# Studies on the β-Turn of Peptides. VIII.[1] β-Turn Preferences of *N*-Acetyltetrapeptide Methylamides Related to the β-Turn Part of Gramicidin S

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*N*-Acetyl-L-Leu–X–L-Pro–L-Val methylamides (**IIa**: X=D-Ala, **IIb**: X=Gly, **IIc**: X=L-Ala) were synthesized and subjected to CD and $^1$H NMR measurements to study the β-turn preferences of these peptides. Temperature dependences of L-Val$^4$–NH proton chemical shifts in $CD_3OH$ solution were in the order of **IIa**<**IIb**<**IIc**, suggesting that the populations of the β-turn conformer with 4→1 hydrogen bonding were in the order of **IIa**>**IIb**>**IIc**. This order of β-turn preferences coincides with the order of the magnitudes of the Cotton effects in the CD spectra of the chromophoric derivatives, *N*-(2,4-dinitrophenyl)tetrapeptide *p*-nitroanilides, having the same sequences. This agreement supports the validity of the chiroptical method to study turn tendency of tetrapeptide sequences using chromophoric derivatives reported previously.

CD spectra of *N*-(2,4-dinitrophenyl)tetrapeptide *p*-nitroanilides (Dnp–tetrapeptide–pNA's)[2] exhibit characteristic Cotton effects above 250 nm when they take β-turn conformations. Exciton coupling of the electric transition moments of the two chromophores were assumed to explain the spectra, and the magnitudes of the Cotton effects near 310 and 350 nm were shown to reflect well the β-turn preference of the derivatives.[3] In the case of model peptides (Dnp–L-Leu–X–L-Pro–L-Val–pNA; **Ia**: X=D-Ala, **Ib**: X=Gly, **Ic**: X=L-Ala) related to the β-turn part of gramicidin S (GS), β-turn preferences of the tetrapeptide derivatives (**Ia**>**Ib**>**Ic** ≈0) had strong correlation with antibiotic activities of the GS analogs having the same tetrapeptide sequences at their β-turn parts.[3-5]

A question was raised, however, whether such chiroptical method using chromophoric derivatives as mentioned above reveals the conformational characteristics of the original peptide sequences lacking those chromophoric groups. To answer the question we attempted syntheses and conformational analyses of some *N*-acetyltetrapeptide methylamides (Ac–L-Leu–X–L-Pro–L-Val–NHMe; **IIa**: X=D-Ala, **IIb**: X=Gly, **IIc**: X=L-Ala) related to the β-turn part of GS, and the results were compared with those of Dnp–tetrapeptide–pNA (**Ia**—**c**) having the same tetrapeptide sequences.

## Results and Discussion

*Syntheses of Peptides (IIa—c).* *N*-Acetyltetrapeptide methylamides (**IIa**—**c**) were synthesized by usual solution-phase procedures according to the scheme shown in Fig. 1. Boc–D-Ala–L-Pro–OH[6] and H–L-Val–NHMe were coupled by EDC method to afford Boc–D-Ala–L-Pro–L-Val–NHMe (**IIIa**), which was treated with hydrogen chloride in formic acid. H–D-Ala–L-Pro–L-Val–NHMe·HCl (**IVa**·HCl) obtained was coupled with Boc–L-Leu–ONSu to afford Boc–L-Leu–D-Ala–L-Pro–L-Val–NHMe (**Va**), which was converted to Ac–L-Leu–D-Ala–L-Pro–L-Val–NHMe (**IIa**) by deprotection and subsequent treatment with Ac–ONp. Compounds **IIb** and **IIc** were prepared by a similar manner to that described for the synthesis of **IIa**. Purities of the synthetic peptides were confirmed by TLC with three solvent systems, elemental analyses, amino acid analyses, and also 270 MHz $^1$H NMR.

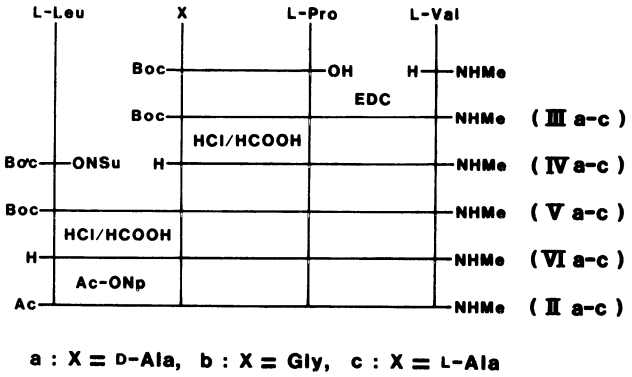


Fig. 1. Synthetic scheme of *N*-acetyltetrapeptide methylamides (**IIa**—**c**).

*CD Measurements.* CD spectra of **IIa**—**c** were measured in $H_2O$ and in $CH_3OH$ solutions (Fig. 2). In $H_2O$ solution, all the CD spectra showed a minimum near 200 nm (Fig. 2A), suggesting that all the Ac–tetrapeptide–NHMe were in random conformations. Certainly, the magnitudes of Cotton effects (**IIa** : **IIb** : **IIc**=2 : 3 : 4) can be explained by difference in the configurations of component amino acid residues. On the other hand, in $CH_3OH$ solution (Fig. 2B), the magnitudes of the Cotton effects were not so dependent on the configuration of second amino acid residues, and the CD minima were shifted to longer wavelength (**IIa**: 204 nm, **IIb**: 202.5 nm, **IIc**: 201 nm). It is of interest to note that the order of CD minima positions of Ac–tetrapeptide–NHMe (**IIa**—**c**) in $CH_3OH$ solution was the same as that of Dnp–tetrapeptide–pNA near 200 nm (**Ia**: 205 nm, **Ib**: 201.5 nm, **Ic**: 200 nm) in $CH_3OH$ solution (Fig. 2C).

*$^1$H NMR Measurements.* $^1$H NMR (270 MHz) of **IIa**—**c** was measured in $H_2O$ and in $CD_3OH$ solutions. All the spectra showed two sets of resonances for most of the protons due to the cis-trans isomerism for the X–L-Pro bonds. The minor set of resonances (<10%) was assigned to the cis isomer from the results of related compounds (**Ia**—**c**).[5] Detailed discussions are described

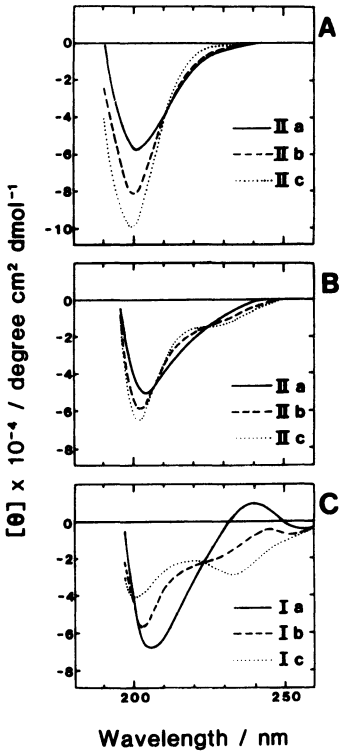


Fig. 2. CD spectra of **IIa**—**c** in $H_2O$ (A) and in $CH_3OH$ (B) solutions and of **Ia**—**c** in $CH_3OH$ (C) solutions.

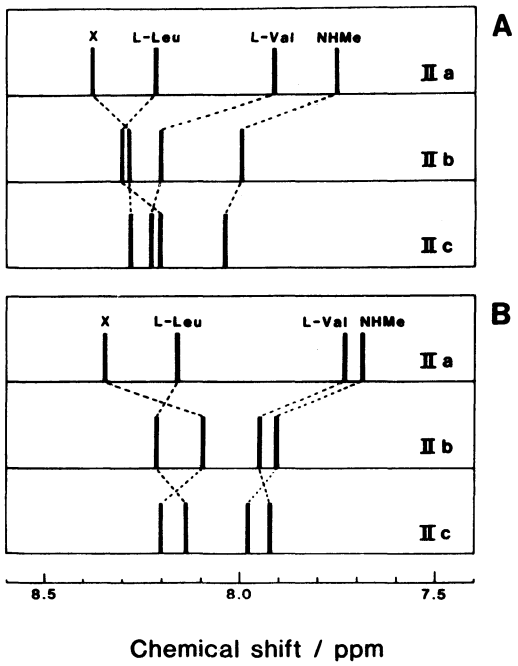


Fig. 3. Chemical shifts of amide protons in major conformer of Ac-L-Leu-X-L-Pro-L-Val-NHMe (**IIa**—**c**) in $H_2O$ (A) and in $CD_3OH$ (B) solutions.

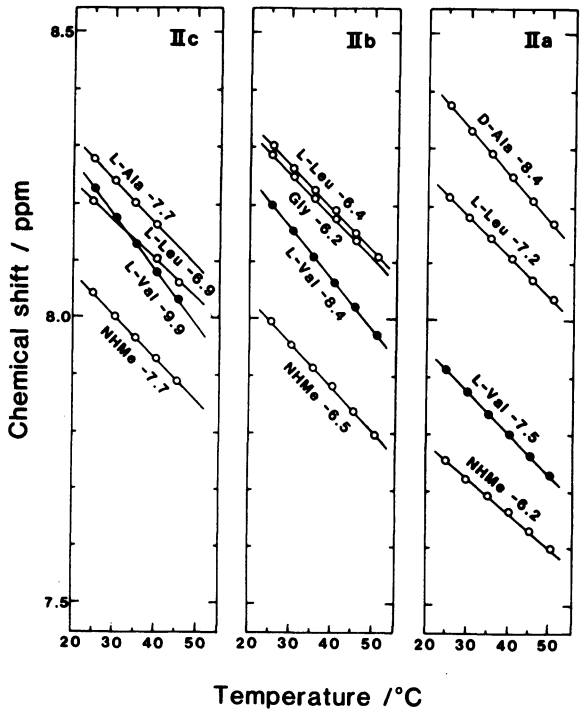


Fig. 4. Temperature dependences of amide proton chemical shifts of major conformers of **IIa**—**c** in $H_2O$ solutions. Numbers on the lines refer to $10^3 \times$ temperature coefficient in ppm $°C^{-1}$.

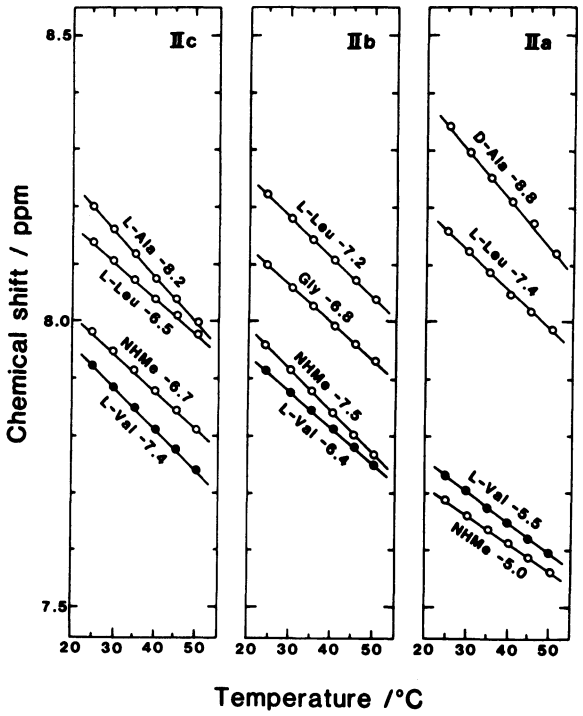


Fig. 5. Temperature dependences of amide proton chemical shifts of major conformers of **IIa**—**c** in $CD_3OH$ solutions. Numbers on the lines refer to $10^3 \times$ temperature coefficient in ppm $°C^{-1}$.

hereafter for the major (trans) conformers.

Chemical shifts of NH protons of **IIa** spread over a wider range as compared with those of **IIc** which showed ordinary values (Fig. 3). The D-Ala residue of **IIa** showed smaller spin-coupling constant ($^3J_{NH-C_\alpha H}$=5.15 Hz) than the other residues (6—8 Hz) in $CD_3OH$ solution, suggesting that the $\phi$ angle of the D-Ala residue was fixed to a certain extent in $CD_3OH$ solution, whereas the L-Ala residue of **IIc** showed such spin-coupling constant (6.25 Hz) as to be typical to free rotation.

These results suggest that **IIa** has some ordered conformation while **IIc** has unordered one.

The temperature dependences of chemical shifts of amide proton resonances are useful for distinguishing between "exposed" and "intramolecularly hydrogen-bonded" amide proton;[7] exposed protons exhibit larger temperature coefficients than hydrogen-bonded amide protons. All the NH proton resonances of **IIa—c** showed large temperature dependence of chemical shifts in $H_2O$ solution (Fig. 4). In $CD_3OH$ solution (Fig. 5), however, L-Val–NH proton of **IIa** showed a little smaller temperature dependence ($-5.5\times10^{-3}$ ppm $°C^{-1}$) than the other NH protons except for NHMe proton, suggesting that the L-Val–NH proton of **IIa** was involved in a weak hydrogen bonding. The fixation of $\phi$ angle of D-Ala residue of **IIa** suggests that the folded conformation of **IIa** in $CD_3OH$ solution is β-turn conformation with 4→1 hydrogen bonding (L-Leu[1]–CO ···HN–L-Val[4]). The L-Val–NH proton of **IIb** also showed smaller temperature dependence than the other NH protons, but the value ($-6.4\times10^{-3}$ ppm $°C^{-1}$) was larger than that of **IIa**. In the case of **IIc**, the L-Val–NH proton showed large temperature dependence ($-7.4\times10^{-3}$ ppm $°C^{-1}$). The results of temperature dependences in $CD_3OH$ solutions suggest that the populations of β-turn conformer with 4→1 hydrogen bond concerned by the L-Val–NH group are in the order of **IIa**>**IIb**>**IIc**≈0, although at a quite lower levels than that of Dnp–tetrapeptide–pNA (**Ia—c**); for example, the L-Val–NH proton of **Ia** showed such a small temperature dependence as $-2.72\times10^{-3}$ ppm $°C^{-1}$ in $CD_3OH$ solution.[3,5] The small temperature dependence shown by the NHMe proton chemical shift of **IIa** in $CD_3OH$ solution suggests the coexistence of another β-turn conformation having L-Pro–L-Val sequence at the internal position, since L-Pro residue has high bend positional potential at the second $(i+1)$ position of β-turn in the table proposed by Chou and Fasman on the basis of statistical analysis of the X-ray crystallographic data of 29 globular proteins.[8]

*β-Turn Preference of Tetrapeptides.* CD and NMR spectra of Ac–tetrapeptide–NHMe (**IIa—c**) did not show such large sequence dependences as those found in Dnp–tetrapeptide–pNA (**Ia—c**).[3,5] However, **IIa** and **IIb** are considered to have some populations of β-turn conformer in $CH_3OH$ solution although they seem to take random conformations in $H_2O$ solution. In addition, the order of population of β-turn conformer (**IIa**>**IIb**>**IIc**≈0) was the same as that of Dnp–tetrapeptide–pNA (**Ia—c**). These agreements suggest that introduction of Dnp and pNA groups amplify the intrinsic β-turn preference of parent tetrapeptide without affecting the order of β-turn preferences by stabilization of the β-turn conformer due to the attractive interaction of the two terminal chromophores. Consequently, CD spectra of Dnp–tetrapeptide–pNA are considered to be available for the study of β-turn preferences of the tetrapeptide sequences. Conformational energy calculation of *N*-acetyldi- or tetrapeptide methylamides also supported the validity of the chiroptical method.[9]

## Experimental

*Syntheses of Peptides.* All the melting points were measured on a Yanagimoto micro melting point apparatus and uncorrected. TLC's were carried out on Merck silica gel 60 $F_{254}$ plates with the following solvent systems: $R_f^1$, $CHCl_3$–$CH_3OH$ (5 : 1, v/v); $R_f^2$, $CHCl_3$–$CH_3OH$–AcOH (95 : 5 : 1, v/v); $R_f^3$, *n*-BuOH–AcOH–pyridine–$H_2O$ (4 : 1 : 1 : 2, v/v). Optical rotations were measured on an Union automatic polarimeter PM-201. Amino acid analyses of peptides were performed by Durrum D-500 amino acid analyzer after hydrolysis in 6 M (1 M=1 mol $dm^{-3}$) HCl at 105 °C for 20 h. Yields and analytical data of synthetic peptides are summarized in Table 1.

*Boc-D-Ala-L-Pro-L-Val-NHMe (IIIa).* To a chilled solution of Boc-D-Ala-L-Pro-OH·DCHA (1.40 g, 3 mmol) and H-L-Val-NHMe·HCl (0.50 g, 3 mmol) in $CHCl_3$ (12 ml)

Table 1. Yields and analytical data of synthetic peptides

| Compound | Yield/% | Mp $\theta_m$/°C | $[\alpha]_D^{23}$[a]/° | Found(Calcd) (%) C | H | N | $R_f^1$ | $R_f^2$ | $R_f^3$ | Amino acid ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| **IIIa** | 81 | 84—89 | −47.6 | 55.96 | 8.32 | 13.75 | 0.59 | 0.25 | 0.74 | Ala:Pro:Val |
| | | | | (56.00 | 8.66 | 13.75)[b] | | | | 1.01:1.04:1.00 |
| **IIIb** | 73 | 135—140 | −85.2 | 55.64 | 8.40 | 14.25 | 0.57 | 0.20 | 0.71 | Gly:Pro:Val |
| | | | | (55.58 | 8.42 | 14.40)[c] | | | | 1.00:1.02:1.00 |
| **IIIc** | 80 | 181—182 | −128.8 | 57.28 | 8.72 | 13.91 | 0.60 | 0.24 | 0.73 | Ala:Pro:Val |
| | | | | (57.27 | 8.60 | 14.06) | | | | 1.00:1.04:1.00 |
| **Va** | 74 | 160—161 | −52.4 | 58.78 | 8.93 | 13.60 | 0.61 | 0.23 | 0.75 | Leu:Ala:Pro:Val |
| | | | | (58.69 | 8.87 | 13.69) | | | | 0.98:1.00:1.04:1.00 |
| **Vb** | 69 | 163—166 | −100.8 | 57.39 | 8.55 | 13.97 | 0.55 | 0.18 | 0.74 | Leu:Gly:Pro:Val |
| | | | | (57.41 | 8.73 | 13.95)[c] | | | | 0.99:1.00:1.03:1.00 |
| **Vc** | 68 | 194—195 | −132.0 | 57.92 | 8.46 | 13.57 | 0.59 | 0.23 | 0.76 | Leu:Ala:Pro:Val |
| | | | | (58.17 | 8.89 | 13.57)[c] | | | | 0.98:1.00:1.04:1.00 |
| **IIa** | 82 | 193—194 | −83.4 | 58.32 | 8.86 | 14.86 | 0.50 | 0.10 | 0.70 | Leu:Ala:Pro:Val |
| | | | | (58.26 | 8.67 | 15.44) | | | | 0.99:1.00:1.04:1.00 |
| **IIb** | 53 | 256—257 | −126.6 | 57.21 | 8.43 | 15.86 | 0.40 | 0.06 | 0.71 | Leu:Gly:Pro:Val |
| | | | | (57.38 | 8.48 | 15.93) | | | | 0.99:1.00:1.04:1.00 |
| **IIc** | 55 | 255—257 | −149.8 | 58.54 | 8.76 | 15.60 | 0.47 | 0.10 | 0.70 | Leu:Ala:Pro:Val |
| | | | | (58.26 | 8.67 | 15.44) | | | | 0.99:1.00:1.05:1.00 |

a) *c* 1, $CH_3OH$. b) 1/2 $H_2O$. c) 1/4 $H_2O$.

was added EDC·HCl (0.58 g, 3 mmol), and the reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. Insoluble materials (DCHA·HCl) were removed by filtration and the filtrate was evaporated under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed successively with 10% citric acid, 4% $NaHCO_3$, and $H_2O$, dried ($Na_2SO_4$), and evaporated. Addition of ether and petroleum ether to the residue gave crystals, which were recrystallized from EtOAc–ether–petroleum ether; yield, 986 mg (81%).

Compounds **IIIb** and **IIIc** were prepared by the similar manner to that described for **IIIa**.

*H–D-Ala–L-Pro–L-Val–NHMe·HCl (**IVa**·HCl).* Compound **IIIa** (398 mg, 1 mmol) was dissolved in 0.1 M hydrogen chloride in formic acid (12 ml). The soultion was allowed to stand for 30 min at room temperature and evaporated. Addition of ether to the residue gave highly hygroscopic crystals, which were used for the next reaction without further treatment; yield, 335 mg (100%); $R_f^1$ 0.06, $R_f^3$ 0.59.

Compounds **IVb**, **c** and **VIa—c** were prepared by a similar manner to that described for **IVa**: **IVb**; $R_f^1$ 0, $R_f^3$ 0.54: **IVc**; $R_f^1$ 0.05, $R_f^3$ 0.53: **VIa**; $R_f^1$ 0.23, $R_f^3$ 0.71: **VIb**; $R_f^1$ 0.10, $R_f^3$ 0.65: **VIc**; $R_f^1$ 0.21, $R_f^3$ 0.70.

*Boc–L-Leu–D-Ala–L-Pro–L-Val–NHMe (**Va**).* To a chilled solution of **IVa**·HCl (335 mg, 1 mmol) and TEA (0.14 ml, 1 mmol) in DMF (10 ml) was added Boc–L-Leu–ONSu (327 mg, 1.2 mmol), and the reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature. A few drops of 1-(2-aminoethyl)piperazine were added to the solution, which was evaporated after being stirred further for 30 min at room temperature. The residue was dissolved in EtOAc and the solution was treated as described for **IIIa**. The crude product was dissolved in $CH_3OH$ (3 ml) and applied to a column (3 cm × 170 cm) of Sephadex LH-20 and eluted with $CH_3OH$. The fractions containing the desired product detected by UV absorption and TLC were collected and evaporated, and the residue was crystallized by additions of ether and petroleum ether and recrystallized from EtOAc–ether–petroleum ether; yield, 379 mg (74%).

Compounds **Vb** and **Vc** were prepared by the similar manner to that described for **Va**.

*Ac–L-Leu–D-Ala–L-Pro–L-Val–NHMe (**IIa**).* To a chilled solution of **VIa**·HCl (224 mg, 0.5 mmol) and TEA (0.11 ml, 0.75 mmol) in DMF (2 ml) was added Ac–ONp (109 mg, 0.6 mmol), and the reaction mixture was stirred for 1 h at 0 °C and overnight at room temperature and evaporated. The residue dissolved in a mixture (5 ml) of $CHCl_3$ and $CH_3OH$ (19 : 1, v/v), was applied to a column (1.8 cm × 30 cm) of silica gel 60 (Merck) and eluted with the same solvent. The fractions containing the desired product detected by TLC were collected and evaporated. The crude product was further purified by column chromatography with Sephadex LH-20 as described for **Va**. The product was recrystallized from $CH_3OH$–EtOAc; yield, 186 mg (82%).

Compounds **IIb** and **IIc** were prepared by the similar manner to that described for **IIa**.

*CD Measurements.* CD spectra were measured on a JASCO J-40A automatic recording spectropolarimeter in a quartz cell of 1-mm path length at the concentration of 0.2 mM at room temperature (23±2 °C).

*$^1H$ NMR Measurements.* $^1H$ NMR spectra were recorded on a Brucker WH-270 spectrometer (270 MHz for $^1H$), equipped with a Brucker B-ST-100/700 temperature control unit at the concentration of 5 mM at 23 °C unless otherwise mentioned. Chemical shifts were measured from the internal standard of DSS. The spectra were assigned by spin-decoupling, H–D exchange of NH protons and saturation transfer (between the two conformers).

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## References

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2) The abbreviations used in this paper are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations: Boc, *t*-butoxycarbonyl; Dnp, 2,4-dinitrophenyl; pNA, *p*-nitroanilide; ONSu, *N*-hydroxysuccinimide ester; ONp, *p*-nitrophenyl ester; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DCHA, dicyclohexylamine; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; TEA, triethylamine, DMF, *N,N*-dimethylformamide; TLC, thin-layer chromatography; GS, gramicidin S.

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