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Synthesis of anthelmintically active *N*-methylated amidoxime analogues of the cyclic octadepsipeptide PF1022A

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Abstract: The *N*-methylated amidoxime analogues of the cyclic octadepsipeptide PF1022A represent novel derivatives with activity against *Trichinella spiralis* Owen and *Nippostrongylus brasiliensis* Lane *in vitro* and against parasitic nematodes in mice and sheep. Some of them show better activity against *Hymenolepis nana* Siebold, *Heterakis spumosa* Schneider and *Heligmosomoides polygyrus* Dujardin in mice than the natural product PF1022A. In particular an improved efficacy against *Haemonchus contortus* Rudolphi and *Trichostrongylus colubriformis* Giles in sheep compared to the potent cyclic octadepsipeptide PF1022A and its mono-thionated derivative has been observed. Here we report on a specific modification at the *N*-methyl amide linkage by using the mono-thionated PF1022A, resulting in novel anthelmintically active backbone analogues of PF 1022A.

Keywords: anthelmintic; *N*-methyl-amidoxime; cyclic octadepsipeptide; PF1022A; thionated depsipeptide; *Haemonchus contortus*; *Trichostrongylus colubriformis*

1 INTRODUCTION

PF1022A (Fig 1; **1**), the active ingredient of the fungus imperfectus *Mycelia sterilia*, is described as a powerful broad-spectrum anthelmintic agent with low toxicity in animals.[1–4] This 24-membered cyclic octadepsipeptide was first isolated from natural sources by Sasaki *et al*[5] and prepared later through different total syntheses.[6–8] Recently it has been shown that PF1022A binds to the amino terminus of a heptahelical, latrophilin-like transmembrane receptor isolated from *Haemonchus contortus* Rudolphi and thereby induces influx of external calcium into cells.[9] This mode of action of an anti-nematodal drug is quite different from the mechanism of action of the known anthelmintics such as benzimidazoles, imidazothiazoles and macrocyclic lactones.[10]

The effects of structural modifications of PF1022A on anthelmintic activity have been extensively studied in order to increase the efficacy of the natural product against parasites. However, the molecular design of

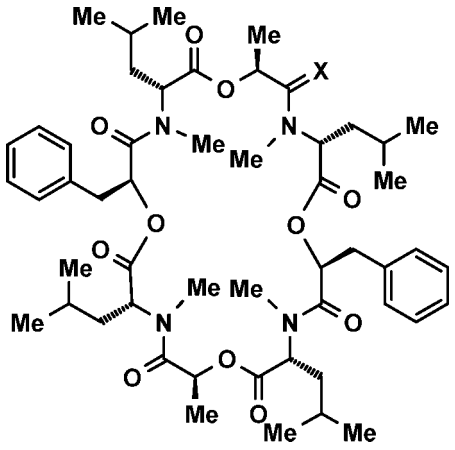

Figure 1. Structures of PF1022A (**1**) and the mono-thionated PF1022A (**2**).

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analogues has focused mainly on variations on the α-amino acids or their *N*-alkyl substituents[11] and on the side chains of the α-hydroxy carboxylic acids.[12,13] Conformationally restricted analogues of PF1022A have been synthesized by fusing five-membered[14] or seven-membered lactam rings[15] onto the macrocycle. Beside aza-analogues of PF1022A in which the α-carbons of the leucine residues are substituted by nitrogen[16] only limited attention has been paid to the cyclic octadepsipeptide backbone. Recently, we have described a useful method for the selective incorporation of one *N*-methylthioamide group into the backbone of PF1022A derivatives,[17] resulting in eg the mono-thionated PF1022A (Fig 1; **2**). For each of the resulting thionated analogues,[18] the anthelmintic activities against relevant intestinal nematodes in livestock were found to be greater than those of the parent cyclic octadepsipeptides. In the light of the latter results, it has been suggested that the asymmetric conformation influences the anthelmintic activity of cyclic octadepsipeptides.[14,18] Novel backbone modifications with strong preference for an asymmetric conformer of cyclic octadepsipeptides may give valuable information about the conformational structure–activity relationships of PF1022A derivatives.

Isosteric replacement of an amide bond in a peptide backbone by an imino peptide bond or an amidine group has been reported, but the concept of *N*-methylated amidoxime or *N*-methylated *N′*-hydroxyamidine analogues of cyclic depsipeptides has not been described in the literature so far. Several methods are known for the synthesis of *N*-substituted amidines.[19] Such a specific backbone modification of chemotactic peptides by exchanging an amide bond for the corresponding amidoxime appears to have a strong influence on the conformation.[20]

In this paper we report an extension of our study on the thionation of cyclic octadepsipeptides,[17,18] wherein the *N*-methyl amide group of PF1022A has been formally replaced by a *N*-methylated amidoxime fragment. The synthesis of these derivatives was undertaken not only as a part of the investigations of structure–activity relationships but also to test the feasibility of using the *N*-methylated thioamide group in cyclic octathiodepsipeptides, such as **2**, to synthesize novel backbone analogues.

2 EXPERIMENTAL

2.1 General

^{1}H and ^{13}C NMR spectra were recorded in deuterochloroform ($CDCl_3$), deuteroacetonitrile (CD_3CN), deuteroacetone (acetone-d_6) or deuterodimethyl sulfoxide (DMSO-d_6) with tetramethylsilane as the internal standard, using a Bruker Avance 600 instrument (600 MHz). Liquid chromatography mass spectra (LC–MS) were obtained on a VG/Fisons Platform 1 with APCI ionization. Reverse phase (RP) high pressure liquid chromatography (HPLC) was performed using a Hewlett-Packard HP 1090 with a column filled with Kromasil C18 (Macherey-Nagel GmbH & Co KG, Düren, Germany). For preparative column chromatography silica gel 60-Merck (40–63 μm) was used. Octanol–water partition coefficients (log *P*) were measured by a HPLC method using reverse phase columns, the general principles of which have been described elsewhere.[21,22]

2.2 Chemicals

The natural product PF1022A (**1**) used for thionation reactions was kindly provided by Meiji Seika Kaisha, Ltd (Tokyo). Mono-thionated PF1022A (**2**) was synthesized from **1** by a route described in the literature, using 2,4-bis(4-phenoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane (Belleau's reagent).[17,18] Hydroxylamine and acyl halides were obtained commercially. *O*-Substituted hydroxylamines were obtained commercially or prepared by either of two general procedures. In one procedure *O*-substituted hydroxylamines were prepared by *O*-alkylation from *N*-hydroxyphthalimide and alkyl/arylalkyl halides followed by hydrazinolysis, or by Mitsunobu reaction[23] of the appropriate alcohol with *N*-hydroxyphthalimide in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD) followed by hydrazinolysis.

2.3 Synthesis

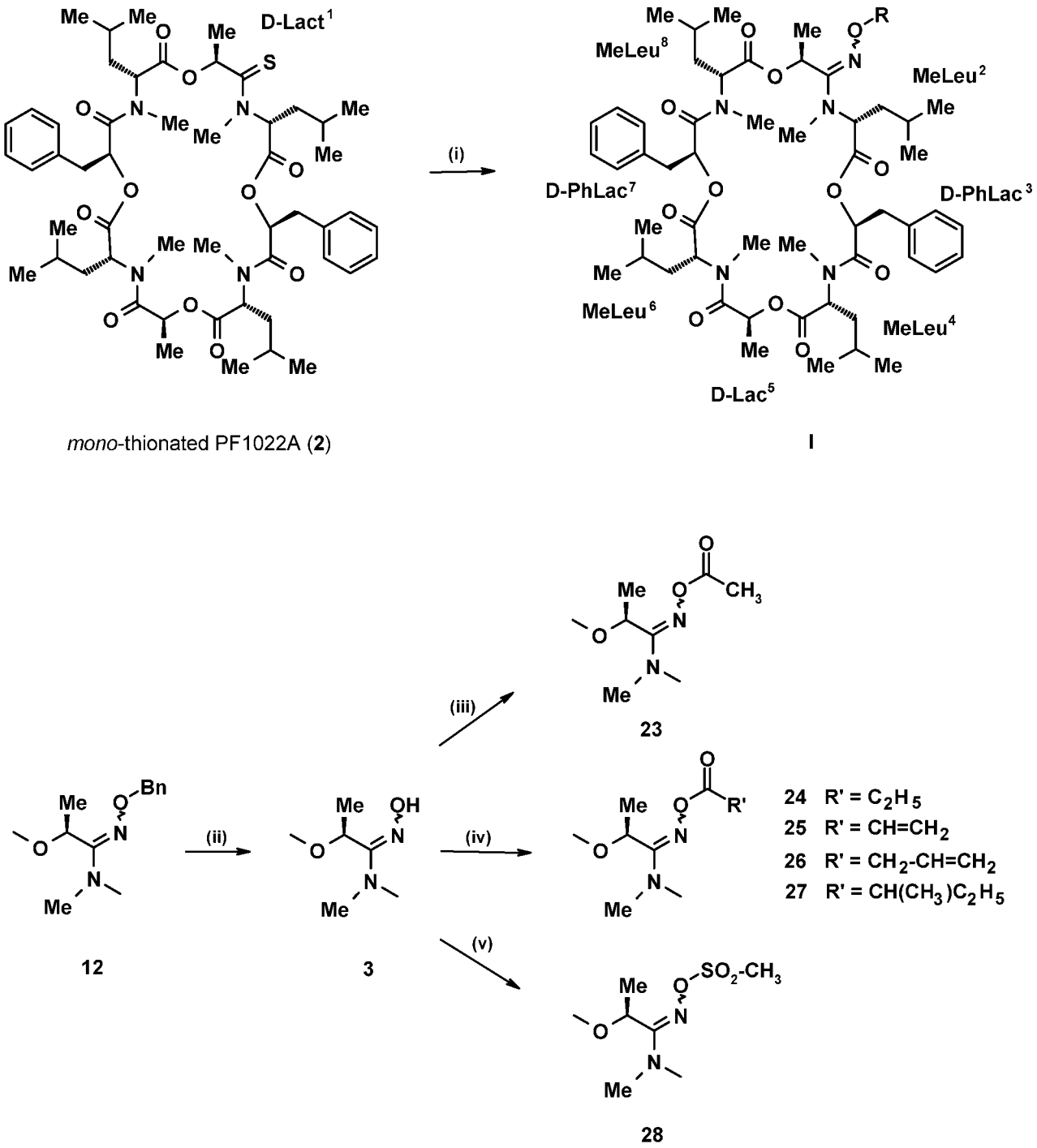
Figure 2 depicts the general synthetic pathway to *N*-methylated amidoxime analogues (general structure **I**), which were prepared by a method reported in the literature.[24,25]

Representative preparations of the *N*-methylated amidoxime analogues (**I**) and their respective transformations into derivatives are given below.

*2.3.1 Cyclo[-*N*-methyl-L-leucinyl-D-(methoxyimino)lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-*N*-methyl-L-leucinyl-D-lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-] (**4**, general procedure for **3** and **5**–**22**)*

To a solution of (**2**)[17] (200.0 mg; 0.20 mmol) in acetonitrile (5 ml) were added *O*-methyl-hydroxylamine hydrochloride ($MeONH_2 \cdot HCl$) (26.2 mg; 0.31 mmol), $Hg(OAc)_2$ (72.5 mg; 0.22 mmol) and *N,N*-diisopropylethylamine (DIEA) (107.4 μl; 1.25 mmol) and the mixture was stirred at room temperature. After 18 h the reaction mixture was treated again with $MeONH_2 \cdot HCl$, (26.2 mg; 0.31 mmol), mercury(II) acetate (72.5 mg; 0.22 mmol), and DIEA (107.4 μl; 1.25 mmol). After 6 h stirring at room temperature, saturated aqueous ammonium chloride solution (20 ml) was added followed by extraction with chloroform (4 × 15 ml). The dried organic phase was evaporated and the residue was chromatographed on silica gel using cyclohexane + acetone (4 + 1 by volume). Yield: 170 mg (85%); *m/z*: 978 $[M]^+$; ^{1}H NMR major conformer: δ 0.79 (d, 3H), 0.86 (d, 3H), 0.86 (d, 3H), 0.86 (d, 3H), 0.86 (d, 3H), 0.88 (d, 3H), 0.94 (d, 3H), 1.03 (d, 3H), 1.03 (d, 3H), 1.45 (d, 3H), 1.46–

1.76 (m, 1H), 1.46–1.76 (m, 2H), 2.85 (s, 3H), 2.87 (s, 3H), 2.90 (s, 3H), 3.04 (s, 3H), 3.06 (d, 2H), 3.09 (d, 2H), 3.15 (d, 2H), 3.15 (d, 2H), 3.64 (s, 3H), 4.40 (dd, 1H), 4.64 (dd, 1H), 5.08 (q, 1H), 5.20 (q, 1H), 5.36 (dd, 1H), 5.36 (dd, 1H), 5.52 (dd, 1H), 5.70 (dd, 1H), 7.23–7.32 (m, 10H); ^{13}C NMR major conformer: δ 29.3, 30.2, 30.9, 31.0 (NCH_3), 53.9, 56.8, 56.9, 59.5 (NCH), 61.3 ($NOCH_3$), 66.9, 68.1, 69.7, 71.1 (OCH), 152.9 (C=N), 170.2, 170.3, 170.3, 170.7, 171.1, 172.5, 172.5 (C=O).



mono-thionated PF1022A (**2**)

I

12 **3** **23** **28**

(i) H_2N-O-R, $Hg(OAc)_2$, DIEA, acetonitrile, r.t.. (ii) H_2 / Pd-C (10%), conc. HCl, methanol, r. t..

(iii) Ac_2O, 70 °C. (iv) R'-CO-Cl, pyridine, 0 °C. (v) Me-SO_2-Cl, pyridine, 0 °C.

Figure 2. Synthetic routes to the novel *N*-methylated amidoxime analogues of PF1022A (**I**).

*2.3.2 Cyclo[-*N*-methyl-L-leucinyl-D-(hydroxyimino)lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-*N*-methyl-L-leucinyl-D-lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-] (**3**)*

Cyclo[-*N*-methyl-L-leucinyl-D-(benzyloxyimino)lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-*N*-methyl-L-leucinyl-D-lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-] (**12**; 400.0 mg; 0.37 mmol) were hydrogenated in methanol (40 ml) in the presence of palladium-charcoal [Pd content: 10% (200 mg)] and concentrated hydrochloric acid (0.7 ml) for approximately 20 min. The reaction mixture was filtered and the solvent was removed under vacuum. The residue was chromatographed on RP-18 material using acetonitrile + water (8 + 1 by volume). Yield: 110 mg (30%); *m/z*: 964 $[M]^+$; ^{13}C NMR (DMSO-d_6) major conformer: δ 153.7 (C=NOH).

*2.3.3 Cyclo[-*N*-methyl-L-leucinyl-D-(acetoxyimino)-lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-*N*-methyl-L-leucinyl-D-lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-] (**23**)*

A mixture of **3** (200.0 mg; 0.20 mmol) in Ac_2O (1.0 ml) was kept at 70 °C for 30 min. Following the addition of saturated sodium hydrogen carbonate solution (15 ml) the mixture was extracted three times with ethyl acetate (3 × 15 ml). After drying over anhydrous magnesium sulfate the solvent was removed under vacuum. The residue was first chromatographed on silica gel using cyclohexane + acetone (10 + 1 by volume) and then on RP-18 using preparative HPLC. Yield: 80 mg (35%); *m/z*: 1006 $[M]^+$; ^1H NMR (CD_3CN) major conformer: δ 0.78 (d, 3H), 0.82 (d, 3H), 0.82 (d, 3H), 0.84 (d, 3H), 0.89 (d, 3H), 0.90 (d, 3H), 0.94 (d, 3H), 0.96 (d, 3H), 1.35 (d, 3H), 1.41 (d, 3H), 1.49 (m, 1H), 1.51 (m, 1H), 1.53 (m, 1H), 1.55 (m, 1H), 1.55 (m, 1H), 1.57 (m, 1H), 1.58 (m, 1H), 1.59 (m, 1H), 1.60 (m, 1H), 1.69 (m, 1H), 1.69 (m, 1H), 1.70 (m, 1H), 1.96 (s, 3H), 2.81 (s, 3H), 2.88 (s, 3H), 2.94 (s, 3H), 2.97 (s, 3H), 3.00 (d, 1H), 3.05 (m, 2H), 3.07 (d, 1H), 3.92 (dd, 1H), 5.04 (dd, 1H), 5.17 (dd, 1H), 5.17 (dd, 1H), 5.34 (q, 1H), 5.46 (dd, 1H), 5.54 (dd, 1H), 5.67 (q, 1H), 7.21–7.32 (m, 10H); ^{13}C NMR (CD_3CN) major conformer: δ 16.6, 17.0 (CH_3), 21.7, 22.6, 23.1, 23.5 (CH_3), 25.5, 25.5, 25.7, 25.7 (CH), 31.7, 32.0, 32.0, 36.4 (NCH_3), 38.0, 38.0, 38.0, 38.1 (CH_2), 38.1, 38.1 (CH_2Ph), 55.3, 55.3, 55.7, 57.6 (NCH), 68.0, 69.2, 72.3, 73.2 (OCH), 128.1, 129.4, 130.6 (Ph—C), 154.8 (C=N), 169.2, 171.1, 171.3, 171.3, 171.7, 171.8, 171.9, 172.2 (C=O).

*2.3.4 Cyclo[-*N*-methyl-L-leucinyl-D-(ethoxycarbonyloxyimino)lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-*N*-methyl-L-leucinyl-D-lactyl-*N*-methyl-L-leucinyl-D-phenyllactyl-] (**24**, general procedure for **25**, **26**, **27** and **28**)*

To a solution of **3** (105 mg; 0.10 mmol) in dry pyridine (5 ml) was added ethyl chloroformate (35.4 mg; 0.32 mmol) at 0 °C and the mixture was stirred at 0 °C for 6 h. The solvent was evaporated and the residue was treated with chloroform (20 ml). The mixture was then extracted with hydrochloric acid (1 M) and twice with saturated sodium hydrogen carbonate solution, and, after drying over anhydrous magnesium sulfate, the solvent was removed under vacuum. The residue was chromatographed on silica gel using cyclohexane + ethyl acetate (1.5 + 1 by volume). Yield: 85 mg (80%); *m/z*: 1036 $[M+H]^+$; ^1H NMR (acetone-d_6) major conformer: δ 0.81 (d, 3H), 0.84 (d, 3H), 0.86 (d, 3H), 0.88 (d, 3H), 0.91 (d, 3H), 0.93 (d, 3H), 0.99 (d, 3H), 1.01 (d, 3H), 1.43 (d, 3H), 1.44 (d, 3H), 1.37–1.53 (m, 2H), 1.58–1.82 (m, 8H), 1.72–1.83 (m, 2H), 2.88 (s, 3H), 3.01 (s, 3H), 3.06 (m, 2H), 3.08 (s, 3H), 3.09 (s, 3H), 3.14 (m, 2H), 4.02 (dd, 1H), 5.14 (dd, 1H), 5.24 (dd, 1H), 5.29 (dd, 1H), 5.45 (q, 1H), 5.54 (dd, 1H), 5.56 (dd, 1H), 5.77 (q, 1H), 7.23–7.37 (m, 10H); ^{13}C NMR (acetone-d_6) major conformer: δ 14.4, 16.0, 16.9, 21.4, 21.5, 21.7, 22.5, 23.1, 23.2, 23.5, 23.6 (CH_3), 25.1, 25.1, 25.3, 25.4 (CH), 31.3, 31.3, 31.7, 36.0 (NCH_3), 37.8, 38.0 (CH_2Ph), 37.4, 37.8, 37.9, 38.1 (CH_2), 54.9, 55.0, 55.3, 57.4 (NCH), 64.6 (OCH_2), 67.5, 68.9, 73.2, 74.8 (OCH), 127.6, 127.6, 129.2, 129.2, 130.4, 130.5 (Ph—C), 154.5 (C=N), 155.1, 168.7, 170.5, 171.0, 171.4, 171.5, 171.5, 171.7 (C=O).

2.4 Biological assays

2.4.1 In vitro experiments

The larvae of the nematode *Trichinella spiralis* Owen were isolated from skeletal muscles and diaphragms of male mice of strain SPF/CFW1, 16–18 g body weight on receipt, and stored in sodium chloride solution (9 g litre^{-1}), supplemented with Canesten (20 μg ml^{-1}). *Trichinella spiralis* larvae were obtained from pepsin-treated tissues from mice 50 days after injection. Twenty larvae per estimation were incubated in a solution (2.0 ml) containing Bacto Casitone, 20, yeast extract 10, glucose 5, KH_2PO_4, 0.8, K_2HPO_4, 0.8 g litre^{-1}, pH 7.2, supplemented with Sisomycin (10 μg ml^{-1}) and Canesten (1 μg ml^{-1}). The test compound (10 mg) was dissolved in dimethylsulfoxide (DMSO; 0.5 ml) and added to the incubation medium to give a final concentration of 100 μg ml^{-1}. The experiment was stopped after 5 days of incubation at 19 °C. Activity was assessed on a scale of 0–3 where 3 = full activity (all larvae dead); 2 = good activity (<100% but > 50% of the larvae dead); 1 = weak activity (>50% of larvae still alive) and 0 = no activity (number of living larvae equals that in the control).[26] Adult nematodes (five males and females each) of *Nippostrongylus brasiliensis* Lane were isolated from the small intestine of female Wistar rats, stored in sodium chloride solution (9 g litre^{-1}), supplemented with Sisomycin (20 μg ml^{-1}) and Canesten (2 μg ml^{-1}). The incubation of each group of male or female worms was performed in 1.0 ml of medium, which was collected for the estimation of the level of activity of the acetylcholinesterase secreted by *N brasiliensis*.

Details of the incubation and determination are described by Rapson *et al.*[27] The level of activity of the test compounds was assessed on a scale 0–3 where 3 = full activity (95–100% enzyme inhibition); 2 = good activity (75–95% inhibition); 1 = weak activity (50–75% inhibition) and 0 = negligible activity (<50% inhibition).

2.4.2 Mixed-parasite infections in mice

For all experiments male mice of strain SPF/CFW1, 16–18 g body weight on receipt, were used. Cages constructed from Makrolon® housed five animals each. The mice were given water and 'Sniff' rat feed 13-mm pellets *ad libitum*. Mice received a mixed inoculation with the tapeworms *Hymenolepis nana* Siebold, and the nematodes *Heligmosomoides polygyrus* (syn *Nematospiroides dubius*) Dujardin, *Heterakis spumosa* Schneider and *T spiralis*.

The infective material from *H nana* was collected from mouse faeces 14–21 days post-infection (PI), third-stage larvae (L_3) of *H polygyrus* were collected from mouse faeces 21 days PI, as described by Martin *et al*,[28] *H spumosa* eggs were obtained from female worms isolated from the mouse colon 35–42 days PI and were then incubated at 27 °C for 3 weeks, and *T spiralis* larvae were obtained from pepsin-treated skeletal muscles and diaphragms of Wistar-W64 rats 20 days PI. The culturing methods are described in Andrews *et al*[29] and in the reference list therein. The mixed-parasite infection was introduced into the mice in a stepwise manner. They were first infected orally with 90 embryonated *H spumosa* eggs, followed after 7 days with 100 *T spiralis* larvae; 27 days later, 60–70 larvae from *H polygyrus* were introduced and the final infection, with 100 *H nana* eggs, followed after a further 2 days. Test compounds (0.1 g) were dissolved or suspended in the emulsifier 'Cremophor' EI (0.2 ml). After addition of distilled water to give a final volume of 5.0 ml, a volume of between 0.1 and 0.5 ml was orally applied per 20 g mouse according to the desired dose in mg kg^{-1} body weight once daily for 4 consecutive days. One mouse received the highest dose of 100 mg kg^{-1}; if anthelmintic activity was observed at this dose, lower dosages of 50 and 25 mg kg^{-1} were tested until anthelmintic activity could no longer be detected. Treatment began on day 46 PI and ended on day 49 PI. After a further 8 days the animals were killed with carbon dioxide and then dissected. *Hymenolepis nana* were isolated from the small intestine and the number of tapeworms was estimated under a microscopically (magnification ×16). *Heterakis spumosa* were isolated from the caecum and the colon, numbers also being determined by microscopy. Removal of the duodenum and dissection of this organ in a compressor, followed by microscopic examination (magnification ×40), allowed the numbers of *H polygyrus* remaining to be determined. The numbers of *T spiralis* present in about 1 cm^2 of abdominal muscle, removed by dissection and compressed between two plastic sheets using a hand press, were determined with the aid of a binocular microscope (magnification ×40). The activity against the three nematodes was evaluated on a scale 0–3 where 3 represents cure (no parasites detectable), 2 effective (<20% of parasites remaining), 1 trace effect (<50% of parasites remaining). Activity against the tapeworm was also evaluated on a scale 0–3 where 3 represents cure (no parasite inclusive scolices detectable), 2 effective (some parasites expelled, tapeworm strobila excreted), 1 trace effect (tapeworm strobila excreted or macerated) and 0 ineffective (all parasites remaining) (see Table 3).

2.4.3 Nematode infections in sheep

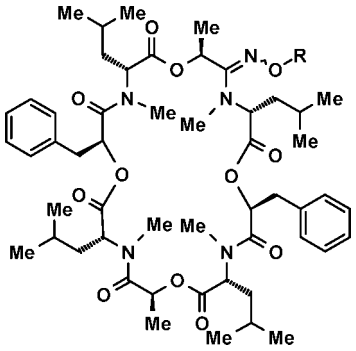
Sheep (*Ovis aries* L, Merino or Schwarzkopf breed, 25–35 kg body weight) were infected experimentally with 5000 *Haemonchus contortus* Rudolphi L_3 and treated with the test substance after the end of the prepotency period of the parasite. The test compounds were administered orally in gelatine capsules. In the case of *Trichostrongylus colubriformis* Giles sheep were infected orally with 12 000 L_3, and similarly dosed with test compound. Anthelmintic effects of the test substances were measured as a function of the reduction in the sheep faecal egg count. For the purposes of counting eggs, freshly obtained faeces from experimental animals were prepared using the McMaster method as modified by Wetzel.[30] The egg counts were determined at regular intervals of 3 days before and after treatment. The anthelmintic evaluation was expressed as a function of the egg reduction as follows: 3 = >95%, 2 = 75–95%, 1 = 50–75% and 0 = <50% egg reduction (see Table 4).

3 RESULTS AND DISCUSSION

3.1 Chemistry

In this procedure, the high reactivity of the monothionated PF 1022A (**2**) and of D-Lact[1] thioamide was used together with the high nucleophilicity of hydroxylamine derivatives. Thus, a desulfurization reaction of **2** with *O*-substituted hydroxylamines in the presence of mercury(II) acetate and/or mercury(II) chloride and *N, N*-diisopropylethylamine (DIEA) in acetonitrile afforded the *N*-methylated amidoxime analogues (**4**–**22**).[25] The *N*-methylated amidoxime derivative **3** was prepared directly from the **2** and hydroxylamine in the same manner as above or alternatively was synthesized by simple removal of the benzyl group (Bn) of the *O*-benzyl *N*-methylamidoxime **12** by palladium-charcoal catalyzed hydrogenation in the presence of hydrochloric acid. The *N*-methylated amidoxime derivative **3** was allowed to react with an excess of acetic acid anhydride at 70 °C forming the *O*-acetyl *N*-methylamidoxime **23** in 35% yield. All further *O*-acylations of the hydroxyimino group were carried out with three equivalents of the corresponding alkyl or alkenyl chloroformates in dry pyridine at low temperature to give the *O*-acylated *N*-methylamidoxime derivatives (**24**–**27**).

Table 1A. *N*-Methylated amidoxime analogues of PF1022A (**I**)



No	Structure R	Molecular Formula	Molecular Relative mass	Yield (%)
3	H	$C_{52}H_{77}N_5O_{12}$	964.2	30
4	CH_3	$C_{53}H_{79}N_5O_{12}$	978.2	85
5	C_2H_5	$C_{54}H_{81}N_5O_{12}$	992.2	49
6	CH_2CH_2OH	$C_{54}H_{81}N_5O_{13}$	1008.2	58
7	3-CF_3-Phenoxyethyl	$C_{61}H_{84}F_3N_5O_{13}$	1152.3	65
8	$CH(CH_3)_2$	$C_{55}H_{83}N_5O_{12}$	1006.3	9
9	$CH_2CH=CH_2$	$C_{55}H_{81}N_5O_{12}$	1004.2	36
10	CH_2COMor[a]	$C_{58}H_{86}N_6O_{14}$	1091.3	28
11	CH_2COPyr[b]	$C_{58}H_{86}N_6O_{13}$	1075.3	76
12	Benzyl	$C_{59}H_{83}N_5O_{12}$	1054.3	15
13	3-Cl-Benzyl	$C_{59}H_{82}ClN_5O_{12}$	1088.7	72
14	3-CF_3-Benzyl	$C_{60}H_{82}F_3N_5O_{12}$	1122.3	32
15	4-F-Benzyl	$C_{59}H_{82}FN_5O_{12}$	1072.3	66
16	2,4-Cl_2-Benzyl	$C_{59}H_{81}Cl_2N_5O_{12}$	1123.2	37
17	2-Cl, 6-F-Benzyl	$C_{59}H_{81}ClFN_5O_{12}$	1106.7	15
18	*(S)*-Tetrahydrofur-2-yl-methyl	$C_{57}H_{85}N_5O_{13}$	1048.3	81
19	Fur-2-yl-methyl	$C_{57}H_{81}N_5O_{13}$	1044.3	20
20	Pyrid-2-yl-methyl	$C_{58}H_{82}N_6O_{12}$	1055.3	17
21	2-Cl-Pyrid-5-yl-methyl	$C_{58}H_{81}ClN_6O_{12}$	1089.7	77
22	CH_2CN	$C_{54}H_{78}N_6O_{12}$	1003.2	24
23	$COCH_3$	$C_{54}H_{79}N_5O_{13}$	1006.2	35
24	$COOC_2H_5$	$C_{55}H_{81}N_5O_{14}$	1036.2	80
25	$COOCH=CH_2$	$C_{55}H_{79}N_5O_{14}$	1034.2	59
26	$COOCH_2CH=CH_2$	$C_{56}H_{81}N_5O_{14}$	1048.3	17
27	$COOCH(CH_3)C_2H_5$	$C_{57}H_{85}N_5O_{14}$	1064.3	52
28	SO_2CH_3	$C_{53}H_{79}N_5O_{14}S$	1042.3	38

[a] *N*-Morpholino-carbonylmethyl = CH_2-CO-N⟨O⟩

[b] *N*-Pyrrolidino-carbonylmethyl = CH_2-CO-N⟨⟩

Methanesulfonylation of **3** to the corresponding *O*-methylsulfonyl *N*-methylamidoxime **28** was easily accomplished using three equivalents of methanesulfonyl chloride at 0 °C. The oxime analogues (Fig 2; **I**) were isolated in yields ranging from 9% to 85% after purification by preparative column chromatography.

The *N*-methylated amidoxime analogues (**I**) showed two to four sets of resonances (major and minor sets) in their ^{1}H NMR spectra. In compound **4**, for example, the ratio between these four sets of signals was about 5:1.5:1.5:1 without a dynamic equilibration at 298 K in $CDCl_3$ solution, thus showing that the presence of (*E*/*Z*)-isomers and/or very stable conformers without dynamic inter-conversion in solution. Further spectroscopic analysis of the main conformer using a combination of two-dimensional NMR (^{1}H–^{1}H-TOCSY, ^{1}H–^{1}H-ROESY, ^{1}H–^{13}C-HMQC, ^{1}H–^{13}C-HMBC) techniques in $CDCl_3$ solution showed only the asymmetric conformation, containing a *cis*-amide bond between D-lactic acid (D-Lac^5) and MeLeu^6. The *cis*-amide bond can be identified in the ROESY NMR spectra by observing the ROE contacts between C_α protons of neighbouring MeLeu/D-Lac units. Compared with the C_α protons of MeLeu^{2,4,8} (δ 4.64–5.36) the C_α protons of MeLeu^6 resonates at a higher field strength (δ 4.40). However, analogues **23** and **24**, carrying an *O*-acetyl group or an *O*-ethoxycarbonyl group instead of the *O*-methyl group,

Table 1B. Analytical and spectroscopic data of the *N*-methylated amidoxime analogues of PF1022A (**I**)

No	*APCI-MS*[a]	*HPLC*[b]			
	[M+H]$^+$	*R_t (min)*	*Purity (ratio)*[c] *(%)*	*[^{1}H]NMR for =N—O—Z—CH—R′ δ (ppm)*	*Z*
3	964	17.54; 17.66[d]	93.5 (20/80)	—[e]	—
4	978	18.34	100	3.64 (3H, s)[f]	—
5	993	20.05[g]	—[j]	3.98 (1H, m), 3.88 (1H, m)[f]	—
6	1008	17.58	90.6	3.94 (2H, t)[h]	—
7	1152	19.80	94.5	4.32 (2H, s)[h]	—
8	1006	13.54	100	4.47 (1H, broad)[i]	—
9	1005	20.21, 20.31[g]	—[j]	4.37 (2H, dd)[f,k]	—
10	1091	17.64	92.9	4.74 (1H, d), 4.40 (1H, d)[f]	—
11	1076	17.95	97.8	4.56 (1H, d), 4.39 (1H, d)[f]	—
12	1055	21.23, 21.50[g]	—[j]	5.02 (1H, d), 4.94 (1H, d)[f]	—
13	1088	19.61, 19.78[d]	99.1 (41.5/58.5)	4.93 (2H, m); 4.71 (1H, d), 5.06 (1H, d)[h,l]	—
14	1123	19.50, 19.68[d]	100 (43.1/56.9)	5.03 (2H, m); 5.16 (1H, d), 4.80 (1H, d)[h,l]	—
15	1072	19.48	99.1	4.98 (1H, d), 4.77 (1H, d)[f]	—
16	1124	20.68, 21.16[d]	99.9 (56/44)	5.02 (2H, s), 4.99 (2H, dd)[h,l]	—
17	1106	19.24, 19.44[d]	100 (70.8/29.2)	4.97 (2H, s)[i]	—
18	1049	19.94[g]	—[j]	—[j]	—
19	1044	18.73	100	4.76 (1H, d), 4.54 (1H, d)[i]	—
20	1055	6.46, 6.58[m]	98.8 (58.6/41.4)	5.08 (2H, broad)[f]	—
21	1089	18.87	100	5.02 (1H, d), 4.78 (1H, d); 4.83 (1H, d), 4.72 (1H, d); 4.94 (2H, m)[h,l]	—
22	1003	7.39, 7.49[n]	97.5 (6.5/93.5)	4.55 (1H, d), 4.15 (1H, d); 4.78 (1H, d), 4.32 (1H, d); 4.50 (2H, m)[i,l]	—
23	1006	7.35[n]	98.7	1.96 (3H, s)[i]	CO
24	1036	17.69	95.5	4.08 (2H, q)[h]	CO
25	1037	17.82	93.6	6.96 (1H, dd)[i]	CO
26	1048	17.83	95.0	4.53 (2H, ddd)[i]	CO
27	1064	18.28	95.4	4.58 (1H, m)[h]	CO
28	1042	17.18	100	2.84 (3H, s)[i]	SO_2

[a] Loop or LC-coupling, *m/z* for quasi-molecule ions related to the main isotopes of the elements.

[b] Conditions for analysis: column 125 × 4.0 mm Kromasil 100 C18, 5 μm; eluent, A = 0.1% phosphoric acid, B = acetonitrile; gradient, 10% B (1 min), 10–95% B (17 min), 95% B (6 min); flow rate, 1.5 ml min^{-1}; detection, UV 210 nm.

[c] Ratio of geometrical *(E/Z)*-isomers around the C = N bond.

[d] Retention times of two conformers.

[e] ^{13}C NMR (DMSO-d_6): δ 153.7 (*C*=N—OH) ppm.

[f] $CDCl_3$.

[g] Conditions for analysis: column 125 × 2.1 mm Kromasil 100 C18, 5 μm; temperature 40 °C; eluent, A = 0.1% formic acid in water, B = 0.08% formic acid in acetonitrile; gradient, 10% B (1 min), 10–95% B (17 min), 95% B (6 min); flow rate, 0.5 ml min^{-1}; detection, mass spectrometer; micromass platform II, APCI ion source.

[h] Acetone-d_6.

[i] CD_3CN.

[j] Not determined.

[k] Mixture of four conformers in a ratio of 2:2:1:1; ^{13}C NMR ($CDCl_3$): δ 74.7 (=N—O—*C*H$_2$—), 158.0 (*C*=N—O—) ppm.

[l] Signals of a mixture of conformers in solution.

[m] Conditions for analysis: column 75 × 4.6 mm Kromasil 100 C18, 3.5 μm; eluent, A = 0.1% phosphoric acid, B = acetonitrile; gradient, 10% B (0.8 min), 10–95% B (6.73 min), 95% B (1.57 min); flow rate, 3.0 ml min^{-1}; detection, UV 210 nm.

[n] Conditions for analysis: column 75 × 4.6 mm Kromasil 100 C18, 3.5 μm; eluent, A = water, B = acetonitrile; gradient, 10% B (0.8 min), 10–95% B (6.73 min), 95% B (1.57 min); flow rate, 3.0 ml min^{-1}; detection, UV 210 nm.

exhibited two sets of signals in CD_3CN with a ratio of 83:17 or in acetone-d_6 solution with a ratio of 85:15. Analogue **24**, dissolved in acetone-d_6, exists in two conformations that are in dynamic equilibrium with one another, as can be deduced from the corresponding exchange cross peaks in its NOESY spectrum. Further spectroscopic analysis showed in each case for the minor conformer the asymmetric conformation, containing two *cis*-amide bonds between the oxime of D-lactic acid (D-Lac^1) and MeLeu^2 as well as between D-Lac^5 and MeLeu^6. These observations suggest that the number of conformers and the presence of *cis*-amide bonds in solution depends strongly on the nature of the substituent R in the *N*-methylated amidoxime moiety of the analogues (**I**). When the acyl substituent R, eg in the compounds **23** and **24**, is changed to an alkyl group, as in **4**, a change in flexibility of the PF1022A derivatives occurs. In other words, the larger steric size of the substituted *O*-carbonyl *N*-methylamidoxime moiety in **23** and **24** generally leads to an increased population of the symmetric conformers compared to the asymmetric conformers containing *cis*-amide bond(s).

In the same solvent, mono-thionated PF1022A (**2**) showed a mixture of two asymmetric conformers, at a ratio of 2:1,[18] whereas PF1022A (**1**) exhibited a mixture of the asymmetric and symmetric conformers, at a ratio of 3:1.[14]

Table 2. Anthelmintic activities *in vitro* of the *N*-methylated amidoxime analogues (**I**) in comparison with PF1022A (**1**), mebendazol and ivermectin

	Anthelmintic activity in vitro at 100 μg ml^{-1}	
Compound no	T spiralis	N brasiliensis
PF1022A[a]	3[b,c]	3[b,c]
Mebendazol[d]	2[b]	3[b]
Ivermectin[d]	0[b]	3[b]
	0	3
4	2	2
6	0	2
7	0	0
10	2	2
11	2	1
13	0	0
14	0	0
16	0	2
17	0	0
20	1	0
21	1	0
22	0	1
23	0	1
24	0	0
25	3	0
27	0	1
28	2	1

[a] Results taken from Reference 28.
[b] Concentration: 1 μg ml^{-1}.
[c] 0 = no activity (number of living larvae equals that in the control)[26] 1 = weak activity (>50% of larvae still alive); 2 = good activity (<100% but >50% of the larvae dead); 3 = full activity (all larvae dead).
[d] Commercial product; results taken from Reference 31.

The ^{13}C chemical shifts were measured from heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra, which resulted in the uncertainty of ±0.3 ppm due to the lack of digital points in the f1-dimension. The structural assigments of the macrocycles (**I**) were based on the quasimolecule ion peaks $[M+H]^+$ in the LC mass spectra and characteristic resonances in the ^{1}H NMR spectra assignable of the ^{1}H chemical shifts of the incorporated [=N—O—R] fragment.

The relevant NMR (^{1}H chemical shifts of =N—O—C*H*-R′, =N—O—CO—C*H*-R′, =N—O—SO_2—CH_3 or ^{13}C chemical shifts of *C*=N—O—R) and physical characteristics of the synthesized *N*-methylated amidoxime analogues (**I**) are summarized in Table 1A and B. From the data available, the question as to the presence of geometrical (*E*/*Z*)-isomers around the amidine C=N bond for the *N*-methylated amidoxime analogues (**I**) cannot be answered in detail.

3.2 Anthelmintic activity

Several *in vitro* studies revealed that PF1022A was very active against *T spiralis* and *N brasiliensis* at 1 μg ml^{-1}.[28] In contrast, the new *N*-methylated amidoxime derivatives (**I**) exerted full or only slight anthelmintic activity against either nematode at the high concentration of 100 μg ml^{-1} (Table 2).

In a further step we examined the anthelmintic activities of the *N*-methylated amidoxime analogues (**I**) *in vivo* using the mixed-parasite infection in mice. PF1022A showed full anthelmintic efficacy at the oral dose-rate of 50 mg kg^{-1} against *H spumosa*[28] and a good activity against *H polygyrus*, but there was no detectable effect against the tapeworm *H nana*[27] (Table 3).

Two cyclic depsipeptides (**14** and **23**) displayed

	Anthelmintic activity in mice			
Compound no	H nana	H spumosa	H polygyrus	T spiralis *larvae*
PF1022A[a]	100[b]/0[c]	50/3	25/2	100/0
Mebendazol[d]	—[e]	10/3	100/0	100/3
Ivermectin[d]	—[e]	0.25/2	0.5/2	2.5/0
3	25/0	25/2	25/3	25/0
4	50/3	50/0	50/0	50/0
5	100/3	100/1	100/0	100/0
7	100/2	100/1	100/1	100/0
9	50/3	50/0	50/1	50/0
11	100/3	100/0	100/0	100/0
13	100/3	100/2	100/0	100/0
14	50/0	50/3	50/0	50/0
17	50/3	50/1	50/0	50/0
18	100/3	100/0	100/1	100/0
21	100/3	100/1	100/0	100/0
23	50/0	50/3	50/0	50/0
24	100/0	100/1	100/0	100/0
26	100/3	100/0	100/0	100/0

[a] Results taken from Reference 28.
[b] Dose in mg test substance kg^{-1} body weight.
[c] 0 = <50% egg reduction; 1 = 50–75% egg reduction; 2 = 75–95% egg reduction; 3 = >95% egg reduction.
[d] Commercial product; results taken from Reference 31.
[e] Not determined.

Table 3. Anthelmintic activities of the *N*-methylated amidoxime analogues (**I**) in comparison with PF1022A (**1**), mebendazol and ivermectin

anthelmintic activity against *H spumosa* (full activity at 50 mg kg^{-1}) comparable to PF1022A (**1**), whereas the *N*-methylated amidoxime **3** showed activity against *H polygyrus* (full activity at 25 mg kg^{-1}). The *N*-methylamidoxime **3** was found to be more active against *H spumosa* and *H polygyrus* at 25 mg kg^{-1} than the parent cyclooctadepsipeptide **1**. Furthermore, a limited number of *N*-methylated amidoxime analogues showed anthelmintic activity against the tapeworm *H nana* in the gut (**4**, **9** and **17**, full activity at 50 mg kg^{-1}). In sheep, *N*-methylated amidoxime analogues (**I**) also displayed anthelmintic activity against the sheep nematodes *H contortus* and but only compound **24** against *T colubriformis* (Table 4).

All the *N*-methylamidoxime derivatives (**I**) tested *in vivo* in the mouse model were found to be fully active against the abomasal sheep nematode *H contortus* at 0.10 mg kg^{-1} and up to 0.01 mg kg^{-1}.

3.3 Structure–activity relationships

Replacement of one *N*-methylamide group in PF1022A (**1**) with a *N*-methylamidoxime group, as in **3** ($\log P = 5.86$), increased the anthelmintic activity compared to both PF1022A ($\log P = 5.84$) and its mono-thionated derivative (**2**) ($\log P = 6.19$).[18] The introduction of alkyl groups as substituents R in the backbone *N*-methylamidoxime moiety in the compounds (**I**), such as methyl (**4**), resulted in 25-fold increase activity against *H contortus* in sheep compared with the parent compound PF1022A. When the substituent was heterocyclic and increased in size, as in **19** ($\log P = 7.05$), the spectrum of anthelmintic activity tended to be reduced compared to **4** ($\log P = 6.73$). In the series of the lipophilic *O*-acylated *N*-methylamidoximes **24** ($\log P = 6.23$), **25** ($\log P = 6.32$) and **27** ($\log P = 6.67$), the ethoxycarbonyl group **24** gave the highest ratings against both intestinal nematode species. However, the introduction of an allyloxycarbonyl group (**25**) as an alternative to the ethoxycarbonyl group drastically reduced the anthelmintic efficacy. Nonetheless, the activity of the cyclic octadepsipeptide **27**, containing the branched, racemic (*R,S*)-*sec*-butyloxycarbonyl group at the *N*-methylamidoxime fragment, was somewhat lower. A comparison of the synthesized *N*-methylamidoximes (**I**) containing different [=N-O-R]-fragments revealed a tendency for the anthelmintic activity against the abomasal nematode *H contortus* in sheep related to the substituent R in the following order:

$$CO{-}O{-}C_2H_5,\ CH_3 > H > \text{Fur-2-yl}{-}CH_2 >$$

$$CO{-}O{-}HC{=}CH_2,\ (R,S)\text{-}CO{-}OCH(CH_3)C_2H_5.$$

In summary, the *N*-methylated amidoxime **3**, which has a $\log P$ value comparable with that of the parent compound PF1022A, as well as the more lipophilic derivatives, **4** and **24**, represent the most anthelmintically active compounds in sheep. Finally, the marked increase in anthelmintic activity against the nematode *H contortus* in sheep of both derivatives **4** and **24** can be explained on the basis of their bioactive asymmetric conformation, containing a *cis*-amide bond. This result is in agreement with previous findings.[18]

4 CONCLUSIONS

Several new *N*-methylated amidoxime analogues (**I**) were synthesized using the mono-thionated PF1022A (**2**) as a precursor. The *N*-methylated amidoxime analogues (**I**) represent novel cyclic octadepsipeptide derivatives of the anthelmintic agent PF1022A (**1**) with improved activity in livestock animals. Although a significant increase in lipophilicity should be critical with respect to the bioavailability of cyclic octadepsi-

		Anthelmintic activity in sheep	
Compound no	*Log* P [a]	H contortus	T colubriformis
PF1022A	5.84	0.25[b]/3[c]	0.25/0
		0.01/0	—[d]
mono-thionated PF1022A[e]	6.19	0.10/3	—[d]
Mebendazol[f]	—[d]	20/3	20/3
Ivermectin[f]	—[d]	0.20/3	0.20/3
3	5.86	0.10/3	—[d]
		0.01/2	—[d]
4	6.73	0.01/3	—[d]
19	7.05	0.10/3	—[d]
24	6.23	0.01/3	0.10/3
25	6.32	0.10/2	—[d]
27	6.67	0.10/2	—[d]

[a] Log *P* from HPLC (see Section 2.1).
[b] Dose in mg test substance kg^{-1} body weight.
[c] 0 = <50% egg reduction; 1 = 50–75% egg reduction; 2 = 75–95% egg reduction; 3 = >95% egg reduction.
[d] Not determined.
[e] Results taken from Reference 18.
[f] Commercial product; results taken from Reference 32.

Table 4. Anthelmintic activities and lipophilicities of the *N*-methylated amidoxime analogues (**I**) in comparison with PF1022A (**1**), *mono*-thionated PF1022A (**2**), mebendazol and ivermectin

peptides,[11] in the present study the more lipophilic *N*-methylated amidoxime derivatives, **4** and **24**, proved more potent in their efficacy against the abomasal nematode *H contortus* in sheep than PF1022A. Furthermore, depending on substitution of the backbone *N*-methylamidoxime oxygen in (**I**), anthelmintic activity can be increased. Some of the anthelmintically active *N*-methylamidoxime analogues (**I**) exist in $CDCl_3$, CD_3CN or acetone-d_6 solution as a mixture of symmetric and asymmetric conformers in different ratios, containing a *cis*-amide bond. Formation of the bioactive asymmetric conformers and increased activity of these *N*-methylamidoxime analogues may be attributable to the substituents R (eg steric factors) on the new *N*-methylamidoxime moiety of the compound. In addition, the asymmetric conformation caused by isosteric replacement of a *N*-methylamide bond with a *N*-methylamidoxime enhances the *cis*-amide bond between D-Lac[5] and L-MeLeu,[6] respectively.

Mode of action and tolerance studies have not yet been completed, but studies with PF1022A suggest that the mechanism of action might rely on interference with a latrophilin-like receptor.[9]

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REFERENCES

1 Terada M, Ishih A, Tungtrongchitr A, Sano M and Shomura T, Effects of PF1022A on developing larvae of *Angiostrongylus costariciensis* in mice, with special reference to route, dose and formulation. *Jpn J Parasitol* **42**:199–210 (1993).

2 Kachi S, Terada M and Hashimoto H, Effects of amorphous and polymorphs of PF1022A, a new antinematode drug, on *Angiostrongylus costaricensis* in mice. *Jpn J Parasitol* 77:235–245 (1998).

3 Conder GA, Johnson SS, Nowakowski DS, Blake TE, Dutton FE, Nelson SJ, Thomas EM, Davis JP and Thompson DP, Anthelmintic profile of the cyclodepsipeptide PF1022A *in vitro* and in *in vivo* models. *J Antibiotics* **48**:820–823 (1995).

4 von Samson-Himmelstjerna G, Harder A, Schnieder T, Kalbe J and Mencke N, In vivo activities of the new anthelmintic depsipeptide PF1022A. *Parasitol Res* **86**:194–199 (2000).

5 Sasaki T, Takagi M, Yaguchi T, Miyadoh S, Okada T and Koyama M, A new anthelmintic cyclodepsipeptide, PF1022A. *J Antibiotics* **45**:692–697 (1992).

6 Ohyama M, Iinuma K, Isogai A and Suzuki A, Total synthesis of the anthelmintic cyclodepsipeptide, PF1022A. *Biosci Biotech Biochem* **58**:1193–1194 (1994).

7 Kobayashi M, Nanba T, Toyama T and Saito A, Synthesis and anthelmintic activity of the cyclodepsipeptide, PF1022A. *Annu Rep Sankyo Res Lab* **46**:67–75 (1994).

8 Dutton FE and Nelson SJ, Synthesis of PF1022A, an anthelmintic cyclodepsipeptide. *J Antibiotics* **47**:1322–1327 (1994).

9 Saeger B, Schmitt-Wrede HP, Dehnhardt M, Benten WPM, Krucken J, Harder A, von Samson-Himmelstjerna G, Wiegand H and Wunderlich F, Latrophilin-like receptor from the parasitic nematode *Haemonchus contortus* as target for the anthelmintic depsipeptide PF1022A. *FASEB Journal* **15**:1332–1334 (2001).

10 Harder A and von Samson-Himmelstjerna G, Cyclooctadepsipeptides – a new class of anthelmintically active compounds, *Parasitol Res* **88**:481–488 (2002). DOI: 10.1007/s00436-002-0619-2 (online: 2002).

11 Scherkenbeck J, Harder A, Plant A and Dyker H, PF1022A—a novel anthelmintic cyclooctadepsipeptide. Modification and exchange of the *N*-methyl leucine residue. *Bioorg Med Chem Lett* **8**:1035–1040 (1998).

12 Sakanaka O, Okada Y, Ohyama M, Matsumoto M, Takahashi M, Murai Y, Iinuma K, Harder A and Mencke N, Derivatives of cyclodepsipeptide, PF1022 substance (Bayer AG, Meiji Seika Kaisha, Ltd), *PCT Application* 9 711 064 (1997).

13 Sakanaka O, Okada Y, Ohyama M, Takahashi M, Suzuki K, Murai Y, Iinuma K, Harder A, Mencke N, Jeschke P and Bonse G, Preparation of cyclodepsipeptide PF1022A derivatives as anthelmintics (Meiji Seika Kaisha Ltd, Bayer AG), *PCT Application* 9 837 088 (1998).

14 Scherkenbeck J, Dyker H, Gondol D, Harder A, Plant A and Reichel F, Synthesis, conformational studies and anthelmintic activity of a constrained PF1022A analogue. *Pestic Sci* **55**:457–461 (1999).

15 Dutton FE and Lee BH, Epsilon-lactam analogs of the anthelmintic cyclodepsipeptide PF1022A. *Tetrahedron Lett* **39**:5313–5316 (1998).

16 Dyker H, Scherkenbeck J, Gondol D, Goehrt A and Harder A, Azadepsipeptides: synthesis and evaluation of a novel class of peptidomimetics. *J Org Chem* **66**:3760–3766 (2001).

17 Jeschke P, Harder A, von Samson-Himmelstjerna G, Mencke N, Bonse G, Iinuma K and Sakanaka O, Thiodepsipeptides for combating endoparasites and a method for producing the same (Bayer AG), *PCT Application* 9 843 965 (1998).

18 Jeschke P, Harder A, Etzel W, Gau W, Thielking G, Bonse G and Iinuma K, Synthesis and anthelmintic activity of thioamide analogues of cyclic octadepsipeptides such as PF1022A. *Pest Manag Sci* **57**:1000–1006 (2001).

19 Bushey DF and Hoover FC, Synthesis and stereochemistry of amidoximes. *J Org Chem* **45**:4198–4206 (1980).

20 Rao VS, Sauve G, Lajoie G and Belleau B, The solution conformation of chemotactic peptides and their backbone-modified analogs. Nuclear magnetic resonance and computer-modeling studies with active amidoxime analogs of formyl-methionyl-leucyl-phenylalanine. *Spectroscopy (Ottawa)* **4**:153–170 (1985).

21 Unger SH, Cook JR and Hollenberg JS, Simple procedure for determining octanol-aqueous partition, distribution, and ionisation coefficients by reversed-phase high-pressure liquid chromatography. *J Pharm Sci* **67**:1364–1367 (1978).

22 Noble A, Partition coefficients (*n*-octanol-water) for pesticides. *J Chromatogr* **642**:3–14 (1993).

23 Mitsunobu O, The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis*, pp 1–28 (1981).

24 Sauve G, Rao VS, Lajoie G and Belleau B, Backbone-modified oligopeptidic bioregulators. The synthesis and configuration of thioamide, amidoxime, cyanoamidine, and amidrazone analogs of the chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe-OR). *Canad J Chem* **63**:3089–3101 (1985).

25 Jeschke P, Harder A, von Samson-Himmelstjerna G and Bonse G, Cyclo-imino depsipeptides and their utilization in controlling endoparasites (Bayer AG), *PCT Application* 0 076 985 (2000).

26 Jenkins DC and Carrington TS, *In vitro* screening test for compounds active against the parenteral stages of *Trichinella spiralis*. *Tropenmed Parasitol* **32**:31–34 (1981).

27 Rapson EB, Jenkins DC and Chilwan AS, Improved detection of anthelmintic activity in an *in vitro* screen utilizing adult *Nippostrongylus brasiliensis*. *Z Parasitenkd* **73**:190–191 (1987).

28 Martin RJ, Harder A, Londershausen M and Jeschke P, Anthelmintic actions of the cyclic depsipeptide PF1022A and

its electrophysiological effects on muscle cells of *Ascaris suum*. *Pestic Sci* **48**:343–349 (1996).
29 Andrews P, Haberkorn A and Thomas H, Antiparasitic drugs: Mechanisms of action, pharmacokinetics, and *in vitro* and *in vivo* assays of drug activity, in *Antibiotics in laboratory medicine*, ed by Lorian V, Williams & Wilkins, Baltimore, London, Los Angeles, Sydney, pp 282–345 (1986).
30 Wetzel R, Verbesserte McMaster Kammer zum Auszählen von Wurmeiern. *Tierärztliche Rundschau* **11**:209 (1951).
31 Bernt U, Junkersdorf B, Londershausen M, Harder A and Schierenberg E, Effects of anthelminthics with different modes of action on the behavior and development of *Caenorhabditis elegans*. *Fundam Appl Nematol* **21**:251–263 (1998).
32 Rommel M, Eckert J, Kutzer E, Körting W and Schnieder T, in *Veterinärmedizinische parasitologie*, ed by Boch J and Supperer R, Parey Buchverlag Berlin im Blackwell, Wissenschaftsverlag GmbH, pp 263–264 (2000).