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Chimeric cyclodepsipeptides as mimetics for the anthelmintic PF1022A

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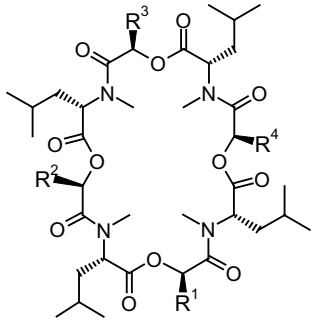
Abstract—In the anthelmintic cyclooctadepsipeptide PF1022A (**1**) didepsipeptide units have been exchanged for the β-turn mimetics (D)-Pro-(L)-Pro and BTD (**7**) in order to elucidate the functional role of the depsipeptide backbone. Compounds **12** and **14** are the first PF1022A analogues in which a substantial part of the PF1022A backbone has been replaced with an improvement of anthelmintical activity. Preliminary structure–activity relationships suggest a symmetric conformation to be the biological active one.

1. Introduction

Nematode infections are a major cause of human morbidity and mortality in the tropics as well as in temperate climates.[1] In animal health, nematode infections play a crucial role in cattle and sheep and are responsible for enormous economic losses.[2] Since the discovery of the highly active anthelmintic macrolides milbemycin and avermectin in the early seventies, reports on potent new classes of anthelmintics have been scarce.[3] One of the most outstanding recently reported anthelmintic is the cyclooctadepsipeptide PF1022A (**1**), the most active member of a novel class of anthelminitic agents.[4] During the past years several total syntheses[5] of PF1022A and manifold structure–activity relationships have been established.[6] Additionally, the biosynthesis of PF1022A has been elucidated[7] and intensive investigations into the mode of action of this novel anthelmintic are underway.[8]

Most of the published PF1022A modifications refer to side-chain alterations of the amino and hydroxycarboxylic acids.[9] Until now only a limited number of backbone modifications such as the exchange of *N*-methylleucine for aza-leucine[10] or the replacement of a leucine for proline or proline related cyclic amino acids have been reported.[11] A common strategy to restrict the number of conformations or to stabilize a particular secondary structural element in a peptide is to replace certain parts of the peptide for rigid, cyclic amino acids or turn mimetics. In this paper we wish to outline the 'conformation-guided' replacement of didepsipeptide



	R^1	R^2	R^3	R^4
PF1022A (**1**)	Me	Bzl	Me	Bzl
PF1022B	Bzl	Bzl	Bzl	Bzl
PF1022C	Bzl	Bzl	Me	Bzl
PF1022D	Me	Me	Me	Bzl
PF1022E	Me	p-OH-Bzl	Me	Bzl
Bassianolide	^{i}Pr	^{i}Pr	^{i}Pr	^{i}Pr

Figure 1.

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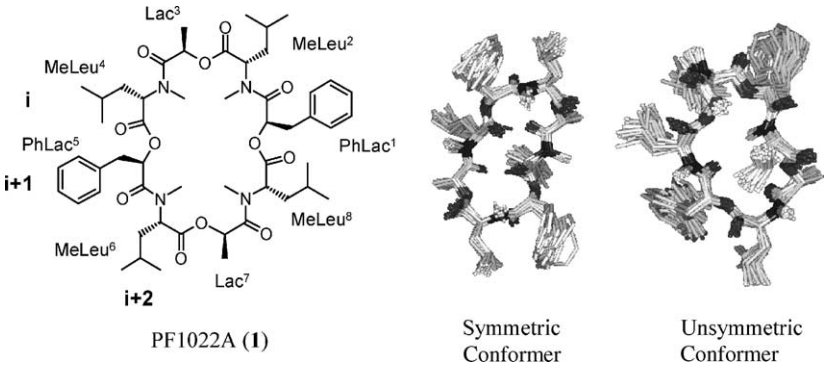


Figure 2.

units for the β-turn mimetics (D)-Pro-(L)-Pro and the bicyclic 'Nagai–Sato' mimetic (BTD, **2**) (Fig. 1).

The structure of PF1022A has recently been determined by single-crystal X-ray analysis.[12] Despite the occurrence of different space groups, structures determined from crystals obtained from methanol, or acetone were nearly identical. Both structures showed one *cis* amide bond between (L)-MeLeu^2 and (D)-Lac^3, conferring asymmetry on the molecule. However, in solution PF1022A exists as a 3:1 mixture of two conformers, which interconvert very slowly on the NMR time scale.[13] The main isomer corresponds to an asymmetric conformer, while the minor isomer can be assigned to a symmetric conformer with all four amide bonds in the *trans* configuration. MD simulations in a H_2O solvent cage using NMR derived NOE's as constraints revealed two type II′ β-turns in the symmetric conformation, containing a (D)-PhLac- and a *N*-methyl-(L)-leucine residue in the central $i+1$ and $i+2$ positions. The two β-loops induce a hydrophobic collapse of the two (D)-PhLac-(L)-MeLeu residues and the side chains of MeLeu^4 and MeLeu^8, which significantly stabilizes the symmetric conformation. The *cis* amide bonds between (D)-Lac^3 and (L)-MeLeu^4 in the asymmetric conformation forces the side chains of MeLeu^2 and MeLeu^4 together, resulting in a NOE between MeLeu^{2}H$_\alpha$ and MeLeu^{4}CH$_3$H$_\delta$. An analogous NOE between MeLeu^{8}H$_\alpha$ and MeLeu^{6}CH$_{3\delta}$ demonstrates that the MeLeu^6 side chain folds into the macrocyclic ring system (Fig. 2).

2. Synthesis

The Nagai–Sato bicyclic dipeptide mimetic (BTD, **2**) was chosen because it shows an almost superimpossible conformation on that of (D)-Phe-(L)-Pro residues in gramicidin S, which is known to be a type II′ β-turn.[14] Additionally, **2** has been used successfully by Alberg and Schreiber[15] as a rigidifying turn mimetic for cyclosporin and by Bartlett and co-workers[16] for a structural mimetic of tendamistat, a 74-residue proteinaceous inhibitor of α-amylase (Fig. 3).

The synthesis of **2** followed the route developed by Bartlett[16] and Schreiber.[15] The two linear hexadepsipeptides **3** and **4** were synthesized from (D)-lactic acid, (D)-

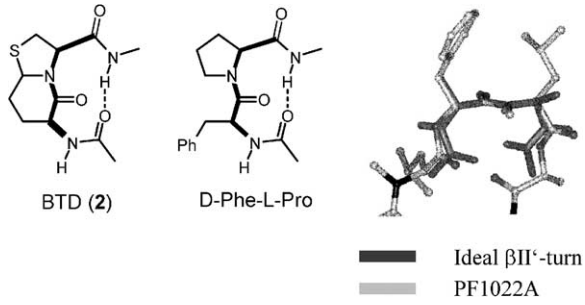


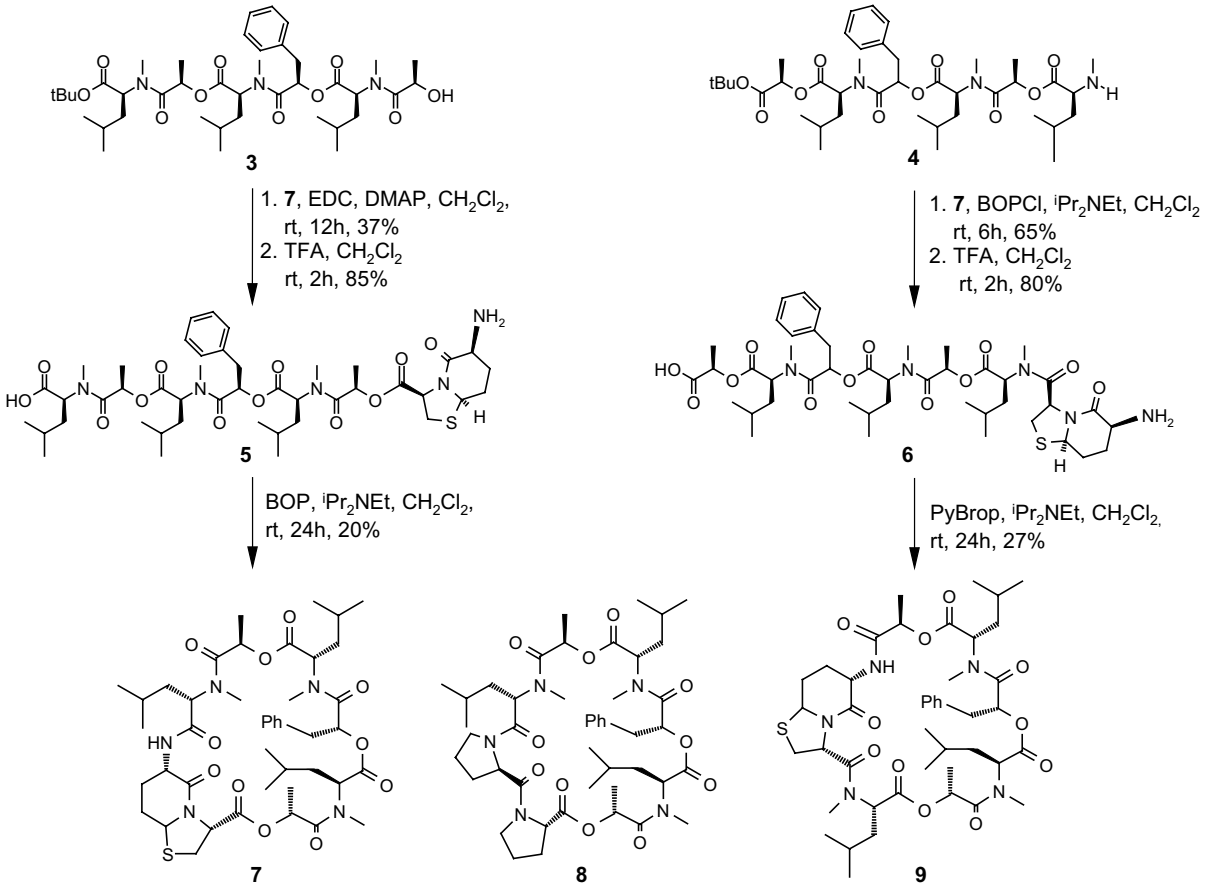
Figure 3.

phenyllactic acid and *N*-methyl-(L)-leucine according to standard procedures.[17] Hexadepsipeptide **4** was coupled with **2** using BOP-Cl as the coupling reagent of choice for N-methyl substituted amino acids.[18] Removal of both the Boc- and ^{t}butyl-group with TFA provided the bicyclic linear PF1022 precursor **6** in 80% yield. Among several coupling reagents tested PyBrop was found to produce the highest yield (27%) of **9** in the entropically critical macrocylization reaction.[19] Formation of the ester bond between **2** and **3** proved to be difficult. Best yields of **5** were obtained with EDC/DMAP. Addition of HOBt did not improve the situation and a two step active ester coupling sequence using the trichlorophenyl ester failed completely. Simultaneous deprotection with TFA and cyclization with BOP afforded the tricyclic PF1022 analogue **7** in sufficient yield.

Similarly to the rigid BTD-mimetic the linear (D)-Pro-(L)-Pro also shows a strong β-turn stabilizing effect. **8** was synthesized analogously to **7** by coupling **3** with the commercially available (D)-Pro-(L)-Pro, followed by protecting group removal and macrocyclization (Scheme 1).

3. Discussion

The chimeric PF1022A analogues **7** and **8** have the turn mimic in the favourable $i+1$, $i+2$ position while in analogue **9** the BTD is shifted to the i, $i+1$ position.[20] NMR data together with MD simulations revealed that all compounds exist in only one conformation, and thus confirm the conformation-stabilizing effect of the (D)-Pro-(L)-Pro and the BTD turn mimetic. In particular



Scheme 1.

the conformation of **8** resembles the symmetric conformation of PF1022A indicating that (D)-Pro-(L)-Pro is an adequate substitute for the (D)-PhLac-(L)-Leu unit in PF1022A.

The synthetic compounds **7**–**9** were tested exclusively in an animal test model, which provides a more realistic picture of the anthelmintic potency compared to in vitro screenings. In order to minimize animal usage in this first screening stage anthelmintic activity was determined only against the sensitive helminth *Haemonchus contortus*.[21] Thus, sheep (Merino or Schwarzkopf breed, 25–35 kg body weight) were infected experimentally with 5000 *H. contortus* L3 larvae and treated with the test substance after the end of the pre-patency period of the parasite.[22] The test compounds were administered orally in gelatine capsules or intravenously, as recently described.[23] Anthelmintic effects of the test substances against *H. contortus* adults were measured as a function of the reduction in faecal egg count. For the purpose of counting eggs, freshly obtained faeces from experimental animals were prepared using the McMaster method as modified by Wetzel and the egg count was calculated per gram of faeces.[24] The egg counts were determined at regular intervals before and after treatment (Fig. 4).

Analogues **7** and **8** containing the mimetics in the centre of the β-turn gave full control of the nematode *H. contortus* even at a dose of 0.01 mg kg^{-1}. Thus, the two

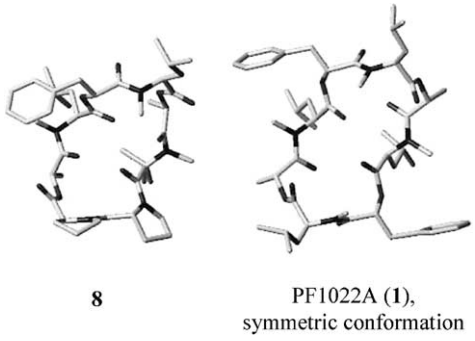


Figure 4.

Table 1.

Compound	Dosage (mg/kg)	Eggs per gram of faeces		Reduction of eggs per gram of faeces after treatment (%)
		Before treatment	After treatment (±SE)	
Control[a]	0	6667	6142 (±364)	8
1	0.1	1833 (±71)	0	100
	0.01	1000 (±189)	573 (±182)	43
7	0.01	1800 (±235)	83 (±21)	95
8	0.01	2500 (±118)	50 (±8)	98
9	0.025	3268 (±1)	1345 (±272)	59
	0.01	1800 (±141)	1638 (±123)	9

[a] Controls were not treated with any compound; egg counting was undertaken at the corresponding times compared to treated animals.

compounds exert a 2 times higher anthelmintic activity compared to PF1022A. Interestingly, compound **9**, in which the turn mimetic has been placed in the unfavourable *i*, *i* + 1 position, was significantly less active against *H. contortus* (Table 1).

Compounds **7** and **8** are the first analogues in which a substantial part of the PF1022A backbone was replaced for other residues with an improvement of anthelmintic activity. The biological results indicate the symmetric conformation might be the biological active one. Thus, structures similar to **7** and **8** may serve as starting points for second generation PF1022 analogues.

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References and notes

1. Bundy, D. A. P.; Silva, N. R. *Br. Med. Bull.* **1998**, *54*, 421–432.
2. Waller, P. J. *Vet. Parasitol.* **1997**, *72*, 391–412.
3. Meinke, P. T. *J. Med. Chem.* **2001**, *44*, 641–659.
4. Sasaki, T.; Takagi, M.; Yaguchi, T.; Miyadoh, S.; Okada, T.; Koyama, M. *J. Antibiot.* **1992**, *45*, 692–697.
5. (a) Dutton, F. E.; Nelson, S. J. *J. Antibiot.* **1994**, *47*, 1322–1327; (b) Ohyama, M.; Iinuma, K.; Isogai, A.; Suzuki, A. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1193–1194; (c) Scherkenbeck, J.; Plant, A.; Harder, A.; Mencke, N. A. *Tetrahedron* **1995**, *51*, 8459–8470; (d) Kobayashi, M.; Nanba, T.; Toyama, T.; Saito, A. *Annu. Rep. Sankyo Res. Lab.* **1994**, *46*, 67–75.
6. Scherkenbeck, J.; Jeschke, P.; Harder, A. *Curr. Top. Med. Chem.* **2002**, *2*, 759–777.
7. Weckwerth, W.; Miyamoto, K.; Iinuma, K.; Krause, M.; Glinski, M.; Storm, T.; Bonse, G.; Kleinkauf, H.; Zocher, R. *J. Biol. Chem.* **2000**, *275*, 17909–17915.
8. (a) Terada, M. *Jpn. J. Parasitol.* **1992**, *41*, 108–117; (b) Chen, W.; Terada, M.; Cheng, J. T. *Parasitol. Res.* **1996**, *82*, 97–101; (c) Saeger, H.-P.; Schmitt-Wrede, M.; Dehnhardt, W. P.; Benten, J.; Krücken, A.; Harder, A.; VonSamson-Himmelstjerna, G.; Wiegand, H.; Wunderlich, F. *FASEB J.* **2001**, *15*, 1332–1334.
9. Scherkenbeck, J.; Harder, A.; Plant, A.; Dyker, H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1035–1040.
10. Dyker, H.; Scherkenbeck, J.; Gondol, D.; Goehrt, A.; Harder, A. *J. Org. Chem.* **2001**, *66*, 3760–3766.
11. (a) Dutton, F. E.; Lee, B. H.; Johnson, S. S.; Coscarelli, E. M.; Lee, P. H. *J. Med. Chem.* **2003**, *46*, 2057–2073; (b) Scherkenbeck, J.; Dyker, H.; Gondol, D.; Harder, A.; Plant, A.; Reichel, F. *Pestic. Sci.* **1999**, *55*, 457–461; (c) Lee, B. H. *Tetrahedron Lett.* **1997**, *38*, 757–760; (d) Lee, B. H.; Dutton, F. E.; Thompson, D. P.; Thomas, E. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 353–356.
12. Kodama, Y.; Takeuchi, Y.; Suzuki, A. *Sci. Rep. Meiji Seika Kaisha* **1992**, *31*, 1–8.
13. Unpublished internal results. See also Ref. 11a.
14. (a) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647–650; (b) Sato, K.; Nagai, U. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1231–1234.
15. Alberg, G. A.; Schreiber, S. L. *Science* **1993**, *262*, 248–250.
16. Etzkorn, F. A.; Guo, T.; Lipton, M. A.; Goldberg, S. D.; Bartlett, P. A. *J. Am. Chem. Soc.* **1994**, *116*, 10412–10425.
17. See literature 6.
18. (a) Shute, R. E.; Rich, D. H. *Tetrahedron Lett.* **1987**, *28*, 3419–3422; (b) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–4343.
19. Coste, J.; Frérot, E.; Jouin, P. *Tetrahedron Lett.* **1991**, *32*, 1967–1970.
20. All compounds gave satisfactory and/or accurate mass data. Characteristical mass data (ESI-MS: *m/z* (%)) of the synthetic cyclodepsipeptides are given below. **7**: 894 (16, $[M + Na]^+$), 889 (10, $[M + NH4]^+$), 872 (100, $[M + H]^+$), 691, 436, 303. **8**: 890 (100, $[M + Na]^+$), 868 (14, $[M + H]^+$), 687 (44). **9**: 894 (39, $[M + Na]^+$), 872 (56, $[M + H]^+$), 783 (62), 733 (100).
21. The results of a more comprehensive biological profiling, including PK measurements and additional pharmacokinetic studies will be published in due time.
22. Shoop, W. L.; Demontigny, P.; Fink, D. W.; Williams, J. B.; Egerton, J. R.; Mrozik, H.; Fisher, M. H.; Skelly, B. J.; Turner, M. J. *Int. J. Parasitol.* **1996**, *26*, 1227–1235.
23. Plant, A.; Harder, A.; Mencke, N.; Bertram, H. *J. Pestic. Sci.* **1996**, *48*, 351–358.
24. Wetzel, R. *Tierärztliche Rundschau* **1951**, *11*, 209.