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Generation of a Small Library of Cyclodepsipeptide PF1022A Analogues Using a Cyclization-Cleavage Method with Oxime Resin

Byung H. Lee,* Fred E. Dutton, David P. Thompson and Eileen M. Thomas

Discovery Research, Pharmacia Animal Health, Kalamazoo, MI 49001, USA

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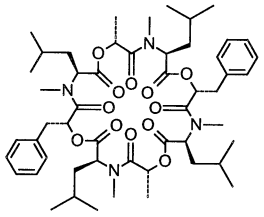
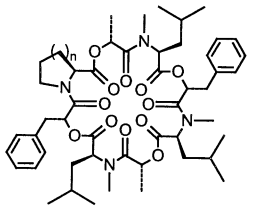
Abstract—*N*-Methyloctadepsipeptides attached to an oxime resin were cyclized by heating them in refluxing ethyl acetate for 2 days to give cyclodepsipeptide PF1022A analogues. By using this method, we generated a small library of PF 1022A analogues (**2**), several of which possessed anthelmintic activity, based on an in vitro assay.

Helminths, especially parasitic nematodes, cause substantial health problems in humans and domestic animals. Currently, three distinct chemical classes are used for broad spectrum control of gastrointestinal nematodes in veterinary medicine: benzimidazoles, imidazothiazoles, and macrocyclic lactones.[1] None of these drugs is ideally suited for all therapeutic situations, and each class has been challenged by the development of drug-resistant nematode strains.[2] Expansion of the anthelmintic arsenal is thus an urgent goal. The potent antiparasitic activity of cyclodepsipeptide (CDP) PF1022A **1** and its analogues was discovered by Japanese scientists.[3] Because PF1022A is unique both structurally and in its mode of action, it represents a promising new class of anthelmintics.



1 (PF1022A)



2a n = 1
2b n = 2

Three different syntheses of PF1022A have been reported.[4] Each synthesis, however, requires multiple steps, which severely limit the development of an SAR in a timely fashion. Recently, combinatorial technology has emerged as a way to rapidly identify and optimize therapeutic agents.[5] Herein we report a solid-phase method for the rapid synthesis of PF1022A analogues and the generation of a combinatorial library. The method utilizes the oxime-funtionalized polystyrene resin developed by Kaiser,[6] which has been used for making cyclic peptides.[7] Previously, we reported that cyclization of *N*-methyl depsipeptides using a solid support.[8]

Using classical synthetic methods, resin-bound tetradepsipeptide **3** was prepared,[8,9] and then linked at its N-terminus with Boc-Leu-Lac-OH, a didepsipeptide, to give hexadepsipeptide **4**[8] (Scheme 1). A second didepsipeptide, Boc-Leu-Plac-OH, was then joined at the N-terminus of **4** to give octadepsipeptide **5**.[8] Removal of the Boc protecting group from peptide **5** gave **6**, which underwent macrolactamization in refluxing EtOAc (2 days) to give CDP **2b**.[8]

Because the ring of the pipecolic acid residue (Pip) adds macrocyclic ring strain, which tends to inhibit cyclization, we prepared in addition to 6 resin-bound octadepsipeptides **7** and **8** and cyclized them using Kaiser's method to determine the optimum position of Pip within the octadepsipeptide chain (Scheme 2). The position found in octadepsipeptide **6** proved optimal, producing both the highest concentration of CDP per milligram of resin and the greatest purity. On this basis we utilized resin-bound tetradepsipeptide **3**,[10] which contains the last four residues of **6**, for the preparation of a CDP library. To further understand the effect of small rings on cyclization, we also prepared depsipeptides **9** and **10**, which contain a proline residue. The

*Corresponding author. Tel.: +1-616-833-6431; fax: +1-616-833-7721; e-mail: bhlee@pharmacia.com

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slightly smaller ring of proline introduced less macrocyclic strain (cf. cyclizations of octadepsipeptides **7** and **10**). It is also less positionally sensitive (cf. cyclizations of octadepsipeptides **9** and **10**).

Having developed chemistry suitable to the application of combinatorial methodology, we prepared eleven didepsipeptides[4c] and used them along with resin-bound tetradepsipeptide **3** to prepare a CDP library consisting of 48 ($8R_1 \times 6R_2$) analogues. The structures of the penultimate products (resin-bound before cyclization) are shown in Scheme 3.

Boc-Leu-Plac-Pip-Lac-O-OxRes (**3**)
↓ TFA
Boc-Leu-Lac-OH ↓ PyBrop / DIEA
Boc-Leu-Lac-Leu-Plac-Pip-Lac-O-OxRes (**4**)
↓ TFA
Boc-Leu-Plac-OH ↓ PyBrop / DIEA
Boc-Leu-Plac-Leu-Lac-Leu-Plac-Pip-Lac-O-OxRes (**5**)
↓ TFA
↓ DIEA
Leu-Plac-Leu-Lac-Leu-Plac-Pip-Lac-O-OxRes (**6**)
↓ EtOAc / Δ
2b

Scheme 1. Synthesis of the cyclodepsipeptides.

Leu-Plac-Leu-Lac-Leu-Plac-Pip-Lac-O-OxRes
6 → CDP **2b**
(CDP 71% pure, 5.1 mg / 50 mg resin)

Leu-Plac-Pip-Lac-Leu-Plac-Leu-Lac-O-OxRes
7 → the related CDP
(CDP < 5% pure)

Pip-Lac-Leu-Plac-Leu-Lac-Leu-Plac-O-OxRes
8 → the related CDP
(CDP < 5% pure)

Leu-Plac-Leu-Lac-Pro-Lac-Leu-Plac-O-OxRes
9 → the related CDP
(CDP 30% pure, 1.2 mg / 50 mg resin)

Leu-Plac-Pro-Lac-Leu-Plac-Leu-Lac-O-OxRes
10 → CDP **2a**
(CDP 41% pure, 2.0 mg / 50 mg resin)

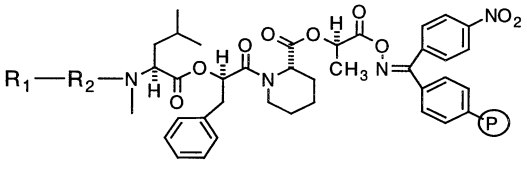
Scheme 2. Determination of optimal pipecolic position.

Table 1 gives the analytical data for this library. The average crude weight of the products is 13 mg and the average concentration is 30%, based on HPLC analysis of each sample. This means that our library produced an average of 3.9 mg of actual product for each sample. The structures of the products were confirmed by electro-spray, mass spectral analysis performed on the appropriate fraction obtained by HPLC.

The highest concentrations of CDPs on resin were obtained when two pipecolic acid residues were present in the octadepsipeptide, namely peptides **24**×**11**–**18**. Simple molecular modeling revealed that the single pipecolic residue of **3** introduces a cisoid turn unfavorable to cyclization. This effect is reversed by the presence of a second pipecolic residue, which introduces a similar, but offsetting, turn that enhances cyclization to produce purities of 16–65%.

Biology

The in vitro activity of the CDP library was assessed using the gastrointestinal nematode, *Haemonchus contortus*.[10] Each CDP was tested at a nominal concentration of 3 μM by adding 2.5 μL plate extract (adjusted in volume with DMSO to give a stock solution of 2.5 mM) to test tubes containing five parasites suspended in 2.5 mL culture medium. Motility levels were recorded at 1 h intervals, for a period of 24 h, using an automated micromotility recording system.[10] Negative controls consisted of *H. contortus* incubates to which were added 0.1% DMSO. Positive controls contained 5 μM PF1022A or 0.1 μM Ivermectin (a widely-used anthelmintic agent), both prepared in DMSO. Results from two separate



R_1	R_2
Leu-Plac-OH (**11**)	Leu-Lac-OH (**19**)
Phe-Plac-OH (**12**)	Phe-Lac-OH (**20**)
Phe-Lac-OH (**13**)	Ala-Lac-OH (**21**)
Ala-Plac-OH (**14**)	Ileu-Lac-OH (**22**)
Ileu-Plac-OH (**15**)	Nleu-Plac-OH (**23**)
Ileu-Lac-OH (**16**)	Pip-Lac-OH (**24**)
Nleu-Lac-OH (**17**)	
Nleu-Plac-OH (**18**)	

Ala=N-methylalanine; Ileu=N-methylisoleucine; Lac=lactate; Leu=N-methylleucine; Nleu=N-methylnorleucine; Phe=N-methylphenylalanine; Pip=pipecolate; Plac=phenyllactate

Scheme 3. Penultimate products, that is resin-bound octadepsipeptides before cyclization to CDPs.

Table 1. Analytical biological data for CDP library

R_2	**19**	**20**	**21**	**22**	**23**	**24**
R_1	Leu-Lac	Phe-Lac	Ala-Lac	Ileu-Lac	Nleu-Plac	Pip-Lac
11	19[a,b]	18	11	13	11	11
Leu-Plac	11.84[c]	13.12	10.67	13.24	15.56	11.71
	27[d]	10	25	10	38	65
	955[e]	989	913	955	1031	939
	93[f]	84	71	89	78	86
12	18	13	21	10	10	12
Phe-Plac	13.34	13.39	11.02	13.50	15.81	11.87
	10	14	18	22	41	48
	989	1023	947	989	1065	973
	85	41	47	63	0	17
13	12	12	12	14	12	18
Phe-Lac	10.76	11.4	8.12	10.88	13.54	9.18
	38	0.25	19	45	54	65
	913	947	871	913	989	897
	66	53	31	60	24	41
14	11	16	10	10	10	15
Ala-Plac	10.75	11.02	8.24	11.01	13.57	9.17
	22	21	20	27	34	54
	913	947	871	913	989	897
	77	53	56	79	15	77
15	8	24	3	12	8	10
Ileu-Plac	13.21	11.02	—	13.58	16.01	11.74
	3	2	0	5	3	16
	—	—	—	—	—	939
	—	—	—	—	—	27
16	14	9	1	12	8	8
Ileu-Lac	10.66	10.94	—	10.86	13.50	8.88
	11	6	0	13	11	43
	897	—	—	879	955	863
	52	—	—	66	48	0
17	22	10	20	12	13	12
Nleu-Lac	10.58	10.93	7.80	10.72	13.39	8.91
	15	20	41	51	34	45
	969	913	837	869	945	853
	72	70	48	60	79	7
18	23	12	3	12	10	11
Nleu-Plac	13.24	13.33	10.86	13.45	15.74	11.74
	17	10	18	15	40	43
	955	999	923	955	1031	939
	8	61	65	4	35	82

[a]CDP **2b**.
[b]Crude weight (mg).
[c]HPLC R_t (min).
[d]Purity (%).
[e]Mass spectra (M + Na).
[f]Motility (% reduction at 24 h).

experiments, four separate culture tubes per study, are shown in Table 1. Results are expressed as % reduction in motility, relative to 0.1% DMSO controls. In these studies, 5 μM PF1022A reduced motility by 83%, and 0.1 μM Ivermectin by 85%. Several products from the CDP library reduced *H. contortus* motility by 80% or more following 24 h incubations.

In conclusion, we have developed a method for the rapid preparation of *N*-methylcyclodepsipeptides using a solid support and generated a small combinatorial library of natural product analogues. Some of these analogues were shown to possess anthelmintic activity, based on results from an in vitro micromotility assay against the gastrointestinal nematode, *H. contortus*.

Acknowledgements

A Genesis RSP 200 (Tecan) synthesizer was used to make the CDP library. We gratefully acknowledge the technical assistance of Gary J. Cleek in the use of this instrument.

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9. Synthesis of **3**. Triphenylphosphine (TPP, 880 mg, 3.36 mmol), Boc-L-pipecolic acid (640 mg, 2.8 mmol), and benzyl L-lactate (0.5 g, 2.8 mmol) were dissolved in Et_2O (20 mL). The resulting mixture was treated with diethyl azodicarboxylate (DEAD) (0.5 mL, 3.17 mmol in 5 mL of Et_2O) at room temperature over 20 min. The mixture was stirred for an additional 1 h and the precipitate removed by filtration. The filtrate was concentrated and the residue purified by silica gel chromatography (10% EtOAc in hexane) to give Boc-Pip-Lac-OBn (0.88 g, 80%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.1–2.3 (m, 18H), 2.8–3.0 (m, 1H), 3.8–4.1 (m, 1H), 4.7–5.2 (m, 4H), 7.2–7.4 (m, 5H). FABHRMS: *m/e* 392.2086 ($C_{21}H_{29}NO_6$ + H requires 392.2073). Boc-Pip-Lac-OBn (7.2 g, 18.39 mmol) was dissolved in CH_2Cl_2 (DCM) containing 10% (v/v) TFA (200 mL). The reaction mixture was stirred for 1.5 h and then slowly poured into saturated $NaHCO_3$ aqueous solution (200 mL) with rapid stirring. The mixture was transferred to a separatory funnel and shaken. The layers were separated, and the aqueous layer extracted with DCM. The organic layers were combined, washed with water, dried ($MgSO_4$), filtered, and concentrated to give Pip-Lac-OBn (5.25 g, 98% yield) as an oil. This was used without further purification. Boc-Leu-Plac-OH (5.0 g, 12.6 mmol) was dissolved in DCM (10 mL) and treated with diisopropylcarbodiimide (DIC) (2.2 mL, 12.6 mmol), dimethylaminopyridine (DMAP) (244 mg, 2 mmol), and Pip-Lac-OBn (2.97 g, 12.4 mmol) at 0 °C. The mixture was slowly warmed to room temperature and stirred for 16 h. The precipitate was removed, and the filtrate concentrated. The residue was purified by silica gel chromato-

graphy (20% acetone in hexane) to give Boc-Leu-Plac-Pip-Lac-OBn as an oil (4 g, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.7–2.4 (m, 27H), 2.6–3.7 (m, 7H), 4.5–5.7 (m, 6H) 7.2–7.5 (m, 10H). FABHRMS: *m*/*e* 667.3608 ($C_{37}H_{50}N_2O_9$ + H requires 667.3594). Boc-Leu-Plac-Pip-Lac-OBn (3.5 g, 5.3 mmol) was dissolved in absolute EtOH (70 mL) and hydrogenolyzed for 17 h at 40 psi over 10% palladium on charcoal (1.0 g). The reaction mixture was flushed with nitrogen, filtered, and concentrated to remove EtOH. The residue was dried under high vacuum to give Boc-Leu-Plac-Pip-Lac-OH (2.9 g, 98%) as a semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8–2.3 (m, 27H), 2.7–3.9 (m, 7H), 4.5–5.8 (m, 4H) 7.2–7.4 (m, 5H). FABHRMS: *m*/*e* 577.3132 ($C_{30}H_{44}N_2O_9$ + H requires 577.3124). $[\alpha]_D = -57.0°$ (*c* 0.97, DCM). Boc-Leu-Plac-Pip-Lac-OH (2.15 g, 3.86 mmol), DMAP (610 mg, 5 mmol), and Kaiser oxime (Novabiochem, 0.91 mmol/g, 2 g, 1.82 mmol) were suspended in DCM (80 mL). The mixture was treated with DIC (0.94 mL, 5.4 mmol) and stirred for 16 h. The resin was washed with DCM (2×25 mL), MeOH (2×25 mL), DMF (2×25 mL), and DCM (2×25 mL). The washed resin was dried in vacuo for 4 h to give **3** (2.67 g, 0.65 mmol/g of resin). To confirm structure and determine purity and resin concentration, a sample of **3** (30 mg) was suspended in DCM (1 mL) containing 10 mg of morpholine. The mixture was stirred for 16 h at room temperature to give the corresponding morpholinopeptide. A small aliquot (2 μL) was removed and analyzed by HPLC (RP 8 column, gradient: 50–90% acetonitrile/H_2O + 0.1% TFA over 20 min), which showed the morpholinopeptide to be 98% pure ($R_t = 6.59$ min). Insoluble material was filtered off from the remaining morpholinopeptide and the filtrate concentrated to give Boc-Leu-Plac-Pip-Lac-morpholine as a thick oil (13 mg, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8–2.3 (m, 27H), 2.5–3.9 (m, 15H), 4.5–5.6 (m, 4H) 7.1–7.4 (m, 5H). FABHRMS: *m*/*e* 646.3710 ($C_{35}H_{51}N_3O_9$ + H requires 646.3703). Similarly cleaved of resin to determine purity were: peptide **4** to give Boc-Leu-Lac-Leu-Plac-Pip-Lac-morpholine, 99% pure ($R_t = 9.12$ min), and peptide **6** to give Boc-Leu-Plac-Leu-Lac-Leu-Plac-Pip-Lac-morpholine, 90% pure ($R_t = 13.34$ min).

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