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Syntheses of Depsipeptide Analogues of the Insect Neuropeptide Proctolin

Jürgen Scherkenbeck,[a,*] Andrew Plant,[a] Frank Stieber,[b] Peter Lösel[a] and Hubert Dyker[a]

[a]*Bayer AG, Central Research, 51368 Leverkusen, Germany*
[b]*Department of Chemistry, University of Dortmund, Germany*

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Abstract—Four depsipeptide analogues of the insect neuropeptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) have been prepared, containing a single ester linkage between Arg[1] and Tyr[2], Tyr[2] and Leu[3], and between Pro[4] and Thr[5], respectively. A didepsipentapeptide containing an ester linkage between Tyr[2] and Leu[3] and between Pro[4] and Thr[5], has also been prepared. The depsipeptide **4** is the first example of a backbone-modified proctolin analogue which shows full myotropic activity. © 2002 Elsevier Science Ltd. All rights reserved.

Insect neuropeptides control almost all physiological processes in insects and thus are potential targets for a novel generation of selective insecticides.[1] Unfortunately, insect neuropeptides suffer from inadequate in vivo efficacy due to poor absorption, lack of transportation, or rapid metabolic degradation. The incorporation of peptide mimetics has been established as a method of circumventing the resulting insufficient bioavailability of the native peptides.[2–7]

Since the insect neuropeptide Proctolin (**1**) was first isolated from extracts of the cockroach *Periplanata americana*[8] and later shown to be a pentapeptide, H-Arg-Tyr-Leu-Pro-Thr-OH (**1**) (RYLPT),[9,10] it has received considerable attention from scientists spanning a range of disciplines (Fig. 1).[11] Proctolin has been identified in species from six orders of insect, as well as other invertebrates, where it has been shown to exert myotropic effects on visceral and skeletal muscle.[11] Proctolin has variously been assigned roles as a neurotransmitter, a neuromodulator, and a neurohormone.[12]

Proctolin has been the subject of structure–activity studies by a number of research groups.[13] The majority of the published data are concerned with the exchange of one or more of the five constituent amino acids with other natural or non-natural α-amino acids,[14] affording a considerable amount of structure–activity relationship information.[15] In only a few cases have proctolin analogues been prepared with modifications to the peptide backbone. For example, the incorporation of α-methyl-L-Tyr,[16] *N*-methyl amino acids,[17] the replacement of the amide bond between Tyr and Leu with the isosteric –CH_2–O–moiety,[18] and cycloproctolin,[19] have been reported.

We were interested in probing the hydrogen bonding requirements in proctolin by replacing one or more of the peptide bonds with peptide bond isosteres or mimics. Furthermore, we speculated that introducing less hydrophilic, protease resistant functionality, might lead to proctolin analogues exhibiting insecticidal activity. We chose to synthesize the following four depsipeptides: H-Arg-ψ[CO–O]-Tyr-Leu-Pro-Thr-OH (**2**) H-Arg-Tyr-ψ[CO–O]-Leu-Pro-Thr-OH (**3**) H-Arg-Tyr-Leu-Pro-ψ[CO–O]-Thr-OH (**4**) H-Arg-Tyr-ψ[CO–O]-Leu-Pro-ψ[CO–O]-Thr-OH (**5**)

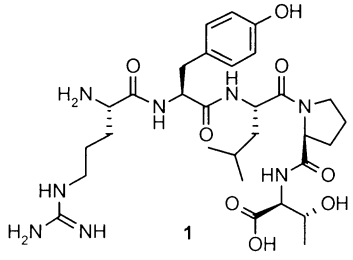

Figure 1. Proctolin (**1**).

*Corresponding author. Tel.: +49-214-307-1157; fax: +49-214-305-0070; e-mail: juergen.scherkenbeck.js@bayer-ag.de

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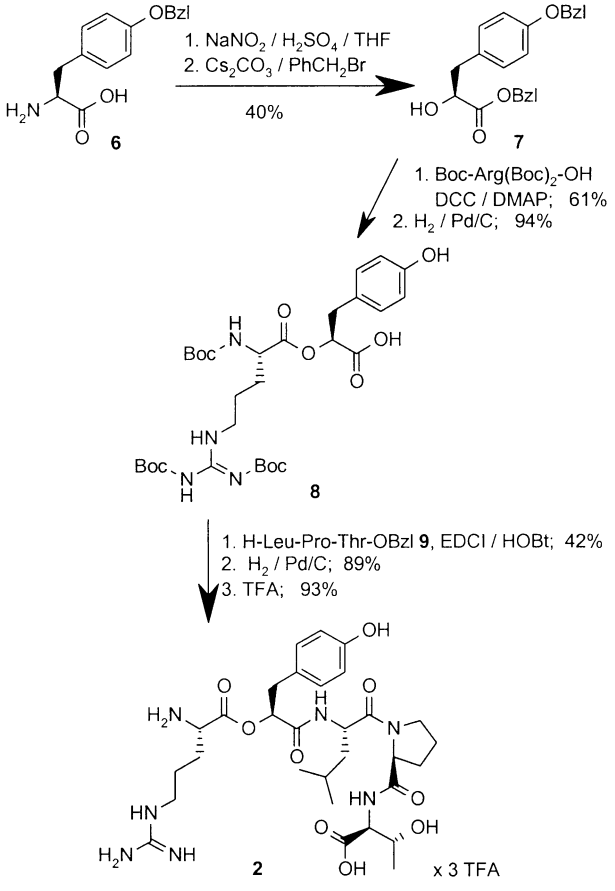
We opted for a strategy employing orthogonal protecting groups, protecting amino and guanidino functionality with the Boc group, and hydroxyl and carboxyl groups were protected as their benzyl ethers and benzyl esters, respectively (vide infra). H-Arg-ψ[CO–O]-Tyr-Leu-Pro-Thr-OH (**2**) which contains an ester linkage between Arg and Tyr was prepared as depicted in Scheme 1.

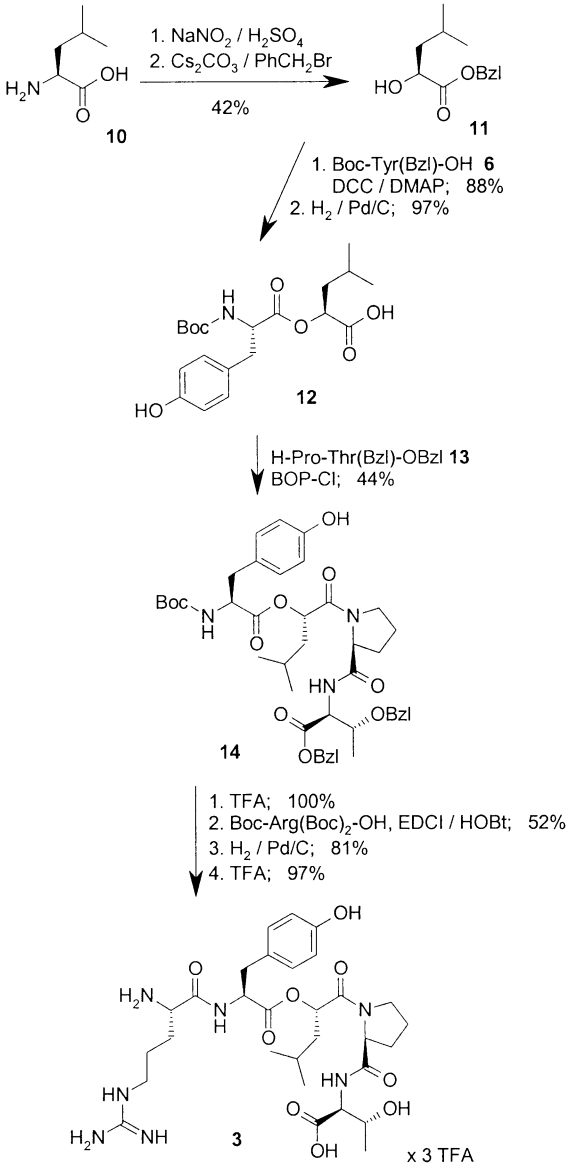
With a slight modification to a procedure from the literature used for analogous α-amino acids,[20] H-Tyr(Bzl)-OH (**6**) was desaminated and hydroxylated to the corresponding α-hydroxycarboxylic acid with overall retention of the desired configuration. Alkylation of the crude product, via its caesium carboxylate,[21] afforded the benzyl ester **7** in 40% yield over two steps. The next step was to couple α-hydroxycarboxylic ester **7** with Boc-Arg(Boc)$_2$-OH. Attempted ester formation using the BOP reagent[22] met with failure, as did the use of water soluble carbodiimide/hydroxybenzotriazole.[23] Fortunately, the DCC/DMAP protocol, according to Gilion and Klausner,[24] afforded the desired depsipeptide in 61% yield. Hydrogenolytic removal of the benzyl protecting groups gave the acid **8** in high yield. This acid was coupled to the tripeptide H-Leu-Pro-Thr-OBzl (**9**), mediated by EDCI/HOBt.

No attempt to optimise the moderate yield of 42% for this fragment coupling was made. The protecting groups were removed in two high yielding steps, firstly cleavage of the benzyl ester by catalytic hydrogenolysis, followed by removal of the three Boc groups on Arg by treatment with trifluoroacetic acid, to afford the first depsipeptide analogue of proctolin **2** (Scheme 1).

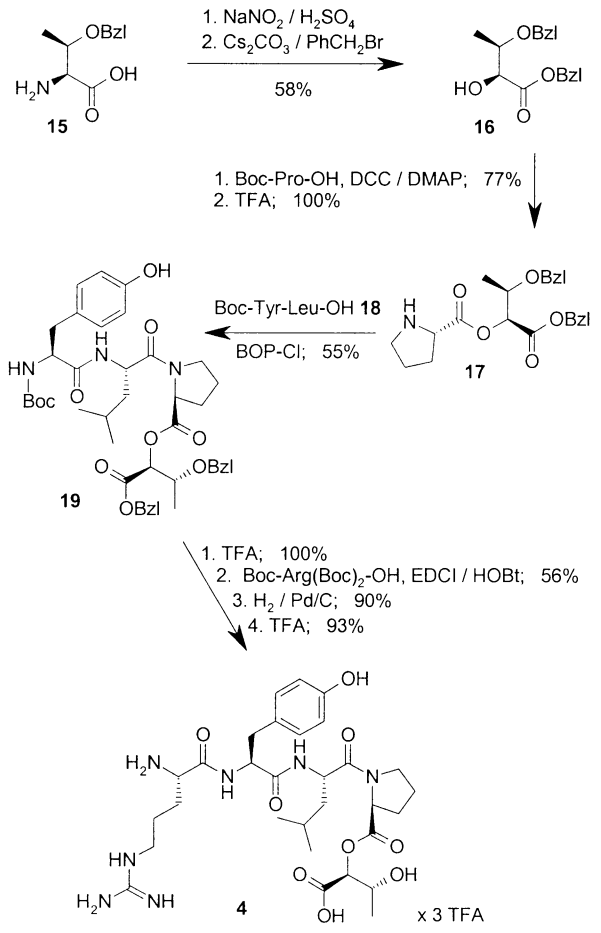
The synthesis of H-Arg-Tyr-ψ[CO–O]-Leu-Pro-Thr-OH (**3**) commenced from H-Leu-OH (**10**), which was converted into the corresponding (*S*)-α-hydroxycarboxylic ester **11** via the tandem desamination-hydroxylation procedure described above. DCC-mediated coupling with Boc-Tyr(Bzl)-OH (**6**) gave the depsipeptide in very good yield. Catalytic hydrogenolytic removal of the benzyl protecting groups afforded the acid **12**. For the key fragment coupling reaction of **12** with the dipeptide H-Pro-Thr-(Bzl)-OBzl (**13**) we chose BOP-Cl, with which we have previously enjoyed success in the synthesis of (cyclic)depsipeptides containing *N*-alkyl amino acids.[25,26]



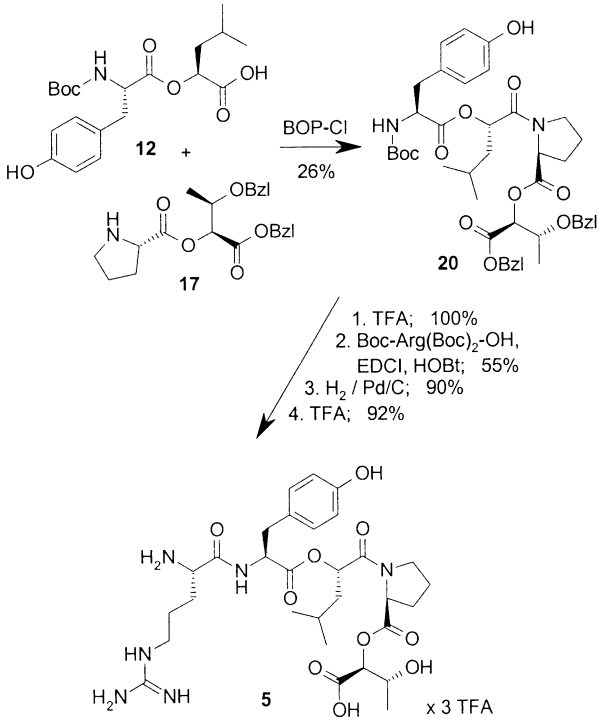
Scheme 1. Synthesis of H-Arg-ψ[CO–O]-Tyr-Leu-Pro-Thr-OH (**2**).



Scheme 2. Synthesis of H-Arg-Tyr-ψ[CO–O]-Leu-Pro-Thr-OH (**3**).



Scheme 3. Synthesis of H-Arg-Tyr-Leu-Pro-ψ[CO–O]-Thr-OH (**4**).



Scheme 4. H-Arg-Tyr-ψ[CO–O]-Leu-Pro-ψ[CO–O]-Thr-OH (**5**).

The N-terminus of tetradepsipeptide **14** was liberated by treatment with trifluoroacetic acid, setting up the final coupling reaction with Boc-Arg(Boc)$_2$-OH, to give the protected pentadepsipeptide. Removal of the protecting groups as described above afforded the depsipeptide analogue **3** of proctolin with an ester linkage between Tyr and Leu (Scheme 2).

H-Arg-Tyr-Leu-Pro-ψ[CO–O]-Thr-OH (**4**) was prepared in a similar fashion to **3**. Key steps were the formation of the ester linkage by DCC-mediated coupling of **16** with Boc-Pro-OH to afford, after deprotection of the *N*-protecting group with trifluoroacetic acid, the didepsipeptide **17**, and subsequent BOP-Cl-mediated coupling of **17** with the dipeptide Boc-Tyr-Leu-OH **18** affording the tetradepsipeptide **19**. *N*-Deprotection and attachment of the N-terminal amino acid Boc-Arg(Boc)$_2$-OH using EDCI/HOBt (56%), and subsequent removal of all protecting groups led to the desired pentadepsipeptide **4** (Scheme 3).

The pentadidepsipeptide H-Arg-Tyr-ψ[CO–O]-Leu-Pro-ψ[CO–O]-Thr-OH (**5**) was constructed from building blocks already employed in the syntheses of **3** and **4**. Thus the BOP-Cl-mediated coupling of H-Pro-ψ[CO–O]-Thr(Bzl)-OBzl (**17**) with Boc-Tyr-ψ[CO–O]-Leu-OH (**12**) afforded the tetradidepsipeptide **20** in modest (unoptimised) yield. The final four steps were identical to those in the abovementioned syntheses of **3** and **4**, giving rise to the final depsipeptide analogue of proctolin (**1**), compound **5**, modified at two positions of the native peptide's backbone (Scheme 4).

Myotropic effects of the new proctolin analogues were assessed in vitro, using an isometric muscle-contraction bioassay employing isolated hindguts from the locust *Locusta migratoria,* based on procedures described by Osborne and co-workers (Table 1).[27]

Introduction of ester functionality between Arg and Tyr (**2**) resulted in a maximal contraction response of approximately two-thirds that recorded for proctolin. This is an interesting, as well as perplexing result when one considers that [NMe-Tyr^2]-proctolin has previously been reported to lack myotropic activity.[16] These conflicting results regarding the importance of hydrogen bonding at this position require further investigation. In contrast compound **3** was found to have 30% of the agonistic activity of proctolin, a value not far removed from that found for [NMe-Leu^3]-proctolin (52%).[17] Compound **4** showed a similar maximal contraction response to proctolin, indicating that hydrogen bonding

Table 1. Preliminary results obtained using a test compound concentration of 1 μmol/L; an arbitrary value of 100% was assigned to proctolin

Compd	Isometric contraction (%)
Proctolin	100
2	66
3	30
4	106
5	27

interaction involving the amide moiety between Pro[4] and Thr[5] is not essential for myotropic activity. Based on NMR studies Osborne et al. had previously postulated an intramolecular hydrogen bond between Thr[4] (donor) and Leu[3] (acceptor), giving rise to an inverse γ-Turn.[17] Based on our findings, it would appear that this conformation is not necessarily the one which interacts with the putative proctolin receptor, leading to an agonistic response. Didepsipeptide **5** showed a similar response to compound **3**. This result can be explained by considering the additive effects of compounds **3** and **4**, and also confirms the conclusions made about the importance of hydrogen bonding interactions at these positions.

In summary, we have synthesised four new analogues of the insect neuropeptide proctolin, containing modifications to the peptide backbone. These novel depsipeptides have been prepared using a building block approach, and employing an orthogonal protecting group strategy.[28] Depsipeptide **4** is the first example of a backbone-modified proctolin analogue which retains full myotropic activity.

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