SYNTHESIS OF PF1022A, AN ANTHELMINTIC CYCLODEPSIPEPTIDE

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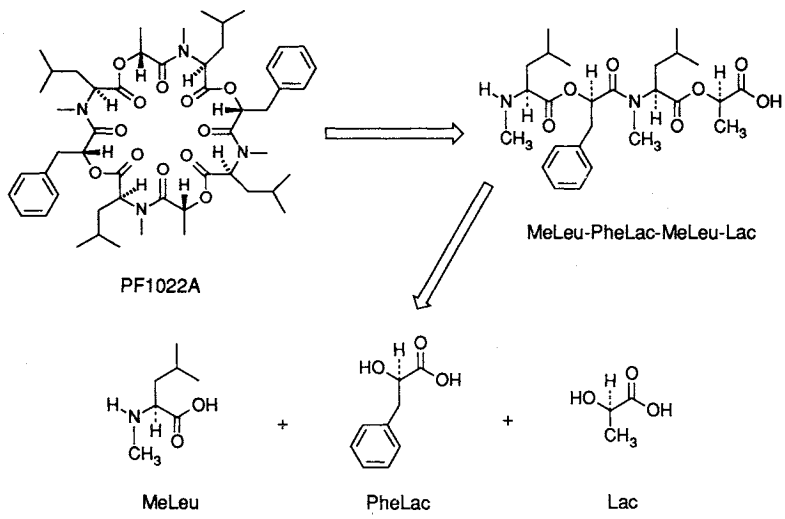
Anthelmintic cyclodepsipeptide PF1022A has been prepared in eleven steps from *N*-Boc-*N*-methyl-L-leucine, benzyl 3-phenyl-D-lactate and benzyl D-lactate.

Recently SASAKI, *et. al.*, reported the isolation and structure determination of the anthelmintic cyclodepsipeptide PF1022A[1]. Our interest in this compound stems from its novel structure and reported broad spectrum anthelmintic activity[2,3]. Because new templates that demonstrate anthelmintic activity are rare, we were interested in evaluating this novel structure in our gerbil model[4],†. To that end, we developed a synthesis of PF1022A.

Results and Discussion

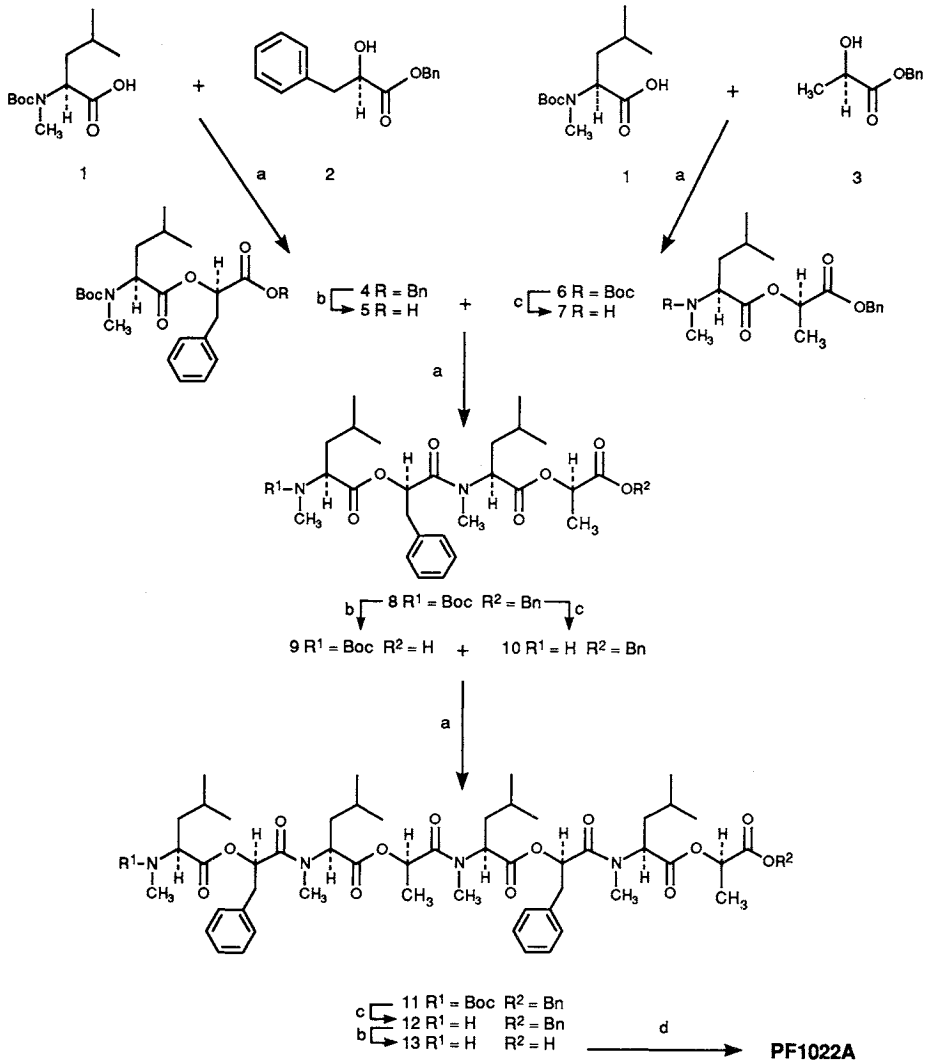
PF1022A contains eight residues: four *N*-methyl-L-leucines (MeLeu), two 3-phenyl-D-lactates (PheLac), and two D-lactates (Lac) (Scheme 1). PF1022A has been shown by NMR to exist in two principal conformations in CD_3OD: an asymmetric form (80%) and a more symmetrical form (20%)[1]. The residues are linked in a pattern which gives PF1022A a two-fold axis of symmetry. This simplifies its synthesis by allowing it to be constructed from any two identical tetradepsipeptide subunits.

Scheme 1. Retrosynthetic analysis of PF1022A.



† Biological data will be reported in a separate paper currently under preparation.

Scheme 2. Synthesis of PF1022A.



Reagents: (a) DCC/DMAP/CH_2Cl_2; (b) 10% Pd/C, H_2 (3 atm), EtOH; (c) 10% TFA/CH_2Cl_2; (d) BOP/NMM/CH_2Cl_2/1 mM.

Of the four unique tetradepsipeptide subunits arising from the four ways in which PF1022A can be retrosynthetically cleaved, we chose the MeLeu-PheLac-MeLeu-Lac subunit (tetradepsipeptide **8**) because of the commercial availability of *N*-Boc-*N*-methyl-L-leucine **1** which enabled us to prepare PF1022A in eleven steps from **1**, benzyl 3-phenyl-D-lactate **2**[5] and benzyl D-lactate **3**[6] (Scheme 2).

Residues were coupled to produce both ester bonds (**1**+**2**→**4** and **1**+**3**→**6**) and amide bonds (**5**+**7**→**8** and **9**+**10**→**11**) in 57~95% yields using dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in methylene chloride. Suspected diastereomers seen in the NMR spectra of **4** and **6** were in fact rotamers which were slowly interconverting on the NMR time scale. The presence of rotamers was indicated by observing the coalescence of seemingly extraneous peaks when samples were heated to 45°C in the spectrometer. NMR revealed a high degree of optical purity for **7** (and by implication

6); here the spectrum was not obscured by rotamers. We determined that most of the extra peaks seen in the spectra of tetradepsipeptide **8** and octadepsipeptide **12** were due to rotamers; the balance were due to diastereomers.

BOC protecting groups were removed (**6**→**7**, **8**→**10** and **11**→**12**) by stirring the substrate in 10% TFA in methylene chloride at room temperature for 30 to 60 minutes (88~97% yields). Benzyl protecting groups were removed (**4**→**5**, **8**→**9** and **12**→**13**) by hydrogenolysis in EtOH using 10% Pd on charcoal in a 5:1 weight ratio of substrate to catalyst (90~96% yields). Hydrogenolysis was complete within two to four hours at 3 atm; when less catalyst was used, reaction time increased dramatically. After coupling tetradepsipeptides **9** and **10**, we found it expedient to remove the Boc protecting group to give **12** before hydrogenolysis of the benzyl ester to give **13**; this allowed isolation of octadepsipeptide **13** as a neutral compound.

Macrocyclic ring closure of octadepsipeptide **13** using DCC gave PF1022A in low yield along with similar products which were not identified. Macrocyclization of octadepsipeptide **13** with benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and *N*-methylmorpholine (NMM) at high dilution in methylene chloride proved more successful. HPLC analysis of the product mixture showed two major components in an 83:17 ratio and trace amounts of two other components. The most abundant component was readily separated by preparative HPLC to give a white powder found to be identical to PF1022A (30% yield) by comparing its ^{1}H and ^{13}C NMR spectra with published spectra[1]. We observed a rotation in methanol of $-101°$ which compares favorably with the reported value of $-102°$. The infrared and ultraviolet spectra were also identical. The large discrepancy in melting points (149~153°; lit. val., ~104~106°) may be due to a difference in crystalline forms.

PF1022A is a good chelator of alkali metal ions as shown by FAB mass spectral data. In addition to the molecular ion at 949 (M+H), ions at 971 (M+Na) (for the sodium chelate) and 1081 (M+Cs) (for the cesium chelate) were detected. These chelates arise as artifacts from mass spectral analysis; sodium and cesium are common residues from instrument calibration. None of the precursors of PF1022A formed chelates upon mass spectrometry.

The other major component was shown by NMR to be a mixture of diastereomers (6% yield). This material gave a mass spectrum virtually identical to that of PF1022A and exhibited the same alkali metal ion chelation ability. Trace amounts of what we presume to be mixtures of other diastereomers were also collected during purification by HPLC; these have not been further analyzed.

Experimental

N-Boc-*N*-methyl-L-leucine was purchased from Bachem. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer. EI mass spectra were obtained on a Finnegan-Matt 8230B mass spectrometer while FAB spectra were obtained on a VG Analytical 70-SE mass spectrometer. Infrared spectra were recorded on a Digilab Model FTS-40 spectrophotometer. A Perkin-Elmer Lambda 7 was used to record UV spectra. Preparative low pressure chromatography was performed using EM Science Silica Gel 60 (230~400 mesh); elution was with 5~40% EtOAc in hexane. Purification of the final product was achieved by HPLC on a Waters Delta Prep 4000 using a 40×200 mm Waters Delta Pak C18 column. Optical rotations were measured at 25°.

Benzyl *N*-Boc-*N*-methyl-L-leucyl-D-lactate **6**

N-Boc-*N*-methyl-L-leucine **1** (2.56 g, 10.4 mmol), benzyl D-lactate **3** (1.88 g, 10.4 mmol) and DCC

(2.15 g, 10.4 mmol) were combined and dissolved in CH_2Cl_2 (50 ml). DMAP (64 mg, 0.52 mmol) was added and the reaction mixture stirred at room temperature for one hour. TLC showed a small amount of unreacted lactate **3**. More leucine **1** (0.45 g) and DCC (0.4 g) were added. After another 40 minutes, TLC showed no starting material. The reaction mixture was filtered to remove the dicyclohexylurea by-product, and the filtrate concentrated to a clear, yellow oil (5.07 g). This was purified by low pressure chromatography to give benzyl *N*-Boc-*N*-methyl-L-leucyl-D-lactate **6** (3.22 g, 76% yield) as a clear, colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (m, 6H), 1.45~1.71 (m, 15H), 2.73 (s, 1.5H), 2.76 (s, 1.5H), 4.74 (m, 0.5H), 4.95 (m, 0.5), 5.15 (m, 3H), 7.35 (m, 5H). EI-MS *m/z* 407 (M, $C_{22}H_{33}NO_6$). *Anal* Calcd for $C_{22}H_{33}NO_6$: C 64.84, H 8.16, N 3.44. Found: C 64.96, H 8.39; N 3.80. $[\alpha]_D$ $-22°$ (*c* 0.49, $CHCl_3$).

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactate **4**

Applying the DCC procedure described for compound **6** to *N*-Boc-*N*-methyl-L-leucine **1** (3.67 g, 14.9 mmol), benzyl 3-phenyl-D-lactate **2** (3.83 g, 14.9 mmol), DCC (3.08 g, 14.9 mmol), DMAP (91 mg, 0.75 mmol) and CH_2Cl_2 (75 ml) gave benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactate **4** as a clear, pale yellow oil (6.87 g, 95% yield) following low pressure chromatography. ^{1}H NMR (300 Mz, $CDCl_3$) δ 0.89 (d, 6H, J=6.09 Hz) 1.35~1.65 (m, 12H), 2.60 (s, 1.5H), 2.65 (s, 1.5H), 3.15 (m, 2H), 4.71 (m, 0.5H), 4.99 (m, 0.5H), 5.12 (m, 2H), 5.26 (m, 1H), 7.25 (m, 10H). EI-MS *m/z* 483 (M, $C_{28}H_{37}NO_6$). $[\alpha]_D$ $-13°$ (*c* 1.0, $CHCl_3$).

Benzyl *N*-Methyl-L-leucyl-D-lactate **7**

Benzyl *N*-Boc-*N*-methyl-L-leucyl-D-lactate **6** (2.55 g, 6.26 mmol) was dissolved in CH_2Cl_2 containing 10% (v/v) TFA (100 ml). The reaction mixture was stirred 50 minutes and then slowly poured into saturated $NaHCO_3$ with rapid stirring. The mixture was transferred to a separatory funnel and shaken. The layers were separated, and the aqueous layer extracted with CH_2Cl_2. The organic layers were combined, washed with water, dried (Na_2SO_4), filtered and concentrated. Drying under high vacuum gave benzyl *N*-methyl-L-leucyl-D-lactate **7** (1.70 g, 89% yield) as a clear, pale-yellow oil. It was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (d, 3H, J=7.00 Hz), 0.92 (d, 3H, J=6.96 Hz), 1.47 (td, 2H, J=1.8, 6.94 Hz), 1.52 (d, 3H, J=7.09 Hz), 1.62 (br d s, 1H), 1.72 (septet, 1H, J=6.73 Hz), 2.35 (s, 3H), 3.27 (t, 1H, J=7.30 Hz), 5.18 (m, 3H), 7.35 (m, 5H).

N-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactic acid **5**

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactate **4** (6.02 g, 12.4 mmol) was dissolved in absolute EtOH (100 ml) and hydrogenolyzed for seven hours at 3 atm over 10% palladium on charcoal (667 mg). The reaction mixture was flushed with nitrogen, filtered through Celite and concentrated to remove EtOH. The residue was taken up in Et_2O, washed with water (4×), dried ($MgSO_4$), and filtered. The filtrate was concentrated and dried under high vacuum to give *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactic acid **5** (4.70 g, 96% yield) as a clear, colorless, viscous oil. This material was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (m, 6H), 1.3~1.9 (m, 12H), 2.5~3.0 (m, 3H), 3.0~3.3 (m, 2H), 4.65 (m, 1H), 4.90 (m, 1H), 5.30 (m, 1H), 7.25 (m, 5H).

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **8**

Applying the DCC procedure described for compound **6** to *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactic acid **5** (2.18 g, 5.53 mmol), benzyl *N*-methyl-L-leucyl-D-lactate **7** (1.70 g, 5.53 mmol), DMAP (34 mg, 0.28 mmol), DCC (1.26 g, 6.08 mmol) and CH_2Cl_2 (40 ml) gave benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **8** as a clear, colorless oil (2.15 g, 57% yield) following low pressure chromatography. It was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (m, 12H), 1.3~1.8 (m, 18H), 2.5~3.0 (m, 6H), 3.0~3.2 (m, 2H), 4.69 (m, 0.5H), 4.96 (m, 0.5), 5.0~5.2 (m, 3H), 5.28 (m, 1H), 5.45 (m, 1H), 7.30 (m, 10H). EI-MS m/z 682 (M, $C_{38}H_{54}N_2O_9$).

N-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid **9**

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **8** (1.15 g, 1.68 mmol) was dissolved in absolute EtOH (100 ml), 10% palladium on charcoal added (200 mg) and the mixture hydrogenolyzed for four hours in accordance with the procedure described for compound **5**. This gave

N-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid **9** (0.90 g, 90% yield) as a clear, colorless, viscous oil. This material was shown by NMR analysis to be reasonably pure and was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (m, 12H), 1.3~1.8 (m, 18H), 2.6~3.3 (m, 8H), 4.6~5.9 (m, 5H), 7.27 (s, 5H). FAB-MS *m/z* 593 (M+H, $C_{31}H_{48}N_2O_9$).

Benzyl *N*-Methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **10**

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **8** (1.20 g, 1.76 mmol) was dissolved in CH_2Cl_2 containing 10% (v/v) TFA (50 ml). The reaction mixture was stirred 30 minutes and then processed following the procedure used for compound **7**. This gave benzyl *N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **10** (0.99 g, 97% yield) as a clear, pale-yellow oil. It was used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.7~1.1 (m, 12H), 1.1~1.9 (m, 10H), 2.24 (s, 3H), 2.93 (m, 3H), 3.11 (m, 2H), 3.23 (t, 1H, J=7.22 Hz), 5.0~5.25 (m, 3H), 5.25~5.4 (m, 1H), 5.4~5.6 (m, 1H), 7.30 (m, 10H). EI-MS *m/z* 582 (M, $C_{33}H_{46}N_2O_7$).

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **11**

Applying the DCC procedure described for compound **6** to *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid **9** (0.88 g, 1.48 mmol), benzyl *N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **10** (0.96 g, 1.65 mmol), DMAP (10 mg, 0.085 mmol), DCC (351 mg, 1.70 mmol) and CH_2Cl_2 (20 ml) gave benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **11** (1.04 g, 60% yield) as a white solid foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.7~1.1 (m, 24H), 1.1~2.0 (m, 27H), 2.5~3.3 (m, 16H), 4.68 (m, 0.5H), 4.96 (m, 0.5H), 5.0~5.6 (m, 9H), 7.27 (m, 15H). $[\alpha]_D$ −69° (*c* 0.98, $CHCl_3$). FAB-MS *m/z* 1157 (M+H, $C_{64}H_{92}N_4O_{15}$). *Anal* Calcd for $C_{64}H_{92}N_4O_{15}$: C 66.41, H 8.01, N 4.84. Found: C 66.12, H 8.09, N 4.96.

Benzyl *N*-Methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **12**

Benzyl *N*-Boc-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **11** (0.99 g, 0.86 mmol) was dissolved in CH_2Cl_2 containing 10% (v/v) TFA (28 ml). The reaction mixture was stirred 30 minutes and then processed following the procedure used for compound **7**. This gave benzyl *N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **12** (0.84 g, 93% yield) as a colorless glass and white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.7~1.1 (m, 24H), 1.1~2.0 (m, 19H), 2.24 (s, 3H), 2.6~3.4 (m, 14H), 5.0~5.7 (m, 9H), 7.27 (m, 15H). FAB-MS *m/z* 1057 (M+H, $C_{59}H_{84}N_4O_{13}$).

N-Methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid **13**

Benzyl *N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactate **12** (0.83 g, 0.78 mmol) was dissolved in absolute EtOH (50 ml), 10% palladium on charcoal added (154 mg) and the mixture hydrogenolyzed for two hours following the procedure described for compound **5** to give *N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid **13** (0.73 g, 96% yield) as an off-white solid foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.6~1.2 (m, 24H), 1.2~2.0 (m, 18H), 2.0~2.7 (m, 3H), 2.7~3.8 (m, 14H), 4.4~5.7 (m, 7H), 7.27 (m, 10H). $[\alpha]_D$ −41° (*c* 0.99, $CHCl_3$). FAB-MS *m/z* 967 (M+H, $C_{52}H_{78}N_4O_{13}$).

Cyclodepsipeptide PF1022A

BOP reagent (0.20 g, 0.45 mmol) was added to a solution of *N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactyl-*N*-methyl-L-leucyl-3-phenyl-D-lactyl-*N*-methyl-L-leucyl-D-lactic acid **13** (0.44 g, 0.45 mmol) in CH_2Cl_2 (460 ml; to give a 10^{-3} M solution) at 0°C followed by *N*-methylmorpholine (50 μl, 46 mg, 0.45 mmol). The reaction mixture was stirred at 0°C for 30 minutes and then at room temperature for 48 hours. The reaction mixture was washed with one-half volume of saturated NH_4Cl, dried ($MgSO_4$) and concentrated to an orange-colored solid foam (592 mg). Upon low pressure chromatography, this

material gave optically crude cyclodepsipeptide PF1022A as a white solid foam (217 mg, 50% yield). HPLC analysis showed two major components in an 83 : 17 ratio. These were separated by preparative HPLC using a Waters Delta-Pak C18 column with a 65~90% gradient elution between 0.1% TFA in CH_3CN and 0.085% TFA in water at a flow rate of 30 ml/minute and detection at 218 nm. The fractions containing the major component were collected and lyophilized to give optically pure cyclodepsipeptide PF1022A (130 mg, 30% yield) as a white powder. The ^1H and ^{13}C NMR spectra were identical with the published spectra of PF1022A[1]. ^1H NMR (300 MHz, CD_3OD) δ 0.6~1.0 (m, 24H), 1.1~1.9 (m, 18H), 2.6~3.1 (m, 14H), 3.21 (s, 4H), 5.0~5.8 (m, 6H), 7.21 (s, 10H). ^{13}C NMR (100 MHz, CD_3OD) δ 17.25, 17.46, 21.10, 21.43, 21.63, 21.68, 23.58, 23.65, 23.67, 23.84, 25.22, 25.60, 25.79, 26.03, 26.10, 26.19, 30.02, 31.12, 31.35, 32.02, 37.38, 37.92, 38.59, 38.63, 38.88, 39.02, 55.45, 55.51, 55.73, 58.62, 68.47, 69.92, 72.33, 72.55, 128.19, 128.29, 128.36, 129.67, 129.73, 129.66, 130.69, 130.73, 130.75, 136.23, 136.50, 170.80, 171.06, 172.07, 172.37, 173.01, 173.09, 173.47, 174.44. $[\alpha]_D$ $-101°$ (*c* 0.97, CH_3OH); lit. val. $-102°$ (0.1, CH_3OH). FAB-MS *m/z* 949 (M+H, $C_{52}H_{76}N_4O_{12}$), 971 (M+Na), 1081 (M+Cs). Additional purification by recrystallization from acetone/hexane gave dense white pellets, mp 149~153°; lit. val.[1] ~104~106° (prisms). Infrared (KBr, cm^{-1}): 1738 (ester), 1651 (amide); lit. val. 1740 (ester), 1660 (amide). UV, λ_{max} (ε (1%, 1 cm, MeOH)): 258 nm (2.5), 264 nm (3.3); lit. val. 257 nm (3.9), 263 nm (2.9). *Anal* Calcd for $C_{52}H_{76}N_4O_{12}$: C 65.80, H 8.07, N 5.90. Found: C 65.56, H 8.13, N 5.89.

The fractions containing the minor component were collected and lyophilized to give a white powder (25 mg, 6% yield). ^1H NMR showed this to consist of a mixture of diastereomers. $[\alpha]_D$ $-49°$ (*c* 1.0, CH_3OH). FAB-MS *m/z* 949 (M+H, $C_{52}H_{76}N_4O_{12}$), 971 (M+Na), 1081 (M+Cs) identical to the mass spectrum of PF1022A.

Conclusion

We have developed a synthesis of the anthelmintic cyclodepsipeptide PF1022A by a convergent route which takes advantage of the symmetry of the molecule. This route consists of eleven steps and is suitable for the preparation of PF1022A in gram quantities. During the preparation of this manuscript, we learned of a synthesis of PF1022A by OHYAMA[7].

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