2), 4.38 (m, 1H, Fmoc α-CH), 4.43 (m, 1H, Pro α-CH), 4.49 (m, 1H, Pro α-CH), 4.63 (m, 1H, Phe α-CH), 4.91 (dd, J = 13.5, 6.6 Hz, 1H, Tyr α-CH), 5.69 (d, J = 9 Hz, 1H, Phe N*H*), 5.75 (d, J = 8.7 Hz, 1H, Tyr N*H*), 6.88 (d, J = 8.1 Hz), 7.06 (d, J = 8.7 Hz), 7.18 (t, J = 8.7 Hz), 7.30 (t, J = 7.2 Hz), 7.39 (t, J = 7.2 Hz), 7.57 (d, J = 7.2 Hz, 2H, Fmoc Ar-CH), 7.75 (d, J = 7.2 Hz, 2H, Fmoc Ar-CH); ^{13}C NMR (75 MHz) δ 21.85 (^{3}Pro γ-CH_2), 24.56 (^{1}Pro γ-CH_2), 27.53 (^{3}Pro β-CH_2), 27.75 (Tyr C(*C*H_3)$_3$), 28.52 (Pro C(*C*H_3)$_3$), 28.74 (^{1}Pro β-CH_2), 38.04 (Phe β-CH_2), 38.44 (Tyr β-CH_2), 46.65 (^{1}Pro δ-CH_2), 46.86 (^{3}Pro δ-CH_2), 52.04 (Tyr α-CH), 53.81 (Phe α-CH), 59.54 (^{1}Pro α-CH), 59.77 (^{3}Pro-α-CH), 65.33 (Fmoc α-CH), 66.80 (Fmoc β-CH_2), 77.99 (Tyr *C*(CH_3)$_3$), 80.96 (Pro *C*(CH_3)$_3$), 119.73 (Fmoc Ar-CH), 123.95 (Tyr Ar-CH), 125.03 (Fmoc Ar-CH), 126.54 (Tyr Ar-CH), 126.87 (Phe Ar-CH), 127.48 (Fmoc Ar-CH), 128.12 (Phe Ar-CH), 129.74 (Fmoc Ar-CH), 129.86 (Phe Ar-*p*CH), 131.11 (Tyr γ-*C*q), 136.25 (Phe γ-*C*q), 141.06 (Fmoc Ar-*C*q), 143.72 (Fmoc Ar-*C*q), 154.02 (Tyr Ar-*C*q-OBu^t), 155.64 (urethane CO), 169.60 (Phe CO), 170.42 (Tyr CO), 171.01 (^{3}Pr CO), 171.13 (^{1}Pro CO); *anal.* C 70.67%, H 7.28%, N 6.36%, calcd for $C_{51}H_{60}N_4O_8 \cdot {}^1/_2H_2O$, C 70.73%, H 6.93%, N 6.47%.

***N*-Fmoc-^{7}Ile-^{6}Ile-^{5}Pro-^{4}Tyr(OBu^t)-^{3}Pro-^{2}Phe-^{1}Pro-OBu^t (13).** *N*-Fmoc-Tyr(OBu^t)-Pro-Phe-Pro-OBu^t (**12**, 2.50 g, 2.92 mmol) was N-deprotected (see above). Simultaneously, *N*-Fmoc-Ile-Ile-Pro-OBu^t (**9**, 1.81 g; 2.92 mmol) was treated with TFA (10 mL) for 1 h, and the solvents were removed (azeotropically) under vacuum. Peptide bond formation led to a faint yellow oil that was purified by chromatography in EtOAc to give the product as a foam that crystallized from EtOAc−hexane (2.50 g, 71%) as a colorless powder: mp 123−125 °C; R_f 0.24 (EtOAc); $[\alpha]^{24}_D$ −80.9° (*c* 0.22, $CHCl_3$); λ_{max} ($CHCl_3$)/nm (log ϵ) 267 (4.29), 289 (3.66), 301 (3.62); IR ν_{max} (KBr)/cm^{-1} 3414, 3306, 2972, 2933, 2877, 1728, 1631, 1508, 1448, 1365, 1236, 1159, 1033, 898, 742; ^{1}H NMR δ 0.81 (t, J = 7.0 Hz, 3H, ^{6}Ile δ-CH_3), 0.85 (d, J = 5.5 Hz, 3H, ^{6}Ile γ-CH_3), 0.85 (t, J = 6.0 Hz, 3H, ^{7}Ile δ-CH_3), 0.95 (d, J = 6.0 Hz, 3H, ^{7}Ile δ-CH_3), 1.14−1.46 (2m, 4H, ^{7,6}Ile γ-CH_2), 1.25 (s, 9H, Tyr C(CH_3)$_3$), 1.38 (s, 9H, Pro C(CH_3)$_3$), 1.59, 1.87 (2m, 2H, ^{7,6}Ile β-CH), 1.87−1.95 (m, 3H, ^{5,3,1}Pro β-CH_2), 1.93−2.17 (m, 6H, ^{5,3,1}Pro γ-CH_2), 2.06−2.17 (m, 3H, ^{5,3,1}Pro β-CH_2), 2.83 (dd, J = 13.0, 6.0 Hz, 2H, Tyr β-CH_2), 2.96 (dd, J = 14.0, 6.6 Hz, 2H, Phe β-CH_2), 3.15 (d, J = 6.0 Hz, 2H, ^{1}Pro δ-CH_2), 3.22 (d, J = 6.0 Hz, 2H, ^{3}Pro δ-CH_2), 3.42−3.55 (m, 1H, ^{5}Pro δ-CH_2), 3.70, 3.90 (2m, 1H, ^{5}Pro δ-CH_2), 4.11 (t, J = 7.0 Hz, 1H, ^{7,6}Ile α-CH), 4.20 (m, 2H, Fmoc β-CH_2), 4.26 (t, J = 8.0 Hz, 1H, ^{6}Ile α-CH), 4.35 (m, 1H, Fmoc α-CH), 4.37 (m, 1H, ^{3}Pro α-CH), 4.48 (bd, J = 8.0 Hz, 2H, ^{5,1}Pro α-CH), 4.76 (t, J = 8.0 Hz, 1H, Phe α-CH), 4.98 (dd, J = 14.0, 6.0 Hz, 1H, Tyr α-CH), 5.53 (d, J = 9.0 Hz, 1H, Phe N*H*), 6.80 (d, J = 8.0 Hz, 1H, Tyr N*H*), 6.85 (d, J = 8.5, 1H, ^{7}Ile N*H*), 7.07 (d, J = 8.0 Hz, 1H, ^{6}Ile N*H*), 7.17 (t, J = 8.0 Hz, 2H, Fmoc Ar-H), 7.21−7.40 (m, 9H, Phe Ar-H and Tyr Ar-H), 7.60 (t, J = 6.0 Hz, 2H, Fmoc Ar-H), 7.72 (t, J = 5.5 Hz, 2H, Fmoc Ar-H), 8.21 (bd, J = 6.0 Hz, 2H, Fmoc Ar-H); ^{13}C NMR δ 10.92 (Ile δ-CH_3), 11.36 (Ile δ-CH_3), 15.07 (Ile γ-CH_3), 15.91 (Ile γ-CH_3), 24.50 (Pro δ-CH_2), 24.53 (Pro δ-CH_2), 24.59 (Pro δ-CH_2), 24.65 (Ile CH_2), 24.77 (Ile CH_2),

27.69 (Pro β-CH_2), 27.84 (Tyr C(CH_3)$_3$), 28.66 (Pro C(CH_3)$_3$), 28.91 (Pro β-CH_2), 30.54 (Pro β-CH_2), 37.89 (Ile β-CH), 38.37 (Ile β-CH), 39.00 (Tyr/Phe β-CH_2), 46.72 (Pro γ-CH_2), 47.08 (Fmoc α-CH), 47.23 (Pro γ-CH_2), 47.86 (Pro γ-CH_2), 52.18 (Tyr α-CH), 52.34 (Ile α-CH), 54.43 (Phe α-CH), 54.70 (Ile α-CH), 58.80 (Pro α-CH), 59.24 (Pro α-CH), 59.69 (Pro α-CH), 65.09 (Fmoc β-CH_2), 77.96 (Tyr *C*(CH_3)$_3$), 80.71 (Pro *C*(CH_3)$_3$), 119.75 (d, J = 6.0 Hz, Fmoc Ar-CH), 124.09 (Tyr Ar-CH), 125.10 (d, J = 12.0 Hz, Fmoc Ar-CH), 126.39 (Phe Ar-CH), 126.89 (Phe Ar-CH), 127.46 (Fmoc Ar-CH), 128.13 (Tyr Ar-CH), 129.72 (Fmoc Ar-CH), 130.04 (Phe Ar-*p*CH), 131.26 (Tyr Ar-γ*C*q), 137.00 (Phe γ-*C*q), 141.16 (Fmoc Ar-*C*q), 143.85 (d, J = 26.0 Hz, Fmoc Ar-*C*q), 153.88 (Tyr Ar-*C*q-OBu^t), 156.19 (urethane CO), 169.47 (Phe CO), 169.94 (Tyr CO), 170.39 (Ile CO), 170.92 (Ile CO), 171.09 (Pro CO), 171.35 (Pro CO), 171.75 (Pro CO); EIMS *m*/*z* calcd 1180.5, found 1180.7; *anal.* C 68.88%, H 7.56%, N 8.40%, calcd for $C_{68}H_{89}N_7O_{11}$, C 69.19%, H 7.60%, N 8.306%.

Phakellistatin 2 (1). Method A. TBTU. *N*-Fmoc-Ile-Ile-Pro-Tyr(OBu^t)-Pro-Phe-Pro-OBu^t (**13**, 0.30 g, 0.254 mmol) was N-deprotected. After removal of solvent, TFA (10 mL) was added to the crude Ile-Ile-Pro-Tyr(OBu^t)-Pro-Phe-Pro-OBu^t at room temperature, and the mixture was stirred for 2.5 h. Excess TFA was removed in vacuo, followed by addition and evaporation of two 5-mL portions of toluene and dichloromethane. To the TFA salt in dichloromethane (170 mL) was added TBTU (0.42 g; 1.7 mmol; 5 equiv) in acetonitrile (5 mL at 0 °C under argon). DIEA (1.7 mL, 1% v/v) was added slowly (~15 min) at room temperature with stirring. Dilution of the heptapeptide corresponded to 1.5 mM. The reaction mixture was stirred for 2 h at ice-bath temperature and at room temperature for 14 days. After removal of solvent in vacuo, a solution of the crude product in CH_2Cl_2 (100 mL) was washed with 10% aqueous citric acid (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and water (10 mL). The combined aqueous phase was extracted with EtOAc (15 mL). The organic solvents were combined, dried, and filtered, and the solvent was removed in vacuo to yield a brown oil (0.59 g). Chromatographic separation with a 25-mm × 20-cm (silica depth) column, elution with hexane−EtOAc−MeOH (4:2:1) under positive pressure, and collection of 5-mL fractions gave phakellistatin 2 (**1**, 117 mg, 56%) as a colorless solid (72% overall).

Comparison of the natural and synthetic specimens of phakellistatin 2 (**1**) by mixture mp, TLC, $[\alpha]_D$, IR, and ^{1}H and ^{13}C NMR (400 MHz, CD_3OD) gave identical results.

Method B. BOP-Cl. The preceding Fmoc and *tert*-butyl ester deprotection reactions (method A) were repeated using heptapeptide **13** (77 mg; 0.065 mmol). Dichloromethane (400 mL) and BOP-Cl (0.37 g; 1.43 mmol; 22 equiv) were added under argon to a dry flask (ice bath). The solution was maintained at ice-bath temperature, and the TFA salt and DIEA (1.82 mL; 10.4 mmol; 160 equiv) in dichloromethane (35 mL) were added slowly (over 1 h) via cannula to the dropping funnel. After addition, the solution was stirred at room temperature under argon for 7 days. The solvent was removed (in vacuo), and the residue was dissolved in EtOAc (50 mL). The EtOAc solution was washed with water (3 × 10 mL), and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined EtOAc extract was washed with saturated aqueous sodium bicarbonate (3 × 5 mL) and brine (10 mL) and dried. Filtration and solvent removal afforded a clear oil (0.17 g). The product was isolated by chromatography (4:2:1 hexane−EtOAc−MeOH) as described in method A to afford the cyclic heptapeptide as a colorless amorphous solid (25.4 mg, 50%), identical (cf. method A) with authentic natural phakellistatin 2.

Method C. PyBroP.[29] The Fmoc and *tert*-butyl ester deprotection reactions (method A) were repeated using the heptapeptide (**13**, 0.10 g; 0.085 mmol). The TFA salt and PyBroP (0.20 g; 0.43 mmol; 5 equiv) were dissolved in dry dichloromethane (100 mL), and the solution was cooled (ice bath) and stirred. DIEA (0.059 mL, 0.34 mmol; 4 equiv) was added (syringe) slowly, and the mixture was allowed to warm to room temperature and then stirred under argon at room temperature for 4 days. The solvent was removed under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with 1 N hydrochloric acid (5 × 20 mL), saturated aqueous sodium bicarbonate (2 × 25 mL), and brine (25 mL). The synthetic phakellistatin 2 was isolated (see method A) as a colorless solid (34.0 mg, 51% yield) that was identical (refer to method A) to the natural specimen (**1**).

Method D. TBTU/HOAt.[30] The linear heptapeptide (**13**, 300 mg; 0.254 mmol) was N-deprotected as described above. After removal of solvent, TFA (10 mL) was added to the crude residue at room temperature, and the mixture was stirred for 2.5 h. Excess TFA was removed in vacuo, followed by evaporation with two 5-mL portions of toluene and dichloromethane. To the TFA salt in dichloromethane (170 mL) was added TBTU (246 mg; 0.762 mmol; 3 equiv) in acetonitrile (5 mL at 0 °C under argon) followed by HOAt (0.108 g, 0.762 mmol, 3 equiv). DIEA (1.7 mL, 1% v/v) was added slowly (~15 min) at room temperature with stirring. Dilution of the heptapeptide corresponded to 1.5 mM. The reaction mixture was stirred for 2 h at ice-bath temperature and at room temperature for 14 days. After removal of solvent in vacuo, a solution of the crude product in dichloromethane (100 mL) was washed with 10% aqueous citric acid (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and water (10 mL). The combined aqueous phase was extracted with EtOAc (15 mL). The organic solvents were combined, dried, and filtered, and the solvent was removed in vacuo to yield a brown oil (0.78 g). Chromatographic separation and elution with hexane−EtOAc−MeOH (4:2:1) under positive pressure gave phakellistatin 2 (**1**, 136 mg, 65%) as a colorless solid identical (see method A) with the natural specimen.

Comparison of Natural and Synthetic Phakellistatin 2 (1). Specimens of both the natural and synthetic phakellistatin 2 were repurified using reversed phase HPLC on a C8 column in methanol−acetonitrile−water (50:50:55) at λ 235 nm. Both separately and in a mixture, the natural and synthetic phakellistatin 2 exhibited the same retention time. When the synthetic specimen was repurified by HPLC to remove two trace impurities, a mixture of the natural and synthetic phakellistatin 2 again showed only one peak in the HPLC recording. Direct comparison of the pure natural and synthetic phakellistatin 2 specimens employing high-field (500 MHz) NMR techniques showed the specimens to be identical; a comparison made by combination of 2.5-mg specimens of each in the same NMR sample tube again showed no structural differences, and the result has been recorded in Table 1.

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References and Notes

(1) (a) For part 399 see: Kalemkerian, G. P.; Ou, X.; Adil, M. R.; Rosati, R.; Khoulani, M. M.; Madan, S. K.; Pettit, G. R. *J. Natl. Cancer Inst.*, submitted. (b) Abbreviations for amino acids and peptides used in this paper are those recommended by the IUPAC−IUPAB Commission of Biochemical Nomenclature (*Eur. J. Biochem*. **1984**, 138, 9−37) and: Pettit, G. R. *Synthetic Peptides*; Elsevier Scientific Pub. Co.: Amsterdam, The Netherlands, 1982; Vol. 6 and preceding volumes. Additional abbreviations are as follows: Fmoc, fluoren-9-ylmethoxycarbonyl; DMF, *N*,*N*-dimethylformamide; DEA, *N*,*N*-diethylamine; DIEA, *N*,*N*-diisopropylethylamine; TAEA, tris(2-aminoethyl)amine; TFA, trifluoracetic acid; HOBt, 1-hydroxybenzotriazole; DEPC, diethyl phosphorocyanidate; BOP-Cl, *N*,*N*-bis(2-oxo-3-oxazolidinyl)-phosphinine chloride; HOAt, 1-hydroxy-7-azabenzotriazole; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; PyBroP, bromotrispyrrolidinophosphonium hexafluorophosphate; NMP, *N*-methyl 2-pyrrolidinone.

(2) (a) Yeung, B. K. S.; Nakao, Y.; Kinnel, R. B.; Carney, J. R.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem*. **1996**, *61*, 7168−7173. (b) Pettit, G. R.; Tan, R.; Herald, D. L.; Cerny, R. L.; Williams, M. D. *J. Org. Chem*. **1994**, *59*, 1593−1595. (c) Bewley, C. A.; He, H.;

Williams, D. H.; Faulkner, D. J. *J. Am. Chem. Soc.* **1996**, *118*, 4314−4321. (d) Pettit, G. R.; Tan, R.; Ichihara, Y.; Williams, M. D.; Doubek, D. L.; Tackett, L. P.; Schmidt, J. M. *J. Nat. Prod.* **1995**, *58*, 961−965. (e) Mau, C. M. S.; Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1996**, *61*, 6302−6304.

(3) (a) Pettit, G. R.; Cichacz, Z.; Barkoczy, J.; Dorsaz, A.-C.; Herald, D. L.; Williams, M. D.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Brune, D. C. *J. Nat. Prod.* **1993**, *56*, 260−267. (b) Pettit, G. R.; Herald, C. L.; Boyd, M. R.; Leet, J. E.; Dufresne, C.; Doubek, D. L.; Schmidt, J. M.; Cerny, R. L.; Hooper, J. N. A.; Rützler, K. C. *J. Med. Chem.* **1991**, *34*, 3339−3340.

(4) (a) Pettit, G. R.; Gao, F.; Cerny, R. L.; Doubek, D. L.; Tackett, L. P.; Schmidt, J. M.; Chapuis, J.-C. *J. Med. Chem.* **1994**, *37*, 1165−1168. (b) Pettit, G. R.; Tan, R.; Williams, M. D.; Tackett, L.; Schmidt, J. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*(12), 2869−2874.

(5) (a) Pettit, G. R.; Tan, R.; Gao, F.; Williams, M. D.; Doubek, D. L.; Boyd, M. R.; Schmidt, J. M.; Chapuis, J.-C.; Hamel, E.; Bai, R.; Hooper, J. N. A.; Tackett, L. P. *J. Org. Chem.* **1993**, *58*, 2538−2543. (b) Pettit, G. R.; Gao, F.; Doubek, D. L.; Boyd, M. R.; Hamel, E.; Bai, R.; Schmidt, J. M.; Tacketet, L. P.; Rützler, K. *Gazz. Chim. Ital.* **1993**, *123*, 371−377.

(6) (a) Pettit, G. R.; Holman, J. W.; Boland, G. M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2411−2416. (b) Pettit, G. R.; Taylor, S. R. *J. Org. Chem.* **1996**, *61*, 2322−2325.

(7) (a) Jiang, J.; Li, W.-R.; Joullie, M. *Synth. Commun.* **1994**, *24*, 187−195. (b) Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, *33*, 4557−4560.

(8) For a review, see: Fields, G. B.; Noble, R. I. *Int. J. Pept. Protein Res.* **1990**, *35*, 161−214.

(9) (a) Tung, R. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1986**, *51*, 3350−3354. (b) For an exception, see: Fischer, P. M.; Solbakken, M.; Undheim, K. *Tetrahedron* **1994**, *50*, 2277−2288.

(10) (a) Bodanszky, M. *Principles of Peptide Synthesis*, 2nd ed.; Springer-Verlag: New York, 1993; pp 186, 106−107. (b) Hoeg-Jensen, T.; Jakobsen, M. H.; Holm, A. *Tetrahedron Lett.* **1991**, *32*, 6387−6390. (c) Atherton, E.; Sheppard, R. C. In *The Peptides: Analysis, Synthesis, Biology*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: San Diego, 1987; Part C, Vol. 9, pp 1−38.

(11) (a) Pettit, G. R.; Srirangam, J. K.; Singh, S. B.; Williams, M. D.; Herald, D. L.; Barkoczy, J.; Kantoci, D.; Hogan, F. *J. Chem. Soc., Perkin Trans. 1* **1996**, 853−858. (b) Hamada, Y.; Rishi, S.; Shioiri, T.; Yamada, S.-I. *Chem. Pharm. Bull.* **1977**, *25*, 224−230.

(12) Valerio, R. M.; Alewood, P. F.; Johns, R. B. *Synthesis* **1988**, *10*, 786−789.

(13) Carpino, L. A.; Sadat-Aalaee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*, 1673−1675.

(14) Benoiton, N. L. *Int. J. Pept. Protein Res.* **1994**, *44*, 399−400.

(15) Haver, A. C.; Smith, D. D. *Tetrahedron Lett.* **1993**, *34*, 2239−2242. Benoiton, N. L.; Kuroda, K. *Int. J. Pept. Protein Res.* **1981**, *17*, 197−204.

(16) (a) Bodanszky, M. *Principles of Peptide Synthesis*, 2nd ed; Springer-Verlag: New York, 1993; p 232. (b) Jones, J. *The Chemical Synthesis of Peptides*; Clarendon Press: Oxford, 1991; p 93. (c) Goodman, M.; Steuben, K. C. *J. Org. Chem.* **1962**, *27*, 3409−3416.

(17) (a) Zimmer, S.; Hoffmann, E.; Jung, G.; Kessler, H. *Leibigs Ann. Chem.* **1993**, 497−501. (b) Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781−4784. (c) Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, *33*, 4557−4560. (d) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, G. *Tetrahedron Lett.* **1989**, *30*, 1927−1930.

(18) Kim, M. H., Patel, D. V. *Tetrahedron Lett.* **1994**, *35*, 5603−5606.

(19) Castro, B.; Dormoy, J. R. *Tetrahedron Lett.* **1973**, 3243−3246. Coste, J.; Frerot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967−1970.

(20) Carpino, L. A.; Elfaham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561−3564. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(21) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788−798. Pettit, G. R. *Synthetic Peptides*; Elsevier Publishing Co.: Amsterdam, The Netherlands, 1976; Vol. 4.

(22) Monks, A.; Scudiero, P.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Boyd, M. R. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.

(23) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Xu, J.-P.; Boyd, M. R.; Cichacz, Z.; Kamano, Y.; Schmidt, J. M.; Erickson, K. L. *J. Org. Chem.* **1995**, *60*, 8257−8261.

(24) Mechnich, O.; Kessler, H. *Lett. Peptide Sci.* **1997**, *4*, 21−28.

(25) De Selms, R. C. *J. Org. Chem.* **1968**, *33*, 478−480.

(26) Williamson, D. A.; Bowler, B. E. *Tetrahedron* **1996**, *52*, 12357−12372.

(27) Azev, Y.; Mokrushina, G. A.; Postovskii, I. Ya.; Sheinker, Yu. N.; Anisimova, O. S. *Chem. Heterocycl. Compds.* **1976**, 1172−1176.

(28) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(29) (a) Sefler, A.; Kozlowski, M. C.; Guo, T.; Bartleett, P. A. *J. Org. Chem.* **1997**, *62*, 93−102. (b) Kurome, T.; Inami, K.; Inoue, T.; Ikai, K.; Takesako, K.; Kato, I.; Shiba, T. *Tetrahedron* **1996**, *52*, 4327−4346.

(30) Ehrlich, A.; Heyne, H.-U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831−8838.

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