Pergamon

Tetrahedron Letters, Vol. 38, No. 5, pp. 757-760, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(96)02454-9

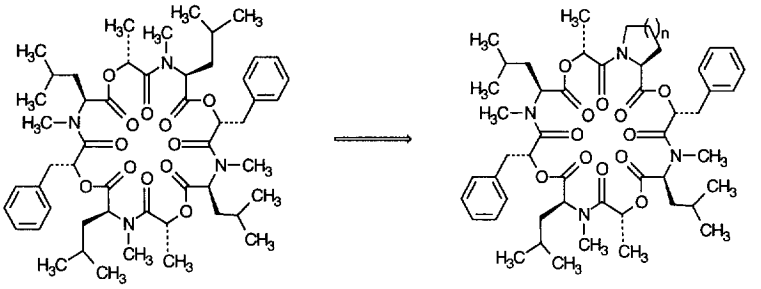
Solid-Phase Synthesis of Cyclooctadepsipeptide PF1022A Analogs using a Cyclization-Cleavage Method with Oxime Resin

Byung H. Lee

Animal Health Discovery Research, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49001

Abstract: *N*-Methyloctadepsipeptides attached to an oxime resin were cyclized by heating them in refluxing ethyl acetate for 2 days to give cyclooctadepsipeptide PF1022A analogs.

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 PF1022A and its analogs 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. Three different syntheses of this cyclooctadepsipeptide have been reported.[4] Each synthesis, however, requires multiple steps, which



PF1022A (1)

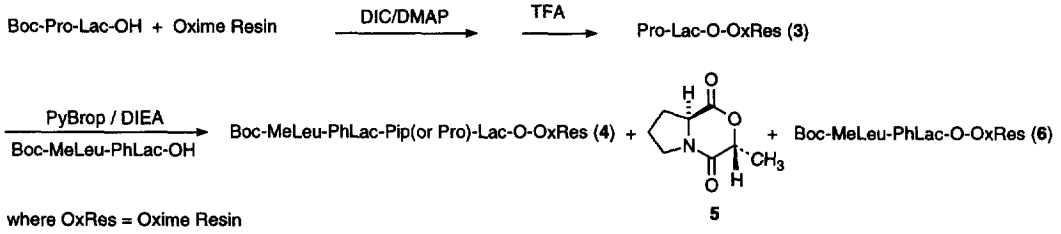
(2) a ; n = 2
b ; n = 1

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 analogs 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] We believe that our cyclization of *N*-methyl peptides and depsipeptides using a solid support is novel. Although DeGrado's group has described[7d] cyclization of peptides containing

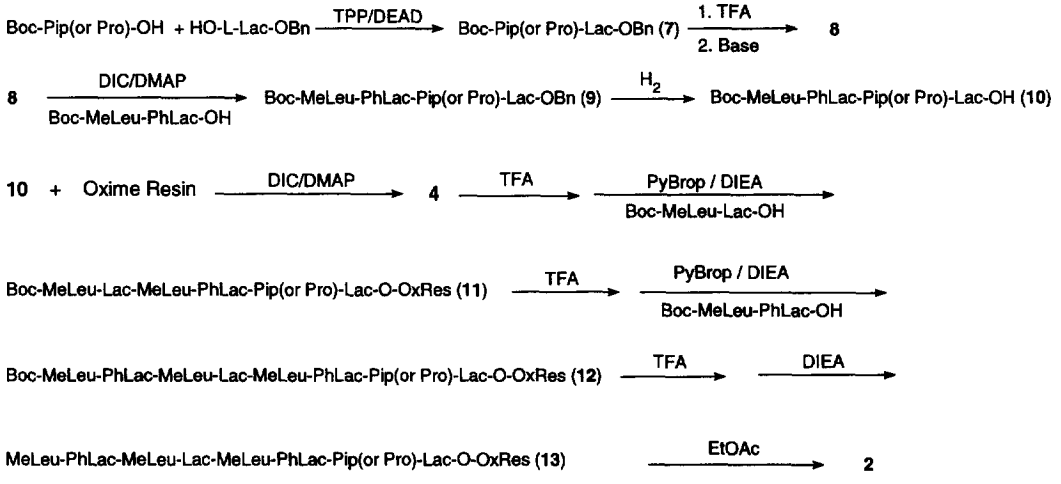
N-methyl substituents, the cyclization itself always took place at a primary amino group.

PF1022A is comprised of eight residues: four *N*-methyl-L-leucines (MeLeu), two 3-phenyl-D-lactates (PheLac), and two D-lactates (Lac) in a floppy, 24-membered ring with alternating amide and ester bonds. To make this molecule more rigid and thereby possibly enhance biological properties we introduced a proline and a pipecolic acid residue in place of the usual leucine residue to produce analogs **2a** and **2b**.

To accomplish our synthetic goal and at the same time build a diverse combinatorial library, we prepared the resin-bound didepsipeptide **3**. This was coupled with Boc-MeLeu-PhLac-OH [PyBrop, DMF, diisopropylethyl amine (DIEA), 1.5 h] giving resin-linked **4**, but in only 50% yield. Under the conditions of the coupling reaction, **3** underwent an intramolecular displacement from the oxime resin[7e] to give morpholinedione **5**. The liberated oxime-resin then coupled with Boc-MeLeu-PhLac-OH to give **6**.



We eliminated this problem by preparing tetradepsipeptide **10** and coupling it to the resin to give **4**. Thus, *N*-Boc-L-Pipecolic acid (Pip) [or proline] was coupled with benzyl L-lactate using triphenyl phosphine (TPP) and diethylazodicarboxylate (DEAD)[4b] to give the corresponding didepsipeptide **7**[8] with the appropriate stereochemistry. This was deprotected with trifluoroacetic acid (TFA)/methylene chloride (DCM) to give the free amine **8**, which was then condensed with Boc-MeLeu-PhLac-OH[4c] using diisopropyl carbodiimide(DIC)/*N*-dimethylaminopyridine (DMAP) to give tetradepsipeptide **9**.[9] Hydrogenolysis of compound **9** gave **10**. Compound **10** was coupled (DIC/DMAP/DCM) with oxime resin to give the resin-linked tetradepsipeptide **4**.[10] This was deprotected with TFA/DCM (25%, 1 h), followed by coupling with Boc-MeLeu-Lac-OH[4c] [PyBrop, DMF, diisopropylethyl amine (DIEA), 1.5 h] to give the



resin-bound hexadepsipeptide **11**.[11] Subsequent deprotection of **11** and coupling with Boc-MeLeu-PhLac-OH gave **12**, which was deprotected and basified to give resin-bound octadepsipeptide **13**. The resin was cleaved from peptides **4**, **11**, and **12** using morpholine[12] to give the free peptides which were at least 90% pure by HPLC analysis. Resin-bound peptide **13** was heated in refluxing ethyl acetate[13] for 2 days to give either **2a** (56% pure, 9.2 mg/50 mg resin by HPLC analysis, 48% from **6**) or **2b** (55% pure, 5.8 mg/50 mg resin by HPLC analysis). Other solvents such as THF, carbon tetrachloride, acetonitrile, dioxane, dioxane with acetic acid, DCM, DCM with acetic acid, toluene, DMF, and *n*-butanol did not give the desired cyclized product. Ethyl acetate-like solvents such as methyl propionate, propyl acetate (**2a**; 47% pure, 5.0 mg/50 mg resin by HPLC analysis) and formyl propionate did not give better results.

In conclusion, we have developed a method for the rapid preparation of *N*-methylcyclodepsipeptides using a solid support and in the process generated a combinatorial library of natural product analogs.

REFERENCES

1. Lynn, R. C. *Georgis' Parasitology for Veterinarians*; W. B. Saunders Co.: Philadelphia, 1995, 247.
2. Prichard, R. *Veterinary Parasitology* **1994**, *54*, 259.
3. Sasaki, T.; Takag, M.; Takasi, Y.; Miyadoh, S.; Okada, T.; Koyama, M. *J. Antibiotics* **1992**, *45*, 692.
4. (a) Ohyama, M. *Biosci. Biotech. Biochem.* **1994**, *58*, 1193. (b) Scherkenbeck, J; Plant, A; Harder, A; Mencke, N. *Tetrahedron* **1995**, *31*, 8459. (c) Dutton, F. E.; Nelson, S. J. *J. Antibiotics* **1994**, *47*, 1322.
5. (a) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233. (b) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385.
6. (a) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1980**, *45*, 1295. (b) DeGrado, W. F.; Kaiser, E. T. *J. Org. Chem.* **1982**, *47*, 3258. (c) Sasaki, T.; Kaiser, E. T. *J. Org. Chem.* **1991**, *56*, 3159.
7. (a) Ösapay, G.; Profit, A.; Taylor, J. W. *Tetrahedron Lett.* **1990**, *31*, 6121. (b) Xu, M.; Nishino, N.; Mihara, H.; Fujimoto, T.; Izumiya, N. *Chemistry Lett.* **1992**, 191. (c) Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany G.; Albercio, F. *Tetrahedron Lett.* **1993**, *34*, 1549. (d) Jackson, S.; DeGrado, W.; Dwivedi, A.; Parthasarathy, A.; Higley, A.; Krywko, J.; Rockwell, A.; Markwaldo, J.; Wells, G.; Wexler, R.; Mousa, S.; Harlow, R. *J. Am.Chem. Soc.* **1994**, *116*, 3220. (e) Nishino, N.; Xu, M.; Mihara, H.; Fujimoto, T. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 991.
8. 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 diethyl ether (20 mL). The resulting mixture was treated with DEAD (0.5 mL, 3.17 mmol in 5 mL of diethyl ether) 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% ethyl acetate in hexane) to give **7a** (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). HRMS(FAB): *m/e* 392.2086 ($C_{21}H_{29}N_1O_6$ + H requires 392.2073).
9. Boc-MeLeu-PhLac-OH (5.0 g, 12.6 mmol) was dissolved in methylene chloride (10 mL) and treated with DIC (2.2 ml, 12.6 mmol), DMAP (244 mg, 2 mmol), and compound **8a** (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 chromatography (20%

acetone in hexane) to give **9a** 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). HRMS(FAB): *m/e* 667.3608 ($C_{37}H_{50}N_2O_9$ + H requires 667.3594).

10. Compound **10a** (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 x 25 mL), MeOH (2 x 25 mL), DMF (2 x 25 mL), and DCM (2 x 25 mL). The washed resin was dried *in vacuo* for 4 h to give **4a** (2.67 g, 0.65 mmol/g by cleavage reaction with morpholine).
11. Resin-bound peptide **4a** (250 mg, 0.16 mmol) was suspended in TFA/DCM (25%, 4 mL). The mixture was stirred for 1 h at room temperature, washed with DCM (2 x 25 mL), MeOH (2 x 25 mL), DMF (2 x 25 mL), and DCM (2 x 25 mL). The washed resin was dried *in vacuo* for 1 h to give resin-bound peptide **4a** having a free amino group. This resin was suspended in DMF (4 mL) and treated with Boc-L-MeLeu-D-Lac-OH (170 mg, 0.54 mmol), DIEA (0.2 mL), and PyBrop (Novabiochem, 250 mg, 0.54 mmol). The mixture was stirred at room temperature for 1.5 h. The resin was washed with DCM (2 x 25 mL), MeOH (2 x 25 mL), DMF (2 x 25 mL), and DCM (2 x 25 mL). The washed resin was dried *in vacuo* for 4 h to give resin-bound peptide **11a** (257 mg).
12. In a typical resin cleavage reaction, resin-bound peptide **4a** (30 mg) was suspended in DCM (1 mL) containing 10 mg of morpholine. The mixture was stirred for 16 h at room temperature. A small aliquot (2 μL) was removed for HPLC analysis [RP 8 column; gradient: 50 to 90% acetonitrile/H_2O + 0.1% TFA over 20 min, R_t of the corresponding mopholine amide (98% pure) is 6.59 min]. Insoluble material was filtered off and the filtrate was concentrated to give Boc-MeLeu-PhLac-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). HRMS(FAB): *m/e* 646.3710 ($C_{35}H_{51}N_3O_9$ + H requires 646.3703). Similarly: Boc-MeLeu-Lac-MeLeu-PhLac-Pip-Lac-morpholine (from **11a**); R_t = 9.12 min (99% pure, RP 8 column; gradient: 50 to 90% acetonitrile/H_2O + 0.1% TFA over 20 min); Boc-MeLeu-PhLac-MeLeu-Lac-MeLeu-PhLac-Pip-Lac-morpholine (from **12a**); R_t = 13.34 min (90% pure, RP 8 column; gradient: 50 to 90% acetonitrile/H_2O + 0.1% TFA over 20 min).
13. In a typical cyclization reaction, resin-bound peptide **13a** (100 mg) was suspended in ethyl acetate (4 mL) and the mixture was refluxed for 48 h. Insoluble material was filtered off and the filtrate was concentrated. Preparative thin layer chromatography of the residue (silica gel, 30% acetone in hexane) afforded product **2a** (16 mg) as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8-2.4 (m, 39H), 2.6-3.4 (m, 15H), 3.5-5.8 (m, 8H) 7.1-7.4 (m, 10H). HRMS(FAB): *m/e* 955.5039 ($C_{51}H_{72}N_4O_{12}$ + Na requires 950.5044). $[\alpha]_D$ = -74.1° (c 0.48, $CHCl_3$). R_t = 11.23 min (RP 8 column, gradient: 50 to 90% acetonitrile/H_2O + 0.1% TFA over 20 min). Compound **2b** (8 mg) was isolated from 100 mg of the resin as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.8-2.4 (m, 37H), 2.7-3.3 (m, 15H), 3.5-6.0 (m, 8H) 7.2-7.4 (m, 10H). HRMS(FAB): *m/e* 941.4905 ($C_{50}H_{70}N_4O_{12}$+ H requires 941.4888). R_t = 9.62 min (RP 8 column, gradient: 50 to 90% acetonitrile/H_2O + 0.1% TFA over 20 min).

(*Received in USA* 5 *November* 1996; *revised* 6 *December* 1996; *accepted* 9 *December* 1996)