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Synthesis and anthelmintic activity of substituted (*R*)-phenyllactic acid containing cyclohexadepsipeptides

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Abstract—The substituted (*R*)-phenyllactic acid containing cyclohexadepsipeptides (CHDPs) represent novel enniatin derivatives with strong in vivo activities against the parasitic nematode *Haemonchus contortus* Rudolphi in sheep. 2D NMR spectroscopic analysis revealed for the substituted (*R*)-phenyllactic acid containing CHDPs one major conformer with an unsymmetrically folded conformation lacking a *cis*-amide bond. A correlation between the substitution pattern and its anthelmintic activity was found. Here we report on a simple total synthetic pathway of the precursor for this particular type of CHDPs and an efficient modification of the benzylic side chain (*R*-PhLac[2]).

Parasitic nematodes cause significant problems to the health and life of many plants and animals, and also of humans. Gastrointestinal nematodes like *Haemonchus contortus* Rudolphi occur worldwide and parasitize the abomasus of domestic animals such as cattle and sheep.[1] Therefore, the search of novel anthelmintic drugs plays an important role in veterinary medicine[2] because a serious problem is the emerging resistance of parasites towards traditional anthelmintics such as benzimidazole derivatives, levamisole and macrocyclic lactones.[3] The 24-membered cyclooctadepsipeptides (CODPs) represent the most promising substance class within the newly described anthelmintics in recent years.[4] This class constitutes a large family of peptide-related compounds derived from 2-hydroxy-(*R*)-carboxylic acids (*R*-HyCar) and *N*-methyl-(*S*)-amino acids (MeXaa) joined by amide and ester linkages. The broad chemical variation of the potent anthelmintic PF1022A[5] led to *semi*-synthetic derivatives containing as (*R*)-HyCar one or two (*R*)-4-*N*-morpholino-phenyl-lactic acids (*R*-4-*N*-MorPhLac) like **1**[6] and emodepside (Bay 44-4400) **2**[7], respectively. The latter is highly active against a broad spectrum of intestinal and extraintestinal nematodes such as filarial parasites and is commercialized as Profender® (2005, Bayer HealthCare Animal Health) in combination with praziquantel.[8]

To obtain more insight into the anthelmintic efficacy of the structurally closely related 18-membered cyclohexadepsipeptides (CHDPs), the so-called enniatins, we became interested in the preparation of semi-synthetic enniatin structures with regard to their efficacy against *H. contortus* in sheep.[9] Recently, the replacement of one *N*-methyl-(*S*)-isoleucine (MeIle) of the naturally occuring enniatin A by *N*-methyl-(*S*)-alanine (MeAla), as exemplified by **3**, has been reported to be 10-fold more active than the natural enniatins against *H. contortus*. A correlation between the nature of different CHDP major conformers and their anthelmintic activities was described.[10]

Structure of the cyclooctadepsipeptides (**1**–**2**) and enniatins (**3**–**7**).

It was found that those CHPDs with strong in vivo activity exist in $CDCl_3$ solution as one major conformer either with one *cis*-amide bond or with an unsymmetrically folded conformation lacking a *cis*-amide bond like **3**.

Keywords: Cyclohexadepsipepdides; CHDPs; Total synthesis; Anthelmintics; Parasitic nematode *Haemonchus contortus*; Conformers; *cis*-Amide bond.
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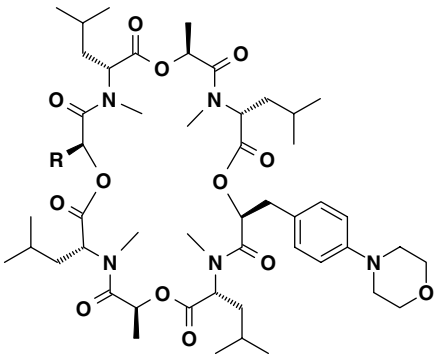
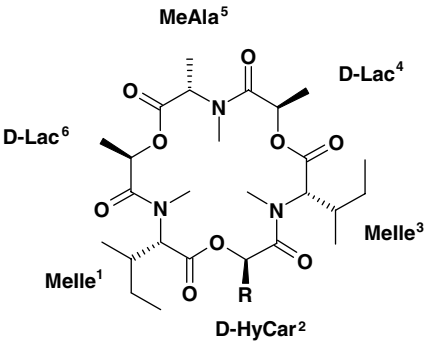


1 R = Me
2 R = 4-*N*-Mor-benzyl
Emodepside (Bay 44-4400)



3 R = Me
4 R = benzyl
5c R = 4-NO_2-benzyl
6a R = 2-NH_2-benzyl
6b R = 3-NH_2-benzyl
6c R = 4-NH_2-benzyl
7 R = 4-*N*-Mor-benzyl

In order to better understand the effect of the unique (*R*)-4-*N*-MorPhLac moiety within the 18-membered macrocycle in connection with its biological activity, we have now focussed our attention on the 2-position of the CHDP **3**. In fact, we found the 2-position in **3** is not important for its high binding affinity. Therefore, as part of our ongoing efforts to find novel anthelmintic drugs, we started to investigate CHDP derivatives of **3** containing substituted 2-hydroxy-(*R*)-phenyllactic acids (*R*-PhLac) in 2-position such as 2-hydroxy-(*R*)-4-nitrophenyllactic acid (*R*-4-NO_2PhLac) and 2-hydroxy-(*R*)-2-, 3- or 4-amino-phenyllactic acids (*R*-2-, 3- or 4-NH_2PhLac), respectively.[11] In this paper, we report the total synthesis of a (*R*-PhLac[2])-containing CHDP and its efficient modification of the benzylic side chain with respect to the 4-*N*-morpholino substitution.

The method for preparing the CHDP **4** involved formation of the depsipeptide hexamers (**10**–**12**)[12] from three dimeric fragments by a [2 + 4]-fragment condensation reaction, for example, by using the N-terminal protected didepsipeptides (**8**) and the O-terminal protected tetradepsipeptide fragment **9**, in a convergent strategy as already described by Jeschke et al.[7] Several further methods are known for syntheses of CHDPs.[13]

The macrocyclization was accomplished by ring closure of the N- and O-terminal deprotected hexadepsipeptides (**12**) under high dilution conditions using the phosphonium coupling reagent bis(2-oxo-3-oxazolidinyl)-phosphonic chloride (BOP-Cl) and *N*,*N*-diisopropylethylamine (DIEA), affording the CHDP **4**[14] as shown in Scheme 1. Subsequent nitration of **4** with 98% fuming nitric acid resulted in a (4:1:1) mixture of enniatins **5a**–**c** containing (*R*)-PhLac fragments *mono*-nitrated in 2-, 3- and 4-positions, from which the (*R*)-4-NO_2PhLac derivative **5c**[15] was isolated by preparative HPLC (Scheme 2).

Hydrogenation of the mixture **5a**–**c** in the presence of 20% $Pd(OH)_2$/C in ethanol afforded the amino analogues **6a**–**c**[16] as mixture of enniatin isomers (4-NH_2PhLac/3-NH_2PhLac/2-NH_2PhLac = 4:1:1) in 67% yield, which can be separated from each other by preparative HPLC or in larger amounts (up to 4.0 g) by Craig distribution (ethyl acetate/*n*-heptane/DMF/H_2O = 4:6:5:5). Finally, the *N*-morpholino ring closure forming the (*R*)-4-*N*-MorPhLac enniatin **7**[17] was carried out by reductive alkylation of **6c** with 2,2′-oxy-bis[acetaldehyde], prepared in situ from 2,5-dihydrofuran by ozonolysis, and sodium cyanoborohydride.[18]

The structural assignments of all CHDPs were based on the molecule ion peaks $[M]^+$ in the EI mass spectra and characteristic resonances in the ^{13}C NMR spectra where all fragments could be assigned.

The single crystal X-ray structure of the CHDP **5c** was determined using MoK_α-radiation as X-ray source (see Fig. 1).[19]

Sheep (*Ovis aries* L, Merino or Schwarzkopf breed, 25–35 kg body weight) were infected experimentally with 5000 *H. contortus* Rudolphi L_3 larvae and treated with the test substance after the end of the prepatency period of the parasite. The test compounds were administered orally in gelatine capsules. Anthelmintic effects of the test substances were measured as a function of the reduction in faecal egg counts. For the purpose of counting eggs, freshly obtained faeces from experimentally infested animals were prepared using the McMaster method as modified by Wetzel.[20] The egg counts were determined at regular intervals before and after treatment. The anthelmintic evaluation was expressed as a function of the egg reduction as follows: $3 \geqslant 95\%$, 2 = 75–95%, 1 = 50–75% and $0 = \leqslant 50\%$ egg reduction.

The CHDP **4** exhibited a 2:1 mixture of conformers in $CDCl_3$ because of the benzyl side chain. On the other hand, the CHDP **5c** and **6a**–**c**, **7** showed a 3:1 and 2:1 mixture of conformers in $CDCl_3$, the appropriate main