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Syntheses of Analogues of the Insect Neuropeptide Proctolin Containing an Oxazole Ring as an Amide Bond Replacement

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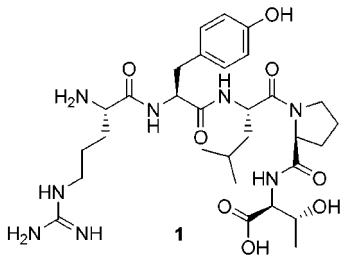
ABSTRACT



Three oxazole analogues of the insect neuropeptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) have been prepared, containing a single oxazole between Tyr and Pro and between Pro and Thr, respectively. A compound containing an oxazole moiety between Tyr and Pro and between Pro and Thr has also been prepared. All compounds have been tested for myotropic activity.

Biologically active peptides often suffer from inadequate in vivo efficacy as a result of 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.[1−6] As part of a study directed toward the synthesis of novel analogues of the insect neuropeptide proctolin (**1**), we prepared compounds modified in their peptide backbones with the aim of increasing their lipophilicity and decreasing their susceptibility to metabolic degradation while maintaining myotropic activity. If such compounds could be prepared, they should serve to better evaluate the potential of proctolin-derived structures for their application as insecticides.

Herein we wish to report the synthesis of proctolin analogues containing an oxazole ring in place of one or more peptide bonds. There is literature precedent for the utility of oxazole peptide mimetics.[7−9]

We chose to synthesize the following three oxazole-containing peptides: H-Arg-Tyr-Leu-Pro-ψ[oxazole]-Thr-OH (**2**), H-Arg-Tyr-ψ[oxazole]-Ser-Pro-Thr-OH (**3**), and H-Arg-Tyr-ψ[oxazole]-Ser-Pro-ψ[oxazole]-Thr-OH (**4**). It is important to note that in compounds **3** and **4**, for reasons of preparative expedience, the amino acid leucine has been replaced by serine. [Ser^3]-proctolin is a known compound

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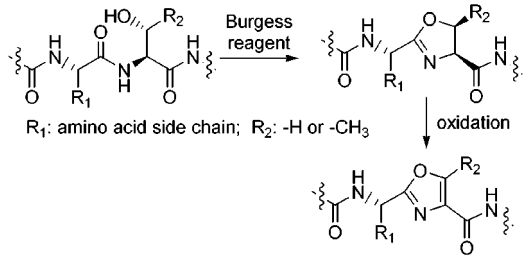
and, although myotropically less active than proctolin itself, has been shown to exhibit a considerably higher membrane affinity for receptors in muscle tissue of the locust hindgut, thus representing an interesting structure for receptor blocking studies.[10]

Initially, we evaluated two distinct routes potentially applicable to compounds **2**−**4**. First, synthesis of an appropriately protected precursor peptide containing a β-hydroxy-α-amino acid moiety, cyclodehydrative oxazoline formation, and oxidation to the resulting oxazole (Scheme 1). This approach had the added attraction of also providing

Scheme 1

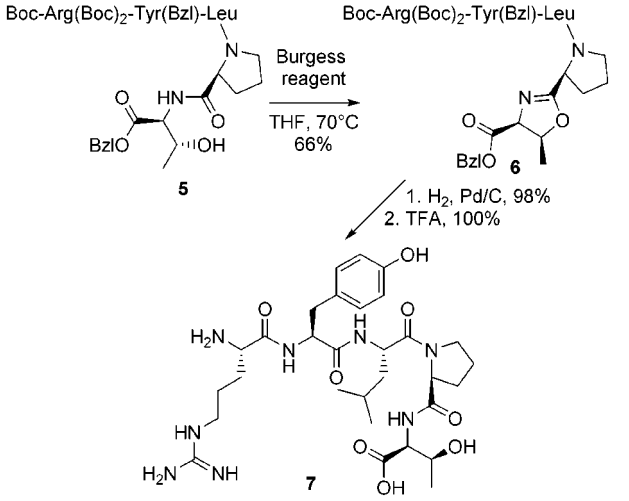


oxazoline-containing proctolin analogues.

Thus, Boc-Arg(Boc)$_2$-Tyr(Bzl)-Leu-Pro-Thr-OBzl (**5**) was prepared using standard solution-phase peptide synthesis (C → N terminus sequential amino acid coupling using EDCI/HOBt). Applying the pioneering work of Wipf,[11] this protected pentapeptide was treated with the Burgess reagent[12,13] to afford the oxazoline **6** in 66% yield, following chromatographic purification (Scheme 2). However, following catalytic hydrogenolysis to remove the benzyl ether and benzyl ester groups, respectively, Boc-Arg(Boc)$_2$-Tyr-Leu-Pro-allo-Thr-OH was the only product isolated. Allo-threonine has the (S)-configuration at the β-carbon atom. Subsequent removal of the Boc groups using trifluoroacetic acid afforded the fully deprotected peptide H-Arg-Tyr-Leu-Pro-allo-Thr-OH (**7**) as its trifluoroacetic acid salt, in 98% yield following lyophilization. As far as we are aware, [allo-Thr^5]-proctolin has not been previously reported, and we were able to confirm its structure by 2D NMR studies. These findings can be explained by oxazoline formation taking place with concomitant inversion of configuration at the threonine β-carbon atom, consistent with literature precedent.[14,15] Adventitious moisture was probably responsible for the hydrolysis of the oxazoline ring, giving rise to the allo-threonine moiety in the product.

Because of the difficulties in manipulating some of the oxazolines, we decided to pursue an alternative approach to

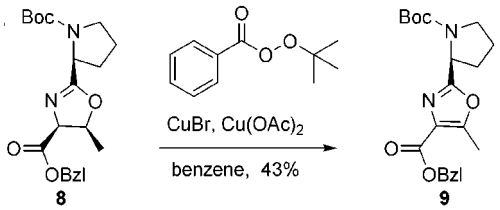
Scheme 2



compounds **2**−**4**, namely, a convergent synthesis via amide bond formation of appropriately protected oxazole building blocks.

Many methods have been reported for the synthesis of the oxazole ring system.[16] Many are not compatible with the functionality present in (protected) amino acids and peptides, particularly with respect to racemization. For the preparation of the oxazole fragment found in **2** we initially investigated the cyclodehydration of a suitably protected dipeptide, containing a β-hydroxy-α-amino acid moiety, followed by oxidation of the resulting oxazoline to the corresponding oxazole. A variety of oxidation reagents were tested for the transformation of Boc-Pro-ψ[oxazoline]-Thr-OH (**8**) to the corresponding oxazole **9**. The best result, affording **9** in 43% yield was obtained using CuBr/Cu(OAc)$_2$/perbenzoic acid *tert*-butyl ester (Scheme 3).[17] Other reagents such as DBU/

Scheme 3



CCl_4/acetonitrile/pyridine,[18] $CuBr_2$/DBU/HMTA,[19,20] and DDQ,[21,22] gave less satisfactory results. We consistently

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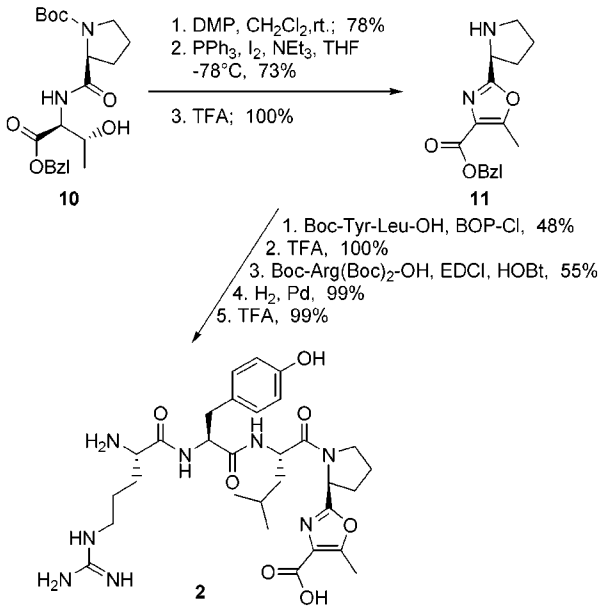
observed significantly inferior results for the 5-substituted heterocycles (threonine-derived) as compared to the unsubstituted (serine-derived) analogues (vide infra).

We eventually turned to an alternative protocol previously reported by Wipf.[23] (Scheme 4). Oxidation of the dipeptide

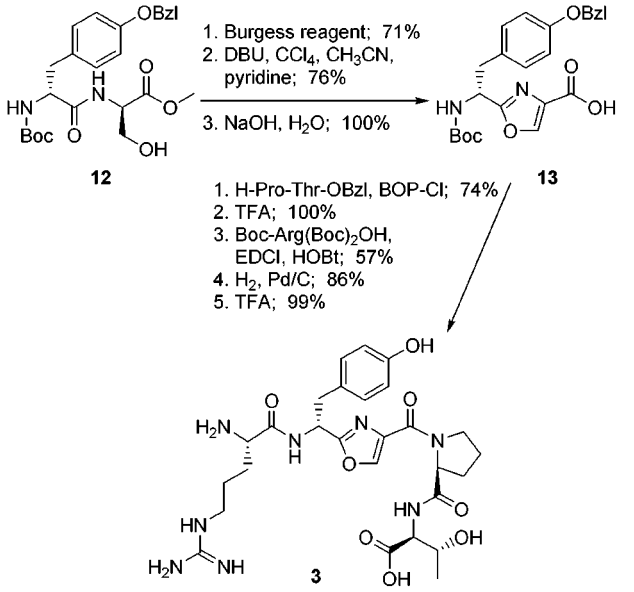
Scheme 4



Boc-Pro-Thr-OBzl (**10**) with the Dess−Martin periodane (DMP)[24,25] gave the corresponding β-keto ester in good yield. This compound underwent cyclodehydration on treatment with triphenylphosphine, iodine, and triethylamine to afford the oxazole, also in good yield. Cleavage of the Boc-group with TFA gave the N-deprotected Pro-Thr mimetic **11**, completing the three-step sequence from Boc-Pro-Thr-OBzl (**10**) in an overall yield of 50%. Fragment coupling of oxazole **11** with Boc-Tyr-Leu-OH mediated by BOP-Cl afforded the modified tetrapeptide in moderate yield. BOP-Cl has previously proved to be the reagent of choice in our laboratories for coupling reactions of *N*-methylamino amino acids and peptides.[26,27] Removal of the N-terminal protecting group and water-soluble carbodiimide-mediated coupling of the resulting amine functionality with Boc-Arg(Boc)$_2$-OH gave the desired protected pentapeptide in 55% yield. Cleavage of the orthogonal protecting groups in two high yielding steps afforded the target compound **2**.

Proctolin analogue **3** contains a serine-derived oxazole. The dipeptide **12** was transformed to the oxazoline using the Burgess reagent. Applying the protocol reported by Barrish et al.,[19] we were able to oxidize the oxazoline to the oxazole in good yield, without observing hydrolysis of the former heterocycle (Scheme 5). Hydrolysis of the ester

Scheme 5



functionality and subsequent BOP-Cl-mediated coupling of the acid **13** with H-Pro-Thr-OBzl gave the tetrapeptide in 74% yield. Applying the final four-step sequence as described for **2** afforded the second oxazole-containing proctolin analogue **3** in 36% overall yield from **13**.

The synthesis of the proctolin analogue **4** containing two oxazoles was accomplished utilizing the synthons **11** and **13**, whose preparation is described above. Coupling of these two fragments using BOP-Cl afforded the pseudo-tetrapeptide **14** in good yield. As with the syntheses of **2** and **3**, the

Table 1. Preliminary Results Obtained Using Test Compound Concentration of 1 μmol/L

compound	isometric contraction (%)[a]
proctolin	100
2	0
3	11
4	5
7	100

[a] An arbitrary value of 100% was assigned to proctolin.

same final four steps were straightforward and high yielding, producing the final target compound **4** (Scheme 6).

Myotropic effects of the new proctolin analogues were assessed in vitro, using an isometric contraction assay

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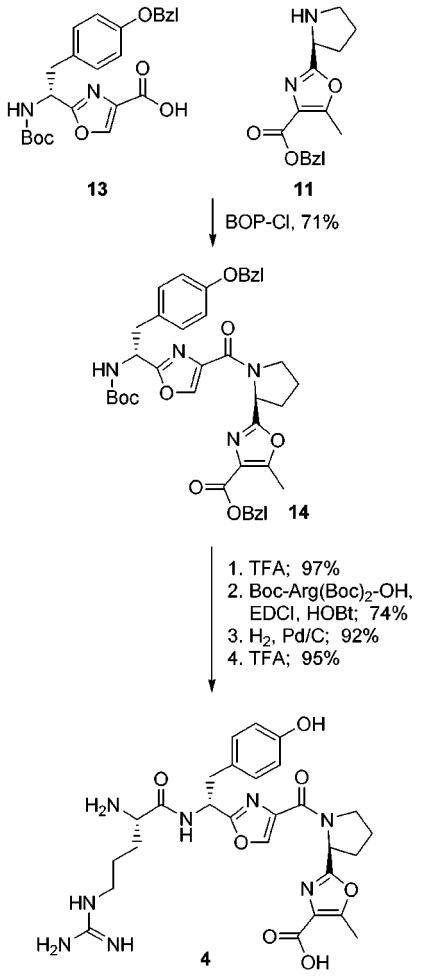
Scheme 6



employing isolated hindguts from the locust *Locusta migratoria*, based on procedures described by Osborne and coworkers.[28]

All three oxazole-containing analogues of proctolin exhibited negligible myotropic activity. Although the incorporation of this heterocycle should lead to more lipophilic and metabolically stable compounds compared to proctolin, the conformational constraints imposed have resulted in loss of myotropic activity.

More interestingly, [allo-Thr^5]-proctolin **7** is comparable in myotropic activity to proctolin itself. This result demonstrates for the first time that the absolute configuration at the Thr^5 β-carbon atom is unimportant with respect to myotropic activity, suggesting that hydrogen bonding interactions involving the Thr^5 hydroxy group are not significant with regard to the biologically active conformation.

In summary, we have synthesized three new backbone-modified analogues of the insect neuropeptide proctolin, incorporating the oxazole ring system as a replacement for the peptide bond. These novel peptides have been prepared using a building block approach and employing orthogonal protecting groups. Furthermore, as a spin-off from these studies we have prepared the previously unknown [allo-Thr^5]-proctolin, whose myotropic effect on the hindgut of *L. migratoria* is comparable to that of proctolin.[29]

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Supporting Information Available: Experimental procedures and analytical data (NMR, MS, HRMS). This material is available free of charge via the Internet at http://pubs.acs.org.

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