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# Chemical investigation of the medicinal mushroom *Ganoderma* cf. *thailandicum* and identification of its major antioxidant component

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**ABSTRACT**: *Ganoderma thailandicum* (Polyporaceae), described in 2019, is a species in the medicinal mushroom genus *Ganoderma*. Its morphological characteristics are partially similar to the popular Chinese medicinal mushroom Lingzhi (species in the *Ganoderma lucidum* complex). To examine the potential as a new medicinal species, *Ganoderma* cf. *thailandicum*, strain CPN001, was artificially cultivated and the fruiting bodies were chemically investigated. Nine major chemical constituents of the $CH_2Cl_2$ extract, including ganomycin B (a meroterpene) and eight lanostane triterpenoids, were isolated and identified. Among the major constituents, ganomycin B showed significant antioxidant activity in the DPPH radical scavenging assay ($IC_{50}$ 7.5 µM), while it was noncytotoxic to normal cells (Vero). The isolated yield of ganomycin B was calculated to be 1.9% of the $CH_2Cl_2$ extract and 0.060% of the dried mushroom material, higher compositions than those of other *Ganoderma* species.

**KEYWORDS**: *Ganoderma thailandicum*, Thai medicinal mushroom, antioxidant activity, meroterpenoid, ganomycin

## INTRODUCTION

Polypores of the genus *Ganoderma* are medicinal mushrooms [1, 2]. *Ganoderma* spp. have been used in traditional Chinese medicine (TCM) for hundreds of years in Asian countries. In particular, *Ganoderma* species in the *Ganoderma lucidum* complex [3], including *G. lucidum*, *G. lingzhi*, *G. sinense*, and *G. sichuanense*, are popular traditional Chinese medicines known as Lingzhi and have been widely used in supplement products [4]. Lingzhi has been used for the treatment of migraine, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, diabetes, hypercholesterolaemia, nephritis, dysmenorrhoea, constipation, lupus erythematosis, hepatitis, and cardiovascular problems [5]. It is claimed to possess anti-cancer (including leukaemia), anti-ageing, and anti-microbial/viral activities including anti-human immunodeficiency virus (HIV) [1, 5–7]. Chemical investigations of *Ganoderma* species have been extensively curried out, which revealed that this genus is a prolific sources of bioactive compounds [1, 8]. In particular, polysaccharides and triterpenoids have been considered as key chemical constituents related to the various medicinal properties of Lingzhi [5].

*Ganoderma thailandicum* T. Luangharn, P.E. Mortimer, S.C. Karunarathna & J.C. Xu was described in 2019 for type strains collected from Nakhon Si Thammarat Province, Thailand [9]. This species is closely related in phylogeny with *Ganoderma casuarinicola* J.H. Xing, B.K. Cui & Y.C. Dai, which was described one year in advance using type strains from China [10]. These two species were placed in separate clade with that of the species in the *G. lucidum* complex [3]. There has been no report on the chemical investigations of *G. thailandicum*, while our research group reported isolation of lanostane triterpenoid from artificially cultivated fruiting bodies of *G. casuarinicola* (produced using a strain from Thailand: TBRC-BCC 78460) [11].

As part of a research program focused on the medicinal utilization of mushroom resources in Thailand, we have been searching for novel bioactive compounds from medicinal and edible basidiomycetes, on rare *Ganoderma* species [12–14]. In a screening of extracts from various *Ganoderma* spp. for antioxidant activity using DPPH radical scavenging assay, an extract of artificially cultivated *G.* cf. *thailandicum* showed highest activity ($IC_{50}$ 142 µg/ml) among the ten different species. This *Ganoderma* mushroom was growing on a living *Salix* cf. *babylonica* (Salicaceae) tree located in front of the Chaipattana Foundation Highland Agricultural Research and Development Project building in Fang District, Chiang Mai Province, Thailand. To evaluate the potential of *G. thailandicum* in the devel-

opment of medicinal products based on the antioxidant properties as "Lingzhi Thai", an authentic chemical investigation was conducted by collaboration with the Chaipattana Foundation.

## MATERIALS AND METHODS

### General experimental procedures

UV spectra were recorded on a JASCO V-730 spectrophotometer (JASCO Corporation, Tokyo, Japan). FTIR spectra were acquired using a Bruker ALPHA spectrometer (Bruker Corporation, Billerica, MA, USA). NMR spectra were recorded on Bruker Avance III HD 400 MHz and 500 MHz spectrometers (Bruker Corporation). ESITOF mass spectra were measured using a Bruker micrOTOF mass spectrometer. Silica gel 60H (Supelco/Merck KGaA, Darmstadt, Germany) was used for column chromatography. Preparative HPLC was performed on a Waters system (Waters Corporation, Milford, MA, USA) with a SunFire Prep C18 OBD column (19 × 250 mm, 10 μm).

### Fungal material

A natural specimen of Lingzhi mushroom growing on a willow tree (*Salix* cf. *babylonica*, family Salicaceae) in Pong Nam Ron Subdistrict, Fang District, Chiang Mai Province, was collected and identified by the Highland Agriculture Research and Development Project team, The Chaipattana Foundation, on June 7, 2019. The reference specimen discovered was not retained; however, the living culture was successfully isolated and preserved (original strain code: CPN001). Using this strain, the mushroom was artificially cultivated, and the reference specimens was deposited at the BIOTEC Bangkok Herbarium & Fungarium (BBH), National Science and Technology Development Agency (NSTDA). The living culture was later deposited at the Thailand Bioresource Research Center as TBRC 21164 on August 28, 2025. This fungus was classified as *Ganoderma* cf. *thailandicum* (Polyporaceae) based on the ITS rDNA gene sequence data (GenBank accession number: PX309337).

### Cultivation of *G. thailandicum* fruiting bodies

The mushroom cultivation was conducted by the Chaipattana Foundation. The mycelia of *G.* cf. *thailandicum* (strain CPN001) were maintained on potato dextrose agar (PDA; HiMedia, Maharashtra, India) at 25 °C for 7 days. The agar was sectioned into 1 × 1 cm plugs, and each plug was placed into five glass bottles (7 × 17 cm) containing sterile millet. Following a 7-day incubation at 25 °C, spawn from the bottles were used as inoculum for cultivation in 100 substrate bags. Each plastic bag contained approximately 800 g of substrate, comprising 91.2% (w/w) parawood sawdust, 0.9% (w/w) calcium oxide, 0.9% (w/w) pumice, 6.4% (w/w) rice bran, 0.5% (w/w) calcium sulfate, and 0.2% (w/w) magnesium sulfate. The cultivation ingredients were purchased from local suppliers in Thailand. The bags were incubated in the cultivation room at 25–35 °C for 15 days to promote mycelial growth, after which they were opened. An extra 45 days were required for the induction and development of the fruiting bodies. The fruiting bodies, measuring 70–75 mm in diameter, were procured from all substrate bags and subsequently harvested for further analysis.

### Extraction and isolation

Dried fruiting bodies (1.5 kg) were cut into small pieces and macerated in $CH_2Cl_2$ (15 l) at room temperature for 7 days. The mixture was filtered, and the filtrate was concentrated under reduced pressure. This extraction procedure was repeated one more time ($CH_2Cl_2$, 10 l) to obtain a $CH_2Cl_2$ extract as a dark brown gum (47.0 g). The $CH_2Cl_2$ extract (30.0 g) was subjected to column chromatography (CC) on silica gel (5.8 × 14 cm, acetone–hexane, step gradient elution 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0, then with acetone) to obtain nineteen fractions, Fr-1–Fr-19. Fr-1–Fr-6 (total 9.52 g) were mainly composed of lipids. Fr-7 (436 mg) was identified as **2**. Fr-8 (1.18 g) was subjected to silica gel CC (5.8 × 10 cm, EtOAc–hexane, 30:70) to furnish **3** (528 mg). Fr-9 (3.65 g) was fractionated by preparative HPLC on a reverse phase column using a MeCN–$H_2O$ gradient (75:25 to 100:0 over 45 min; flow rate 8 ml/min) to furnish **3** (391 mg) and **4** (549 mg). Fr-10 (1.26 g), Fr-11 (2.96 g), and Fr-12 (1.09 g) were fractionated by preparative HPLC (MeCN–$H_2O$, gradient from 60:40 to 100:0 over 50 min) to furnish **1** (573 mg), **5** (431 mg), and **6** (942 mg). Fr-13 (1.44 g), Fr-14 (0.508 g), and Fr-15 (1.59 g) were also fractionated by preparative HPLC (MeCN–$H_2O$, gradient from 50:50 to 100:0 over 50 min) to furnish **7** (797 mg) and **8** (516 mg). Fr-16 (1.59 g) was further purified by preparative HPLC (MeCN–$H_2O$, gradient from 40:60 to 100:0 over 60 min) to furnish **9** (752 mg). No characteristic secondary metabolite was obtained from Fr-17–Fr-19.

*Ganomycin B* (**1**): colorless amorphous solid; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 297 (3.64) nm; IR (ATR) $\nu_{max}$ 3324, 1684, 1502, 1450, 1231 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.73 (1H, d, $J$ = 8.3 Hz, H-6′), 6.63 (1H, d, $J$ = 2.7 Hz, H-3′), 6.62 (1H, dd, $J$ = 8.3, 2.7 Hz, H-5′), 6.02 (1H, t, $J$ = 8.7 Hz, H-2), 5.06 (1H, m, H-10), 5.04 (1H, m, H-6), 3.66 (2H, d, $J$ = 8.7 Hz, H-1), 2.31 (2H, t, $J$ = 7.4 Hz, H-4), 2.14 (2H, m, H-5), 2.00 (2H, m, 9), 1.92 (2H, m, H-8), 1.67 (3H, s, H-12), 1.58 (3H, s, H-13), 1.54 (3H, s, H-14); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 173.1 (C, C-15), 149.1 (C, C-1′), 148.9 (C, C-4′), 141.5 (CH, C-2), 136.5 (C, C-7), 131.3 (C, C-11), 130.8 (C, C-3), 124.3 (CH, C-10), 124.1 (C, C-2′), 122.5 (CH, C-6), 117.1 (CH, C-3′), 117.0 (CH, C-6′), 115.0 (CH, C-5′), 39.6 ($CH_2$, C-8), 34.3 ($CH_2$, C-4), 31.8 ($CH_2$, C-1), 27.3 ($CH_2$, C-5), 26.6

($CH_2$, C-9), 25.7 ($CH_3$, C-12), 17.7 ($CH_3$, C-13), 16.0 ($CH_3$, C-14); HRMS (ESI-TOF, negative ion mode) $m/z$ 343.1918 $[M-H]^-$ (calcd for $C_{21}H_{27}O_4$, 343.1915).

### DPPH radical scavenging assay (antioxidant activity)

The antioxidant activity of the isolated compounds was investigated by the DPPH radical scavenging assay [15]. In brief, different concentrations with the maximum dose of 400 µg/ml of the compounds were prepared in DMSO and mixed with DPPH (0.1 mM in EtOH). The mixtures were gently shaken in the dark at room temperature for 30 min. Then, the absorbance was measured at 517 nm. The antioxidant activity was calculated according to the following equation: Inhibition % $= [(A_0 - A)/A_0] \times 100$, where inhibition % is the percentage of inhibition of DPPH radicals, $A_0$ is the absorbance of negative control (DMSO with DPPH), and $A$ is the absorbance of the compound with DPPH. Ascorbic acid was used as a standard antioxidant. The concentration which is able to inhibit 50% DPPH radicals was reported as $IC_{50}$. Ascorbic acid was used as positive control ($IC_{50}$ 68 µM).

### Cytotoxic activities

The cytotoxic activity to Vero cells (African green monkey kidney fibroblasts, ATCC CCL-81) was evaluated using the green fluorescent protein microplate assay [16]. Ellipticine was used as positive control ($IC_{50}$ 0.79 µg/ml). The cytotoxic activities against cancerous cells, including MCF-7 (ATCC HTB-22) and NCI–H187 (ATCC CRL-5804), were evaluated using the resazurin microplate assay (REMA) [17]. Doxorubicin ($IC_{50}$ 5.4 µg/ml) and tamoxifen ($IC_{50}$ 5.9 µg/ml) were used as positive controls for MCF-7 cells. Doxorubicin ($IC_{50}$ 0.031 µg/ml) and ellipticine ($IC_{50}$ 1.6 µg/ml) were used as positive controls for NCI-H187 cells. All cell lines were obtained from ATCC (Manassas, VA, USA). All positive controls and standard drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA), except ellipticine, which was purchased from EMD Millipore Corporation (Temecula, CA, USA)

### Antitubercular and antimalarial activity assays

Antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) was evaluated using the green fluorescent protein microplate assay [18]. Rifampicin (MIC 0.0125 µg/ml) and isoniazid (MIC 0.0469 µg/ml) were used as positive controls. Antiplasmodial activity against *Plasmodium falciparum* K1 (MRA-159; BEI Resources, VA, USA), was performed using the microculture radioisotope technique [19]. Dihydroartemisinin ($IC_{50}$ 3.2 nM) and chloroquine ($IC_{50}$ 0.32 µM) were used as positive controls. All positive controls and standard drugs were obtained from Sigma-Aldrich.

## RESULTS AND DISCUSSION

The fungus *G.* cf. *thailandicum*, strain CPN001, was successfully cultivated by applying factory production protocols for the Lingzhi medicinal mushroom. This fungus exhibits rapid growth, yielding fruiting bodies in 60 days, in contrast to 90 days for commercial Lingzhi strains. It can tolerate daily temperature variations in its cultivation region. It can be cultivated as a commercially viable mushroom for its spores, as it generates twice the number of spores compared to commercial Lingzhi. On the other hand, the size of fruiting body was smaller than Lingzhi. Therefore, *G.* cf. *thailandicum* was shown to be suitable for mushroom mass production by factory cultivation.

Dried mushroom material was extracted with $CH_2Cl_2$. The extract was subjected to silica gel CC (acetone–hexane), and the fractions were further separated by silica gel CC (acetone–hexane or EtOAc–hexane) and preparative HPLC (reverse phase column, MeCN–$H_2O$) to furnish nine major chemical constituents (**1**–**9**) with >1% composition of the extract. The isolated yields were calculated as compositions in the extract (Fig. 1). The structure of compound **1** was elucidated on the basis of the spectroscopic data (1D and 2D NMR, IR, UV) and HRMS (ESI-TOF), and identified as the known geranylhydroquinone meroterpenoid, ganomycin B [20]. All other major compounds (**2**–**9**) were identified as known lanostane triterpenoids: ganoderol A (**2**) [21], ganodermadiol (**3**) [22], (24*E*)-15$\alpha$,26-dihydroxy-3-oxolanosta-7,9(11),24-triene (**4**) [23], (24*E*)-3$\beta$,15$\alpha$,26-trihydroxylanosta-7,9(11),24-triene (**5**) [11], (24*E*)-3-oxo-7$\alpha$,15$\alpha$,26-trihydroxylanosta-8,24-diene (**6**) [11], ganocasuarin E (**7**) [11], applanoxidic acid F (**8**) [24], and ganocasuarin F (**9**) [11]. It should be noted that all these compounds (**1**–**9**) were previously isolated from cultivated fruiting bodies of *G. casuarinicola* [11]. These results are consistent with the close phylogenetic relation between the two species.

The isolated compounds (**1**–**9**) were evaluated for antioxidant activity in the DPPH radical scavenging assay. Among these, ganomycin B (**1**) showed significant activity with an $IC_{50}$ value of 7.5 µM (2.6 µg/ml). Lanostane triterpenoids **2**–**9** were inactive ($IC_{50}$ >400 µg/ml) in this assay.

Ganomycin B (**1**) was isolated in good yield from *G.* cf. *thailandicum*, which was calculated to be 1.9% of the $CH_2Cl_2$ extract, and 0.060% of the dried mushroom material. Ganomycin B (**1**) was first isolated from natural fruiting bodies of *G. pfeifferi* [20]. According to the experimental descriptions of this original report, the isolated yield of **1** was calculated to be 0.55% of the extract, and 0.022% of the air-dried mushroom material. As described above, we previously investigated the chemical constituents of artificially cultivated fruiting bodies of *G. casuarinicola* [11]. The isolated

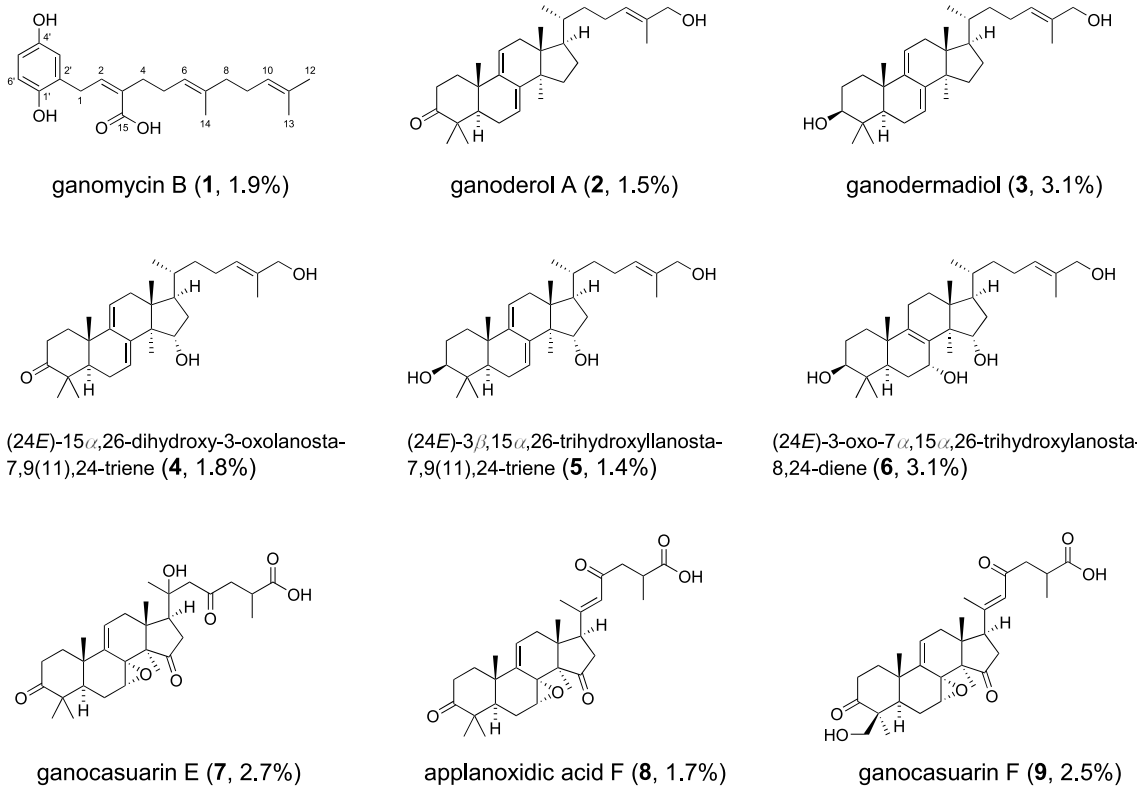


**Fig. 1** Major constituents (composition >1%) of the extract from *G.* cf. *thailandicum* fruiting bodies.

**Table 1** Biological activities of ganomycin B (**1**) and positive controls.

| Compound | Antioxidant activity (DPPH; $IC_{50}$, μM) | Antitubercular activity (*M. tuberculosis* H37Ra; MIC, μg/ml) | Antimalarial activity (*P. falciparum* K1; $IC_{50}$, μM) | Cytotoxic activities ($IC_{50}$, μM) | | |
|---|---|---|---|---|---|---|
| | | | | NCI-H187 cells | MCF-7 cells | Vero cells |
| Ganomycin B (**1**) | 7.5 | 12.5 | >29 | 39 | >145 | >145 |
| Ascorbic acid | 68 | – | – | – | – | – |
| Rifampicin | – | 0.0125 | – | – | – | – |
| Dihydroartemisinin | – | – | 0.003 | – | – | – |
| Doxorubicin | – | - - | – | 0.053 | 9.31 | |
| Ellipticine | – | – | – | – | – | 3.21 |

yield of **1** was calculated to be 0.51% of the $CH_2Cl_2$ extract, and 0.011% of the dried fruiting bodies, both of which were lower than the corresponding yields obtained from *G.* cf. *thailandicum*. Ganomycin B (**1**) production by the species in the *G. lucidum* complex are rare and with much lower compositions than those described above [25]. We also previously investigated cultivated fruiting bodies of *G. sichuanense* (produced using a Thai strain TBRC-BCC 55500), which is a currently recognized species corresponding to the TCM "Lingzhi". Ganomycin B (**1**) was not isolated from the mushroom extract.

*Ganoderma* meroterpenoids, including ganomycin B (**1**), are a group of metabolites from *Ganoderma* composed of modified farnesyl-hydroquinone or geranyl-hydroquinone derivatives [26]. Ganomycin B (**1**) represents as one of the simplest (least modified) members in the structural group. There have been reports on antioxidant activities, including the DPPH radical scavenging assay, of *Ganoderma* meroterpenoids [27–29]. However, this is the first report on the antioxidant activity of ganomycin B (**1**).

Ganomycin B (**1**) is reported to exhibit moderate growth inhibition against Gram-positive bacteria [16], and it is also known to inhibit HIV-1 protease ($IC_{50}$ 1.0 μg/ml) [30]. In the present study, ganomycin B (**1**, from *G.* cf. *thailandicum*) was also evaluated for cytotoxicity to Vero cells (African green monkey kidney fibroblasts) and cancer cell-lines, MCF-7 (breast cancer) and NCI-H137 (small-cell lung cancer), antitubercular activity (*M. tuberculosis* H37Ra), antimalarial activity (*P. falciparum* K1). Most notably, ganomycin B

(**1**) was noncytotoxic to noncancerous Vero cells and MCF-7 cells ($IC_{50}$ >145 μM). It showed weak cytotoxicity to NCI-H187 cells ($IC_{50}$ 39 μM). Ganomycin B (**1**) also showed moderate antimycobacterial activity (MIC 12.5 μg/ml), while it was inactive in the antiplasmodial activity assay ($IC_{50}$ >29 μM) (Table 1).

## CONCLUSION

*Ganoderma* cf. *thailandicum* was artificially cultivated and an authentic chemical investigation was conducted, which led to the isolation and identification of nine major chemical constituents. Among them, ganomycin B (**1**) exhibited significant DPPH radical scavenging activity, while it was shown to be noncytotoxic to normal cells (Vero). *G.* cf. *thailandicum* was shown to produce **1** in higher efficacy than other *Ganoderma* species. The present results suggest that it deserves further studies toward the medicinal utilization of this fungal species.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2026.073.

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## Appendix A. Supplementary data

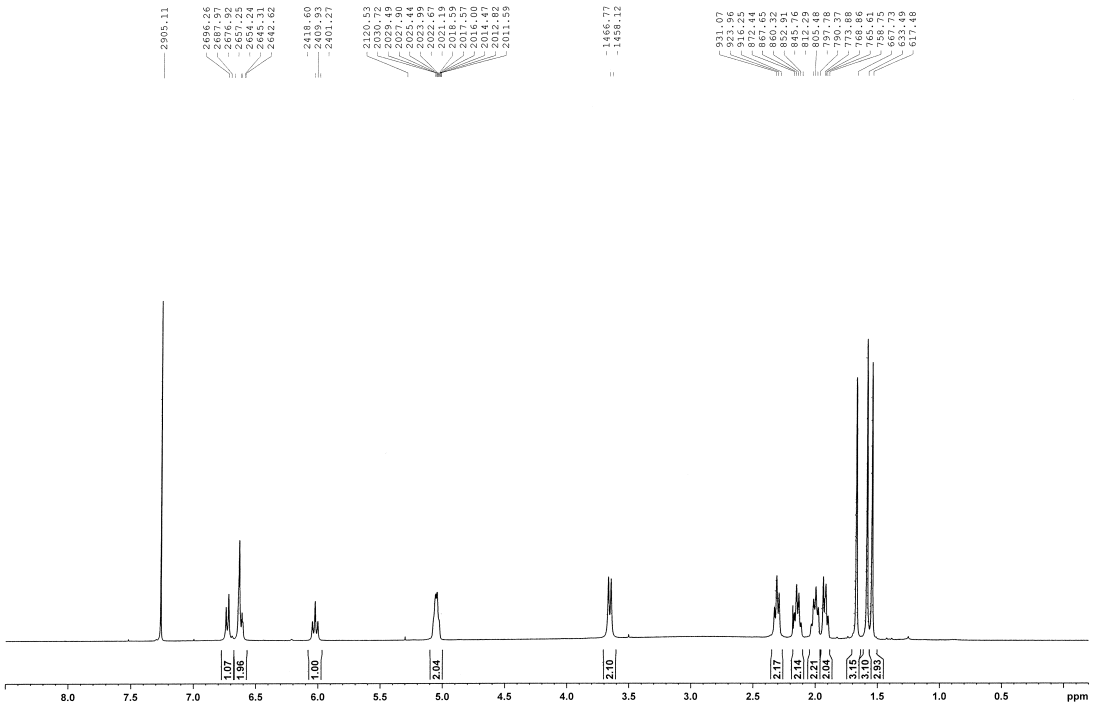


**Fig. S1** $^{1}$H NMR spectrum of ganomycin B ($CDCl_3$, 400 MHz).

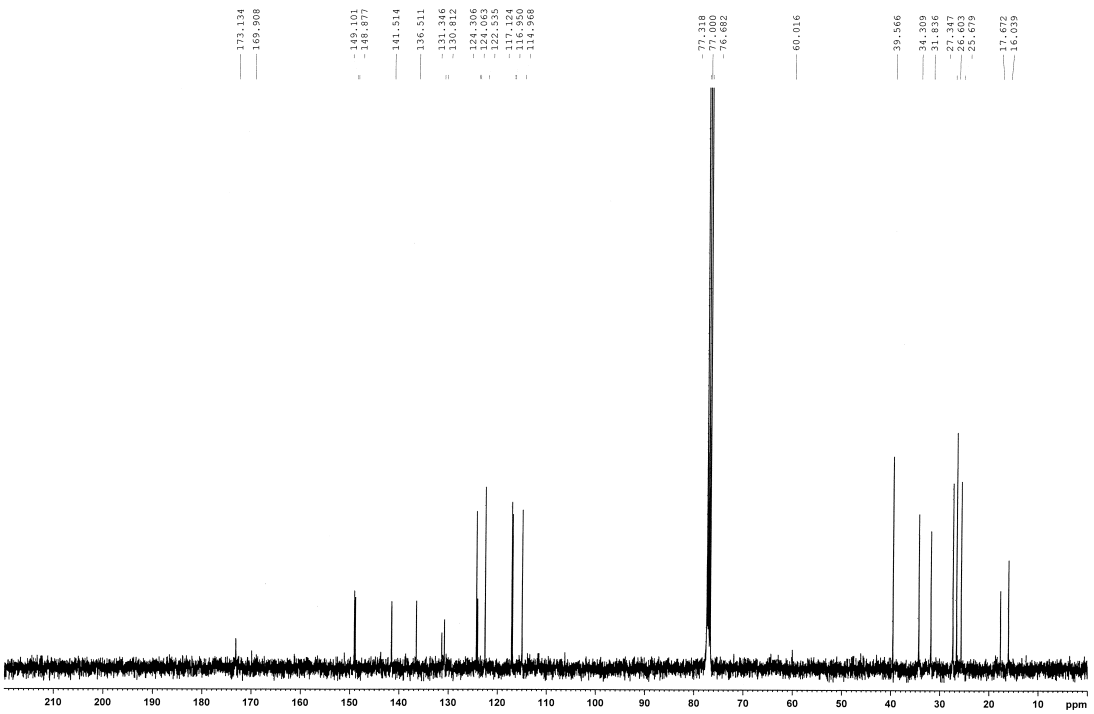


**Fig. S2** $^{13}$C NMR spectrum of ganomycin B ($CDCl_3$, 125 MHz).

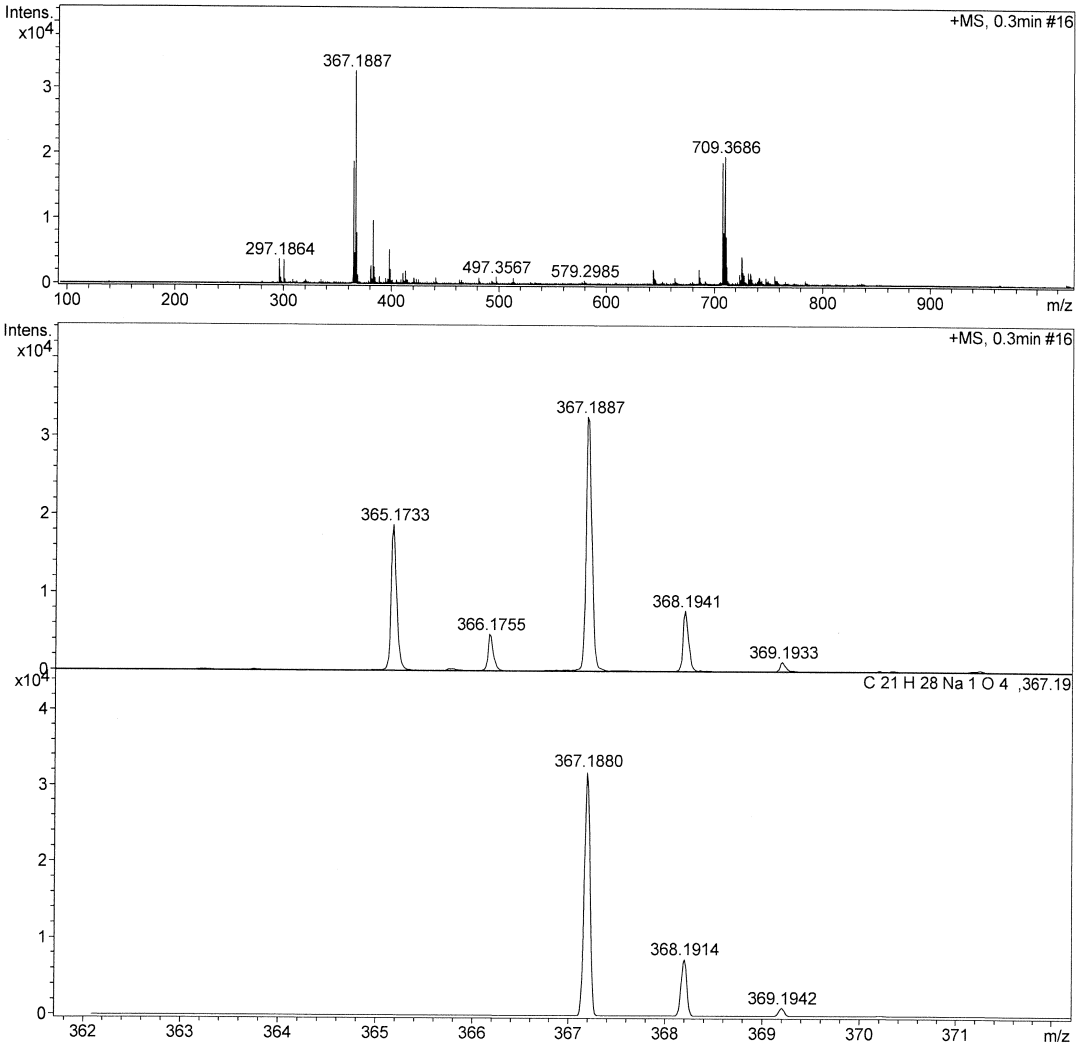


**Fig. S3** HRESIMS of ganomycin B (negative ion mode).