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Functional and Structural Characterization of Drosocin and its Derivatives Linked *O*-GalNAc at Thr[11] Residue

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Antimicrobial peptides have recently gained the much attention because of their ability to make defense system from attacking bacterial infections. Drosocin has been considered as very attractive antibiotic agents because of low toxicity against human erythrocytes and active at the low concentration. We have studied the structure-activity relationship of a glycopeptide drosocin focused on the *N*-acetyl-D-galactoside at Thr[11] residue. Based on the radial diffusion assay, we found that the acetylation of carbohydrate moiety increased the antimicrobial activity and the Pro[10], present in the middle of drosocin plays an important role in the antimicrobial activity. Our results provide a good lead compound for further studies on the design of drosocin-based analogues targeting glyco linked Thr site.

Key Words : Glyco-peptide, Antimicrobial peptide, Drosocin, Structure-Activity Relationship (SAR), Circular Dichroism (CD)

Introduction

The rising number of infections caused by bacterial isolates resistant to conventional antibiotics has lead to an intense search for novel antibiotics.[1] Insects possess an exceptional ability to respond to bacterial challenges, with the most important part of their defense system being rapid synthesis of potent broad-spectrum antimicrobial peptides as a reply to bacterial infections.[2,3] Otvos *et al*. group reported that drosocin and other family members such as pyrrhocoricin appear to exhibit their antimicrobial activity by binding to the bacterial heat shock protein Dnak, preventing chaperone assisted protein folding and inhibiting the related ATPase activity of Dnak.[4] Drosocin is the first characterized *O*-glycosylated antimicrobial peptide.[5,6] Drosocin, isolated from Drosophila melanogaster, is a 19-mer oligopeptide containing three Pro-Arg-Pro (PRP) units and an *O*-glycosylation site at Thr[11] residue by either monosaccharide [2-acetamido-2-deoxy-D-galactopyranosyl(GalNAc)Thr)] or a disaccharide [D-galacto pyranosyl (GalGalNAcThr)]. Drosocin has been considered as a very attractive antimicrobial agent because of low toxicity against human erythrocytes and only active toward Gram-negative bacteria at the low concentration. This prompted a number of studies that subsequently demonstrated the synthesis of drosocin analogues in improving antimicrobial activity.[7,8]

In an attempt to define the drosocin features, the next three kinds of a structure-activity relationship study were undertaken. *First*, the role of the carbohydrate component, especially hydroxyl group, was investigated by varying the monosaccharide. A growing body of evidence[5] suggests that the carbohydrate moiety plays an important role in antimicrobial activity in the order of increasing activity measured by the antimicrobial assay as disaccharide > monosaccharide >> unglycosylated drosocin. *Second*, we have designed the drosocin analogues by replacing the Ser[7] with Thr[7]. In the previous study,[7] it was reported that the simple substitution of Ser[7] with Thr[7] that is susceptible to proteolytic cleavage, revealed the increased in both activity and stability in unglycosylated drosocin analogues. Thus, we have decided to test antimicrobial activity with drosocin linked *O*-GalNAc at Thr[11] residue. *Third*, drosocin consists of 19 amino acid residues, one-third of which are prolines, and contains three characteristic PRP motifs. Proline has a secondary amine and is known to induce local constraints to the peptide sequence. It acts as a secondary structure (α-helix, β-sheet) terminator and induces turn motifs (such as β-turns) into the secondary structure. At present, the significance of Pro in drosocin is unknown, but it may have an important bearing on the three-dimensional structure. Moreover, the NMR data[9] shows that the significant structural changes occur at the residues 10-13 in which Pro[10] was located on the center of drosocin. In an effort to investigate the significance of Pro on antimicrobial activity, we have also synthesized the drosocin analogues by replacing the Pro[10] with Ala[10].

In the present paper, we investigated the antimicrobial activities of four new drosocin glycopeptides (D1, D2, D3

and D4), correlating their efficacy with the native drosocin D. The synthesized drosocin analogues (D-D4) have been assayed against clinically relevant bacteria, *Escherichia coli* (KCTC 1682). Here, we used the radial diffusion assay system, followed recently by antibiotic glycopeptide, formaecin I[10] that has glyco linkage on Thr[11] similar to our drosocin analogues. The radial diffusion assay is suitable as a screening test for measuring the susceptibilities of *E. coli* to drosocin analogues, because it is sensitive and simple and has good reproducibility.[11] The conformational properties of the synthetic glycopeptides (D-D4) were examined by circular dichroism (CD) spectroscopy.

Experimental Section

General. Materials were obtained from commercial suppliers and employed without further purification unless otherwise state. All reactions were carried out under argon atmosphere in oven-dried glassware. CH_3CN and CH_2Cl_2 were distilled under N_2 from CaH_2 and toluene from sodium/benzophenone immediately before use. Analytical TLC was performed on silica gel plate using UV light and/or anisaldehyde stain followed by heating. Flash column chromatography was performed on silica gel 60 (230-400 mesh). ^{1}H NMR spectra was recorded at 500 MHz on a Bruker Avance 500 spectrometer, using $CDCl_3$ and MeOH-d_4 as a solvent. Chemical shift were reported in parts per million (ppm, δ) relative to tetramethylsilane (δ 0.00). HPLC (Younglin, YL 9100) was carried out on a reversed-phase column, which was eluted with CH_3CN in 0.05% aqueous TFA and detected at OD 220 nm.

2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-galactopyranosyl Nitrate (1): Tri-*O*-acetyl galactal (5.20 g, 19.1 mmol) was dissolved in acetonitrile (100 mL) and cooled to - 15 °C. Then ceric ammonium nitrate (31.41 g, 57.3 mmol) and sodium azide (1.87 g, 28.7 mmol) were added. The mixture was stirred for 7 h and then allowed to warm to room temperature overnight. The mixture was diluted with ethyl acetate (100 mL) and washed with H_2O (100 mL × 3). The aqueous layers were extracted with ethyl acetate (50 mL × 2). The organic layers were dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography (*n*-hexanes/EtOAc, 3:1 → 2:1) to give the compound **1** (3.48 g, 48%) as a mixture of anomers.

^{1}H-NMR (500 MHz, $CDCl_3$, major, α-anomer): δ 6.35 (d, 1H, *J* = 4.2 Hz), 5.51 (m, 1H), 5.26 (dd, 1H, *J* = 11.3, 3.2 Hz), 4.37 (m, 1H), 4.17-4.10 (m, 3H), 2.18 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H).

Minor, β-anomer: β 5.60 (d, 1H, *J* = 8.8 Hz), 5.41 (m, 1H), 4.98 (dd, 1H, *J* = 11.3, 3.2 Hz), 4.37 (m, 1H), 4.17-4.10 (m, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H).

2-Azido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl Bromide (2): Azidonitrate **1** (3.82 g, 10.2 mmol) was dissolved in CH_3CN (50 mL) and then added LiBr (4.43 g, 51.0 mmol). The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (50 mL). The aqueous layers were extracted with CH_2Cl_2 (50 mL × 2). The organic layers were dried over Na_2SO_4, filtered, and evaporated to give the crude product **2** (3.70 g, 92%) as a syrup

^{1}H-NMR (500 MHz, $CDCl_3$): δ 6.48 (d, 1H, *J* = 3.9 Hz), 5.52 (m, 1H), 5.36 (dd, 1H, *J* = 10.7, 3.2 Hz), 4.49 (m, 1H), 4.19 (dd, 1H, *J* = 11.4, 6.4 Hz), 4.12 (dd, 1H, *J* = 11.4, 6.8 Hz), 4.00 (dd, 1H, *J* = 10.7, 3.9 Hz), 2.17 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H).

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine-*tert*-butylester (3):** Fmoc-L-threonine-*tert*-butylester (2.5 g, 6.3 mmol), Ag_2CO_3 (3.47 g, 12.6 mmol), and 4 Å molecular sieve powder (3.0 g) were stirred in CH_2Cl_2 and toluene (40 mL, 1:1) at 0 °C for 30 min. Then $AgClO_4$ (1.31 g, 6.3 mmol) was added and stirred for another 20 min. Subsequently, the solution of galactosyl bromide **2** (3.78 g, 9.6 mmol) in CH_2Cl_2 and toluene (20 mL, 1:1) was added slowly and stirred in the dark under argon at room temperature for 15 h. The mixture was diluted with CH_2Cl_2 (100 mL) and filtered over Celite. The filtrate was washed with water (100 mL) and saturated $NaHCO_3$ solution (100 mL). The organic layers were dried over Na_2SO_4. After filtration and evaporation, the residue was purified by column chromatography (*n*-hexanes/EtOAc, 2:1 → 1:1) to give the compound **3** (4.00 g, 89%) as a mixture of α- and β-anomer (5:1).

^{1}H-NMR (500 MHz, $CDCl_3$, major, α-anomer): δ 7.78-7.31 (m. 8H), 5.67 (d, 1H, *J* = 9.5 Hz), 5.49 (d, 1H, *J* = 2.4 Hz), 5.36 (dd, 1H, *J* = 11.2, 3.2 Hz), 5.12 (d, 1H, *J* = 3.6 Hz), 4.51-4.26 (m, 6H), 4.13-4.11 (m, 2H), 3.66 (dd, 1H, *J* = 11.2, 3.6 Hz), 2.16 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.53 (s, 9H), 1.37 (d, 3H, *J* = 6. 5 Hz).

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonine-*tert*-butylester (4):** Fmoc-(Ac_3GalN_3) Thr-O*t*Bu **3** (7.90 g, 11.1 mmol) was dissolved in a mixture of thioacetic acid and dry pyridine (30 mL, 2:1). The mixture was stirred at room temperature for 16h and then the solution was concentrated in vacuo. The residue was purified by column chromatography (*n*-hexanes/acetone, 2:1 → 1:1) to give the compound **4** (6.52 g, 81%) as a white solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.80-7.34 (m, 8H), 5.95 (d, 1H, *J* = 9.8 Hz), 5.49 (d, 1H, *J* = 9.4 Hz), 5.41 (m, 1H), 5.11 (dd, 1H, *J* = 11.3, 2.5 Hz), 4.90 (d, 1H, *J* = 3.5 Hz), 4.64 (m, 1H), 4.42-4.50 (m, 2H), 4.28-4.07 (m, 6H), 2.18 (s, 3H), 2.06 (s, 3H), 2.01 (s, 6H), 1.48 (s, 9H), 1.34 (d, 3H, *J* = 6.2 Hz).

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonine (5):** *tert*-Butylester **4** (2.90 g, 3.99 mmol) was dissolved in a mixture of TFA and H_2O (30 mL, 95:5) and stirred at room temperature for 2 h. The solution was evaporated azeotropically with toluene. The residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:1 +0.5% acetic acid) to give the compound **5** (2.43 g, 91%) as a white solid.

^{1}H-NMR (500 MHz, CD_3OD): δ 7.82-7.31 (m, 8H), 5.40 (d, 1H, *J* = 2.5 Hz), 5.07 (dd, 1H, *J* = 11.5, 3.1 Hz), 4.94 (d,

1H, J = 3.6 Hz), 4.59 (dd, 1H, J = 10.8, 6.5 Hz), 4.47 (dd, 1H, J = 10.8, 6.2 Hz), 4.38 (m, 2H), 4.28 (m, 3H), 4.11 (m, 2H), 2.14 (s, 3H), 2.03 (s, 3H), 1.944 (s, 3H), 1.94 (s, 3H), 1.24 (d, 3H, J = 6.5 Hz); MS (MALDI-TOF) m/z = 764.3 $[M + Na]^+$, 780.3 $[M + K]^+$.

Peptide Synthesis and Purification. Resin loaded with H-Val-substituted Wang resin (0.51 mmol/g) was used as the support. The coupling of Fmoc-amino acids was performed with an equimolar mixture of *O*-Benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBT) and *N*,*N*-diisopropylethylamine (DIEA) as the coupling reagents. The side chain protecting group of amino acids were as follows: Asn, Cys, Gln and His, trityl; Tyr, *tert*-butyl; Arg, 2,2,5,7-pentamethylchroman-6-sulfonyl (Pmc); Glu, *tert*-butoxy. After coupling the last amino acid, the Fmoc-group was removed with 20% piperidine/DMF, and the protected side chains and peptide-resin were deprotected and cleaved with a mixture of TFA-based reagent [trifluoroacetic acid-triisopropylsilane-water (TFA-TIS-H_2O; 95:2.5:2.5)] for 3 h at room temperature, and then precipitated with diethyl ether, and dried in the vacuum. The crude peptides were dissolved in 10% acetic acid and purified by a reversed-phase high-performance liquid chromatography (RP-HPLC) on a preparative (10 × 250 mm) C_{18} Bondapak column using a water-acetonitrile gradient [30-70%/30 min] containing 0.05% trifluoroacetic acid (TFA).

Purity of the purified peptides was checked by a RP-HPLC on an analytical (10 μm, 4.6 × 150 mm) C_{18} Pepmap column using a water-acetonitrile gradient [30-70%/30 min] containing 0.05% trifluoroacetic acid (TFA).

For deacetylation of the carbohydrate protecting group, the peptides were dissolved in 0.1 M NaOH and shaked mixtures for 1 min at room temperature. After complete deprotection, the solution was neutralized with acetic acid and concentrated in vacuo. The peptides were purified by RP-HPLC and identified by MALDI-TOF-Mass analysis.

Antimicrobial Assays (Radial diffusion assay). A radial diffusion assay was used to verify the antimicrobial activity of drosicin and its derivatives. *E. coli* were grown overnight for 18 h at 37 °C in 10 mL of LB broth and then 10 μL of this culture was inoculated into 10 mL of fresh LB and incubated for an additional 3 h at 37 °C to obtain mid-logarithmic phase organisms. The bacteria suspension (2 × 10^6 CFU/mL in LB) was mixed with 0.7% agarose. The mixture was poured into a 10 cm petri dish after rapidly dispersing. A 10-μL samples of the peptides at concentrations of 25, 50, and 100 μM were placed in each circle paper (6mm in diameter) put on the agarose plates and then incubated at 37 °C for overnight. The diameters of the bacterial clearance zones surrounding the circle paper was measured for the antimicrobial activities.

Circular Dichroism (CD) Spectroscopy. The circular dichroism (CD) spectra of the peptides were recorded at 25 °C using a Jasco J-715 CD spectrophotometer (Tokyo, Japan). The samples were scanned at room temperature in a capped quartz cuvette (1-mm path length) cells in the wavelength range of 190-250 nm. The spectra were recorded at a peptide concentration of 100 μg/mL in 10 mM sodium phosphate buffer (pH 7.4), 90% (v/v) trifluoroethanol (TFE) or 30 mM sodium dodecyl sulfate (SDS) micelles. The mean residue ellipticity, [θ], was given in deg·cm^2·dmol^{-1}: [θ] = [θ]obs (MRW/10 × l × c), where: [θ]obs is the ellipticity measured in millidegrees, MRW is the mean residue molecular weight of the peptide, c is the concentration of the sample in mg/mL, and l is the optical path length of the cell in cm. The spectra were expressed as molar ellipticity [θ] v*s.* wavelength.

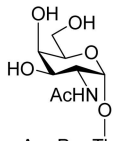
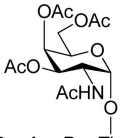
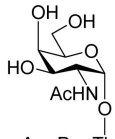
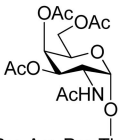
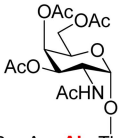
Results and Discussions

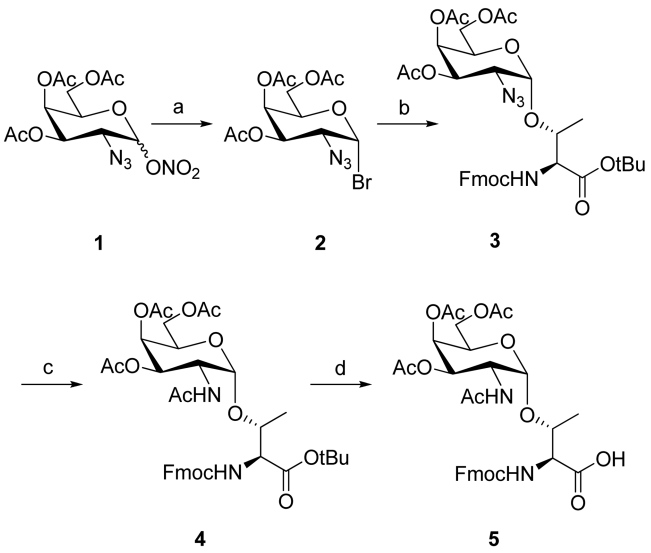
The preparation of glyco-peptides shown in Table 1, was accomplished using solid phase peptide synthesis (SPPS) techniques using the appropriated Fmoc-amino acid and Fmoc-glyco-amino acid. Scheme 1 shows a typical synthesis of GalNAc-Thr building block **5**.[12] Briefly, 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-desoxylgalactopyranosyl nitrate **1** was synthesized using a method similar to the reported by winans *et al.*.[13] The azidonitrate was converted directly into the labile galactosyl bromide **2** with LiBr in CH_3CN in 92% yield. The key glycosylation of Fmoc-threonine-*Ot*Bu by galactosyl donor **2** was performed using the Ag_2CO_3 and the two anomers (α,β-anomers) were separated by column chromatography to yield α-glycoamino acid ester **3**. Reductive acetylation of the azide group was carried out with thioacetic acid to afford the intermediate **4**, which upon deprotection of the *t*Bu group provided the Fmoc-protected building group **5** in good yield. Solid phase peptide synthesis of glycopeptides D-D4 (Table1) using building block **5** was carried out using an Fmoc/*t*Bu strategy. After completion of the assembly, the glycopeptides were cleaved from the resin using a standard cleavage cocktail [trifluoroacetic acid-triisopropylsilane-water (TFA-TIS-H_2O; 95:2.5:2.5)]. The glycosylated peptides were purified by preparative reverse phase HPLC both before and after the cleavage of the glycoside acetyl protection group. The glycopeptides were characterized by MALDI-TOF Mass.

First, our study focused on the effect of hydroxyl group of carbohydrate moiety into antimicrobial activity, because there are significant number of NOE contacts between the sugar moiety and the peptide near glycosylation site.[9] Our SAR study started with the assessment of the antimicrobial activity of the native drosocin D. The antimicrobial activities are summarized in Figure 1(a) and 1(b). The tests showed a marked increase of the antimicrobial activity for D1 (tri-*O*-acetylated drosocin), compare to native one D. This was unexpected in view of the importance of H-bonding between sugar and peptide backbone. At present, we do not have a rational explanation of this unexpected result. But, the reported observation of NOEs between the amide and sugar protons cannot rule out the possibility of strong interaction of backbone peptide with the carbonyl on tri-*O*-acetylated drosocin D1.

Second, a major hurdle in the development of drosocin-

Table 1. Amino acid sequence of native drosocin monosaccharide (D) and its derivatives (D1-D4)

Name	Sequence	M.W
D	 Gly-Lys-Pro-Arg-Pro-**Tyr-Ser**-Pro-Arg-Pro-Thr-Ser-His-Pro-Arg-Pro-Ile-Arg-Val	2402
D1	 Gly-Lys-Pro-Arg-Pro-**Tyr-Ser**-Pro-Arg-Pro-Thr-Ser-His-Pro-Arg-Pro-Ile-Arg-Val	2527
D2	 Gly-Lys-Pro-Arg-Pro-**Tyr-Thr**-Pro-Arg-Pro-Thr-Ser-His-Pro-Arg-Pro-Ile-Arg-Val	2416
D3	 Gly-Lys-Pro-Arg-Pro-**Tyr-Thr**-Pro-Arg-Pro-Thr-Ser-His-Pro-Arg-Pro-Ile-Arg-Val	2541
D4	 Gly-Lys-Pro-Arg-Pro-**Tyr-Thr**-Pro-Arg-**Ala**-Thr-Ser-His-Pro-Arg-Pro-Ile-Arg-Val	2515



Reagents and conditions: (a) LiBr, CH_3CN (b) Fmoc-Thr-OtBu, Ag_2CO_3, $AgClO_4$, CH_2Cl_2/toluene (c) AcSH/pyridine (d) TFA/H_2O (95:5)

Scheme 1. The synthesis of building block **5**.

based antimicrobial agents is the instability of drosocin towards proteolytic stability present in mammalian sera. Visser *et al.* group[7] reported that the simple substitution of Ser^7 with Thr^7 that is susceptible to proteolytic cleavage (Tyr^6-Ser^7), results in drosocin analogue with remarkably enhanced stability with slight increase in the antimicrobial activity. However, this result came from non-glycosylated linear peptide in order to simplify the synthetic procedure. We synthesized the drosocin analogue D2 having *O*-GalNAc at Thr^{11} residue and substituted Ser^7 with Thr^7. In contrast to the non-glycosylated drosocin analogues,[7] our assay results (Figure 1) tell us that the drosocin analogue, D2 showed the slight decrease in activity compared to native, D. But the most promising way is to find the highly active drosocin analogues having Thr^7, that would eventually initiate the further studies in designing novel drosoncin antimicrobial peptides.

Next, based on the above experimental results, we also synthesized the drosocin analogue D3 which has tri-acetylated *O*-GalNAc at Thr^{11} and $Ser^7 \rightarrow Thr^7$ replacement in an effort to increase the antimicrobial activity as observed in D1. Interestingly, acetylation of free hydroxyl group on

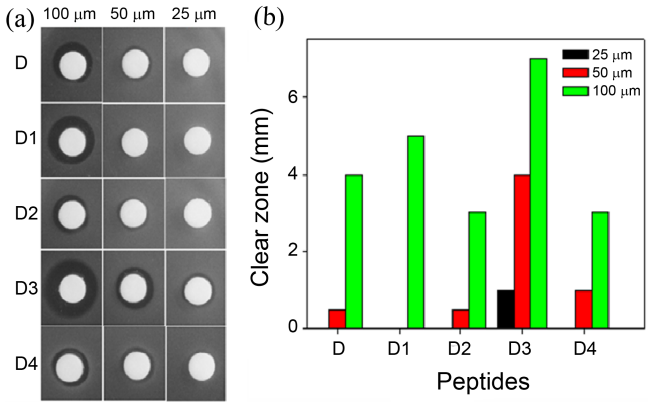


Figure 1. (a) Antimicrobial activity of the peptides determined by radial diffusion assay. *Escherichia coli* (2×10^6 CFU/mL) was incorporated into LB agarose medium. Antimicrobial activity was tested by placing 10-μL peptide samples in each circle paper put on the agarose plates, incubating them for overnight to permit the peptides to diffuse into the agarose. Clear circles show the inhibition of bacterial growth. *E. coli* (KCTC 1682) was supplied from the Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology (KRIBB). (B) The diameters of the clear zones measured after subtracting the diameter of the circle paper (6 mm). (b) Antimicrobial activity of the peptides against *Escherichia coli* in a radial diffusion assay. The diameters of the clear zones were measured after subtracting the diameter of the circle paper (6 mm).

drosocin analogue D2, strongly improved the antimicrobial activity as shown in the Figure 1.

As mentioned earlier, drosocin belongs to the proline-rich family of insect antimicrobial peptides. The current study revealed that the PRP sequence is precede regions of local folded structure.[9] Prolines are required at these sites, not only on the basis of side chain shape but simply because they are the only naturally available *N*-substituted residue. This unusual recognition code has been used to guide design of novel antimicrobial glycopeptides. Thus, the drosocin derivative D4 was synthesized by substituting Pro^{10} with Ala^{10} on D3 and investigated the influence of the antimicrobial activity through disrupting the critical PRP unit in the middle of drosocin sequence. Our result (Figure 1) clearly showed that the Pro^{10} strongly influences the antimicrobial activity as evident from the activity of D4 lower than our most active analogue D3.

We hypothesize that the replacement with the Ala destroys the "glyco-turns" which in turn aids for the correct orientation of drosocin to bind the target membrane receptor. To the best of our knowledge, this is the first report that shows the importance of acetylated derivative and also the presence of Pro^{10} in drosocin analogues, strongly influence the antimicrobial activity.

From the evaluation of the activity discussed above, we concluded that the conformation of the glycopeptides drosocin is a key factor for antimicrobial activity. Therefore, to study the relationship between the activity and the conformation, CD spectra of the synthetic drosocin analogues, D-D4 were measured in sodium phosphate buffer (pH 7.4), 2,2,2-trifluoroethanol (TFE) and in sodium dodecyl sulfate (30 mM SDS) micelles. The CD spectra of drosocin and its analogues are shown in Figure 2. As shown in Figure 2(a) for drosocin D and its analogues (D2-D4), the CD spectrum in sodium phosphate buffer (pH 7.4) was characterized by a broad negative band around 200 nm, characteristic of unordered structure. In 90% TFE (Figure 2(b)), all the glycopeptides exhibited a red-shifting and a broadening of the negative band. This CD spectral pattern can be correlated to the increase of β-turn percentage in the conformational mixture. These findings are in agreement with previous CD study which found that in buffer glycopeptide has no conformational preference but, in 90% TFE, CD spectra of the glycopeptide was consistent with the presence of β-turn.[4,14] The SDS micelle has been used as a model of the negatively charged bacterial lipid membranes, with which cationic glycopeptides first interact. In the SDS condition (Figure 2(c)), all glycopeptides showed the very similar CD spectra that were characterized by a negative band ~205 nm, indicating electrostatic glycopeptides/micelles interaction without effect on the conformation. Since, CD spectroscopy is mainly used to detect the overall secondary structure content, as expected subtle conformational changes may not be detected in our CD spectra.

Conclusion

In summary, we have been described the synthesis, structural features, and antimicrobial activities of drosocin and its analogues linked *O*-GalNAc at Thr^{11} residue. Two of our drosocin analogues in particular, D1 and D3, showed

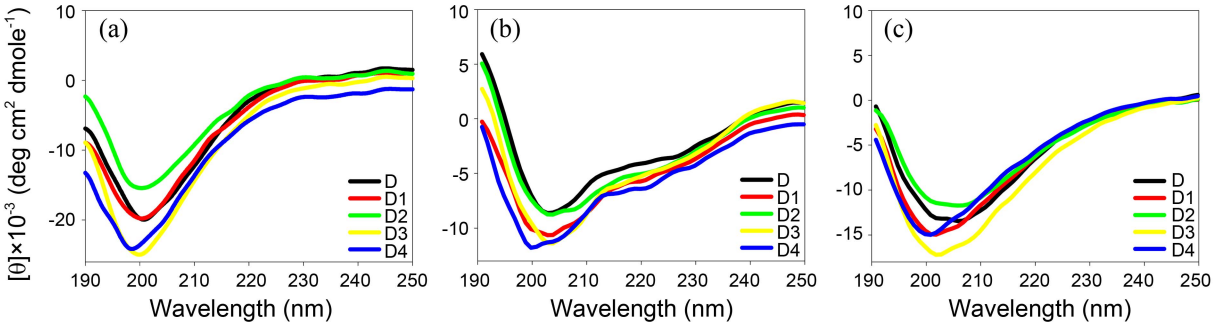


Figure 2. CD spectra of drosocin and its derivatives (a) in sodium phosphate buffer (pH 7.4), (b) 90% TFE, (c) 30 mM SDS.

good antimicrobial activity. These results are the first indication that the acetylation of carbohydrate moiety increased the antimicrobial activity compared to native one. Though, one of our drosocin analogues, D2 showed the decreased in the antimicrobial activity on replacing Ser^7 with Thr^7, We successfully designed the D3 as the most potent glycopeptide compare to the native one. Additionally, our data showed that Pro^{10}, present in the middle of drosocin sequence plays an important role in the antimicrobial activity.

The present results provide a good lead compound for further studies on the design of drosocin-based analogues targeting glyco linked Thr site, utilizing both the retained antimicrobial potency and the increased metabolic stability.

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