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Tetrahedron Letters 45 (2004) 5371–5373
Tetrahedron Letters

Structure and enantioselective synthesis of polyamine toxin MG30 from the venom of the spider *Macrothele gigas*

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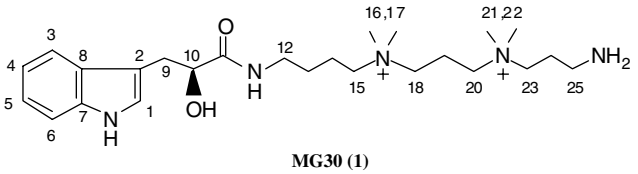
Received 13 April 2004; revised 12 May 2004; accepted 17 May 2004

Abstract—A novel polyamine toxin, named MG30, was isolated from the venom of the spider, *Macrothele gigas*, and its structure was elucidated by two-dimensional NMR and mass analysis. In addition, the enantioselective synthesis of MG30 was achieved to assign its absolute stereochemistry.

1. Introduction

Spider venom contains various kinds of compounds such as proteins, peptides and polyamine toxins. These toxins are of interest as tools for studying neurophysiology and pharmacology. Particularly, polyamine toxins have shown specific activity as glutamate receptor blockers.[1–3]

In the previous report, we described six peptide toxins (Magi 1–6), isolated from the venom of the Hexathelidae spider *Macrothele gigas* collected at Iriomote-Island in Japan.[4] We have also isolated an unknown polyamine compound, named MG30, as a major component of the same spider venom. We report here the structure determination of MG30 using two-dimensional NMR, MS analysis and the enantioselective synthesis of (*R*) and (*S*)-MG30 to assign the absolute configuration of the native MG30.



MG30 (1)

Keywords: Spider; Polyamine toxin; *Macrothele gigas*.
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doi:10.1016/j.tetlet.2004.05.076

2. Results

M. gigas venom was obtained by electrical stimulation of field-collected spiders from the Sonai area in Iriomote, Japan. The crude venom was frozen and stored at −20 °C until use.

The crude venom (70 μL) was resuspended in 0.1% aqueous trifluoroacetic acid (TFA) containing 10% acetonitrile, and the insoluble materials were removed by centrifugation at 14,000*g* for 5 min. The supernatant was purified by the established high-performance liquid chromatography method (C18 ϕ 10×250 mm, linear gradient with 0–60% acetonitrile/H_2O in 0.1% TFA in 60 min, flow rate 2.0 mL/min, UV detection at 220 nm). The polyamine, named MG30, was a major component of the venom to yield 150 μg.

The ^{1}H NMR spectrum of MG30 showed peculiar signals to an indole ring at the low-field region (H-1, 3, 4, 5 and 6). ^{1}H–^{1}H COSY, TOCSY and HMBC analysis of MG30 easily gave the structural information on an indole-3-lactyl part, and the HMBC peaks from H-12 (δ_H 3.05 and 3.17) to C-11 (δ_C 178.8) revealed that another unit connected to the indole-3-lactyl moiety through an amide linkage. ^{1}H–^{1}H COSY spectra led to the connectivities from C-12 to C-15, C-18 to C-20 and C-23 to C-25. This was also supported by TOCSY and HMBC data. The methyl protons of H-16, 17, 21 and 22 (δ_H 2.98 and 3.17) had cross-peaks with δ_C 53.2 or 53.3

Table 1. ^{13}C NMR and ^{1}H NMR spectral data of MG30 in D_2O

Position	δ_C	δ_H (mult., J in Hz)
1	127.6	7.30 (s)
2	111.9	
3	121.8	7.69 (d, 7.8)
4	122.1	7.19 (t, 7.4)
5	124.6	7.27 (t, 7.7)
6	114.5	7.52 (d, 7.8)
7	138.8	
8	130.3	
9	32.2	3.24, 3.29 (dd, 15.0, 5.5)
10	74.4	4.53 (t, 5.5)
11	178.8	
12	40.5	3.05, 3.17 (m)
13	27.9	1.23 (m)
14	21.7	1.37 (m)
15	67.1	3.15 (m)
16,17	53.2 or 53.3	2.98 (s)
18	62.9	3.22 (m)
19	19.3	2.25 (m)
20	63.6	3.37 (m)
21,22	53.2 or 53.3	3.17 (s)
23	65.0	3.46 (m)
24	24.8	2.12 (m)
25	39.5	2.97 (m)

methyl carbons on HSQC. These methyl carbons were assumed to attach to the positively charged nitrogen atoms from the chemical shift values. The methyl protons H-16 and H-17 (δ_H 2.98) showed HMBC correlations with C-15 and C-18 (δ_C 67.1 and 62.9), and H-21 and H-22 (δ_H 3.17) showed HMBC correlations with C-20 and C-23 (δ_C 63.6 and 65.0). These data showed that MG30 has two dimethyldialkyl ammonium salts in the main chain backbone (Table 1).[7]

Mass spectral data for MG30 are presented in Figure 1.[8] The molecular weight of 223.68 $[M]^{2+}$ was obtained by ESI-Q-Tof MS, and MS/MS fragmentation patterns clearly showed that MG30 was composed of a main chain with two dimethyldialkyl ammonium salts acylated by an indole-3-lactyl group. Combining NMR and MS data, the structure of MG30 was elucidated as shown in **1**.

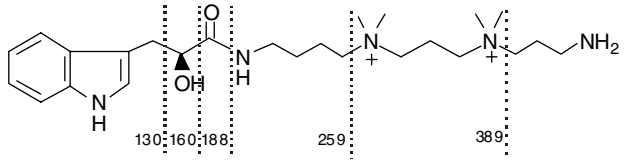
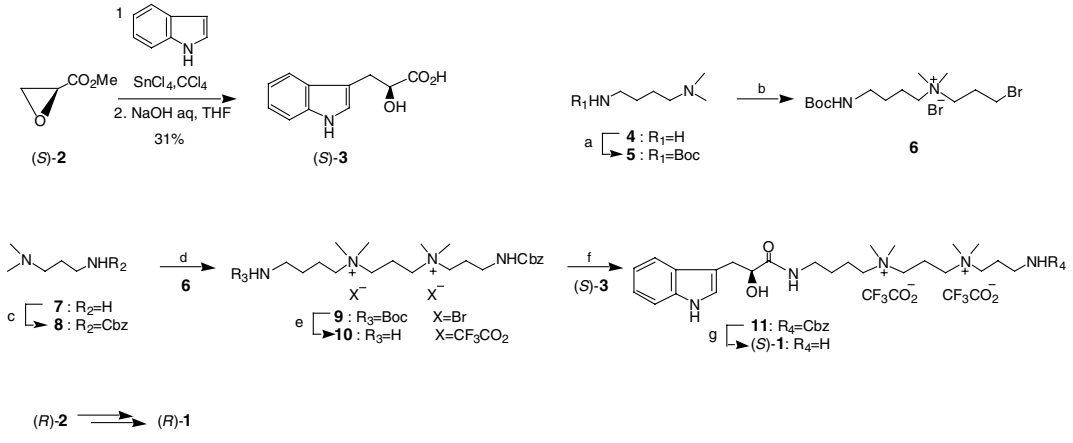


Figure 1. Fragmentation of MG30 by ESI-Q-TOF MS/MS.

In order to determine the absolute configuration of the secondary hydroxy group of MG30, both enantiomerically pure (*R*) and (*S*)-MG30 were synthesized in short steps as shown in Scheme 1. The preparation of the (*S*)-indole-3-lactic acid, (*S*)-**3**, was carried out by coupling of indole and methyl (2*S*)-glycidate with tin tetrachloride in carbon tetrachloride, followed by hydrolysis.[5] The monoprotected polyamine **10** having two quaternary ammonium salts was synthesized step-by-step. Protection of the amine **4** with di-*tert*-butyl dicarbonate (Boc_2O) and subsequent treatment of excess amounts of 1,3-dibromopropane gave the monoammonium salt **6**.[6] Coupling of **6** and **8**, which was prepared by protection of the amine **7** with pyrocarbonic acid dibenzyl ester (Cbz_2O), was performed by treatment with sodium bicarbonate in ethanol at 50 °C to give the bisammonium salt **9**. Subsequent removal of the Boc group with trifluoroacetic acid (TFA) furnished the mono-primary amine **10**. Coupling of **10** with (*S*)-**3** was accomplished by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide (DMF) at room temperature to give the protected MG30, (*S*)-**11**. Deprotection of (*S*)-**11** with thioanisole and trimethylsilyl trifluoromethanesulfonate in TFA gave the desired (*S*)-**1** ($[\alpha]_D$ +3.5 (*c* 0.43, H_2O)). (*R*)-**1** ($[\alpha]_D$ −3.0 (*c* 0.58, H_2O)) was obtained by using (*R*)-**3** instead of (*S*)-**3**.

The CD spectra of the two synthetic enantiomers and the native polyamine are shown in Figure 2.[9] From these spectra, the absolute stereostructure of the native polyamine is (*S*)-**1**.



Scheme 1. Reagents and conditions: (a) Boc_2O, THF, rt (99%); (b) dibromopropane, rt (74%); (c) Cbz_2O, THF, 60 °C (54%); (d) $NaHCO_3$, EtOH, 50 °C; (e) TFA, CH_2Cl_2, rt (2 steps 58%); (f) EDCI, HOBt, DMF, rt; (g) thioanisole, TMSOTf, TFA, 0 °C (2 steps 31%).

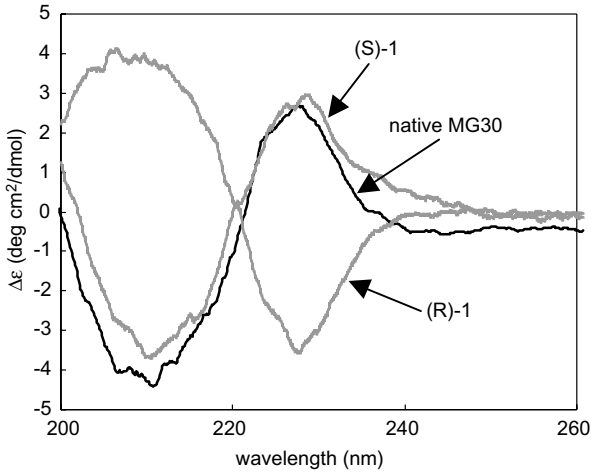


Figure 2. CD spectrum of native MG30, (*R*)-**1** and (*S*)-**1** in H_2O.

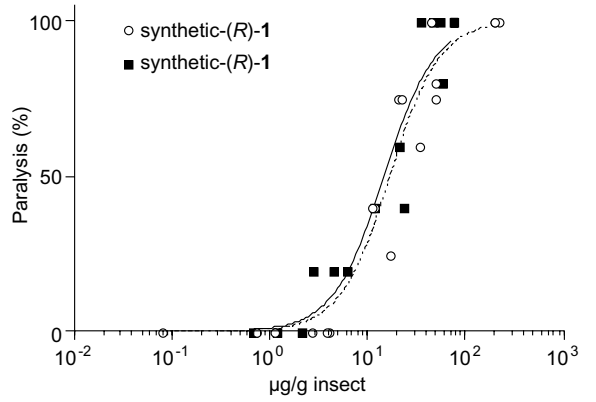


Figure 3. Dose–response curves resulting from injection of synthetic (*R*)-**1** and (*S*)-**1** into crickets.

The preliminary toxicity assay was performed with the synthetic polyamines using crickets (*Gryllus bimaculatus*) (Fig. 3).[10] The polyamines were dissolved in distilled water, and controls were injected with water only. Injections were made using a micro-syringe into crickets between legs 2 and 3. After injection, their paralyses occurred depending on the concentration of the polyamines, and most insects recovered within a few hours. ED50s for paralysis were calculated using the program Prism version 3.0C (Graphpad Software, Inc). The results indicated that the respective enantiomers, (*R*) and (*S*)-**1**, had similar potency.

Acknowledgements

This work was supported in part by a grant from the Research for the Future Program from the Japanese Society for the Promotion of Science (JSPS).

References and notes

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7. NMR spectra were recorded by a DMX-750 spectrometer (Bruker Biospin, Germany) in D_2O using a microcell NMR tube (Shigemi, Japan).
8. Mass spectra were recorded by a Q-Tof mass spectrometer (Micromass, UK).
9. CD spectra were obtained on a JASCO-725 spectropolarimeter (JASCO, Japan). Data were collected at 0.1 nm with a scan rate of 100 nm/min and a time constant of 0.5 s at room temperature using a 0.5 cm path-length cell.
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