

Article

Iso-Partricin, an Aromatic Analogue of Amphotericin B: How Shining Light on Old Drugs Might Help Create New Ones

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Abstract: Partricin is a heptaene macrolide antibiotic complex that exhibits exceptional antifungal activity, yet poor selective toxicity, in the pathogen/host system. It consists of two compounds, namely partricin A and B, and both of these molecules incorporate two *cis*-type bonds within their heptaenic chromophores: 28Z and 30Z. In this contribution, we have proven that partriccins are susceptible to a chromophore-straightening photoisomerization process. The occurring 28Z→28E and 30Z→30E switches are irreversible in given conditions, and they are the only structural changes observed during the experiment. The obtained *all-trans* partricin's derivatives, namely iso-partriccins A and B, exhibit very promising features, potentially resulting in the improvement of their selective toxicity.



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Keywords: partricin; aureofacin; polyene macrolides; photoisomerization; selective toxicity; structural studies; NMR

1. Introduction

Among several groups of clinically used antifungal antibiotics, polyene macrolides should be regarded as the most versatile and promising family of fungicidal agents. They exhibit most of the features of a mythical “ideal drug”, with two major disadvantages depriving them of their rightful place on top of the antifungal pantheon: (1) poor bioavailability, resulting from poor solubility in water; and (2) relatively poor pathogen/host selective toxicity [1]. Although the former problem may be easily overcome by a proper formulation of a drug, the latter is directly related to the molecular mode of action of clinically used polyene macrolides; thus, they require rational modifications to their chemical structure.

The highest antifungal activity is attributed to the polyene macrolides belonging to the heptaenic group. This family may be divided into two subgroups: (1) non-aromatic *all-trans* heptaenes, with amphotericin B (AmB) being “the big star” and still considered to be a golden standard in treatment of systemic fungal infections, and (2) aromatic heptaenes (AHs), incorporating two *cis*-type double bonds in their heptaenic chromophores and an extra structural feature: an alkyl-aromatic side chain, attached to the major macrolactone ring [2,3]. Members of the latter subgroup exhibit up to two orders of magnitude higher antifungal activity than AmB [4], yet none of them is currently recommended for clinical use in treatment of internal mycoses, mainly due to their especially poor selective toxicity and severe side effects.

AHs are often misrepresented in the literature, as the *all-trans* compounds, yet none of the AHs—found in nature to this date, that is—exhibits such a geometry of the chromophore. The most widely known representative of AHs, the candicidin complex (syn. levorin, ascosin, produced by *Streptomyces griseus*), consists of three major components and each

direct daylight. As can be seen in Figure 2, observed number of major components of the partricin complex, illuminated with daylight (red line), has doubled in comparison to the control sample (blue line). Partricins A and B were partially transformed into their isomers, exhibiting the very same molecular masses, yet responding with significantly altered UV–VIS spectra. Since the heptaenic chromophore of polyene macrolides is by far the major contributor to their electronic spectra, it was obvious that structural changes—at least those registered by photonic absorption—must have occurred in this region.

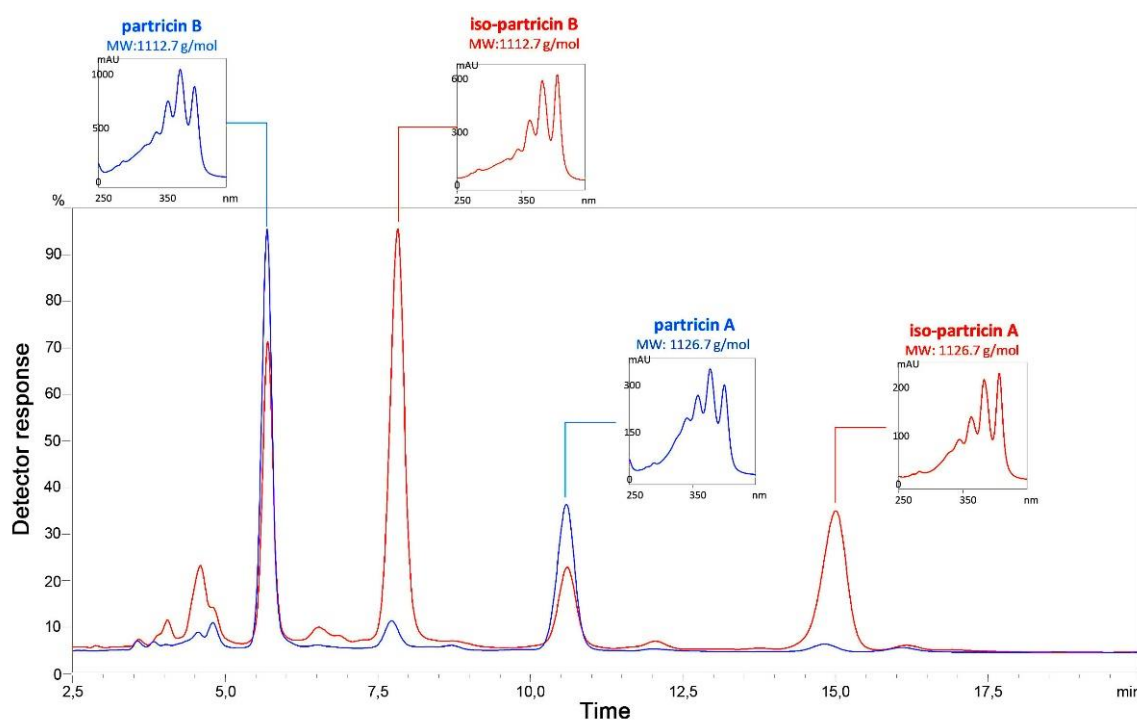


Figure 2. Superimposed HPLC–DAD–ESIMS chromatograms of partricin complex. Red line: sample dissolved and stored at room temperature for 24 h, with an unlimited access to daylight. Blue line: sample dissolved and stored at room temperature in darkness for 24 h (control).

UV–VIS spectra of the native partricins incorporate three absorption maxima at $\lambda_1 = 360$ nm, $\lambda_2 = 378$ nm and $\lambda_3 = 401$ nm, with the one at the medium (λ_2) being of highest intensity (Figure 2). Such an image is generally attributed to a heptaenic system that contains several Z double bonds within [19]. While the positions and number of the Z bonds might not be directly extracted from the antibiotics' electronic spectra, previous studies on partricin A and partricin B have proven that those molecules contain two *cis*-type double bonds at positions 28 and 30 (Figure 1) [8,10].

The absorption maxima observed for iso-partricins A and B were subjects to bathochromic shifts, manifesting at $\lambda_1^* = 364$ nm, $\lambda_2^* = 384$ nm and $\lambda_3^* = 407$ nm. Moreover, the relative intensities of λ_2^* and λ_3^* were inverted in relation to the native compounds (Figure 2). This shape of an electronic spectrum has been previously demonstrated to indicate to the *all-trans* type of heptaenic chromophore, identical to the one of amphotericin B [3]. However, this testimony could not be accepted as a direct structural proof and, therefore, required further verification. Additionally, the remaining structural regions of the iso-molecules must have also been tested for other potential light-induced constitutional alterations. Thus, ^1H NMR was selected as a major tool for the following structural studies.

2.2. NMR Studies on Iso-Partricin A and B

The native partricin complex was derivatized into its N-acetyl methyl ester via series of simple reactions. This procedure, developed at our department, is a well-established approach for the elucidation of constitution and/or stereochemistry of polyene macrolides,

using NMR techniques [5,16,20–22], and, as an option, could be followed in order to facilitate purification of studied antibiotic molecules and enhance their solubility in standard NMR solvents. Later, the resulting methyl ester of N-acetylpartricin (referred to as partricin*) was exposed to moderate UV–VIS radiation of $\lambda = 365$ nm. The chosen reaction wavelength was identical to the one that produced the best results for candidicin D and was not optimized in this study [17]. The progress of a photochemical reaction was traced by using continuous UV–VIS spectra. When further irradiation caused no visible changes in the electronic spectra, methyl ester of N-acetyl-iso-partricin A (referred to as iso-partricin A*) and methyl ester of N-acetyl-iso-partricin B (aka iso-partricin B*) were isolated and purified by using HPLC.

Iso-partricin A* and iso-partricin B* molecules were subjects to a standard set of 2D NMR experiments, consisting of DQF-COSY, TOCSY, HSQC, HMBC and ROESY spectra (please consult Figures S1–S12) [5,16,20–26]. DQF-COSY, TOCSY and HSQC spectra were used to trace proton–proton and proton–carbon connectivities within isolated protonic spin systems, while HMBC and ROESY experiments enabled gluing all the pieces together, due to long ranged heteronuclear couplings and structurally conclusive dipolar couplings between protons. Finally, DQF-COSY and ROESY spectra allowed the definition of the stereostructure of the studied compounds, including absolute configurations of almost all stereogenic centers within the molecules and—most importantly—the geometries of iso-partricin’s A* and iso-partricin’s B* chromophores.

Detailed NMR studies revealed that, in contrary to the NMR data on the partricin A and partricin B methoxycarbonylmethylamide derivatives [8–10], all the $^3J_{H,H}$ coupling constants within the double bonds were no lower than 15.1 Hz and no higher than 15.6 Hz, thus determining the *E* geometry of the entire chromophore systems. The chemical shifts of all the olefinic carbons (C22–C35) ranged between 130 and 137 ppm, which strongly suggested the *E* geometries of all the double bonds within the chromophores, since no shielding γ -effects were observed for the C27/C30 and C29/C32 pairs [9,16,22]. Moreover, rich sets of ROEs between the protons of the C2–C13 and C22–C35 fragments were recorded (Figure 3), as well as two uninterrupted ROE pathways, incorporating even- and odd-numbered olefinic protons (see Appendix A section, Table A1). The stereostructural requirements for all those dipolar couplings might be met only in case of straightening of the heptaenic chromophores to the *all-trans* geometries. Additionally, some of the vicinal proton–proton coupling constants within the C2–C9 fragments have changed in comparison to the *cis-trans* derivatives [9,10], which should be attributed to the alteration of the flexibility of the macrolactone ring systems, resulting from changes in the geometry of the chromophores.

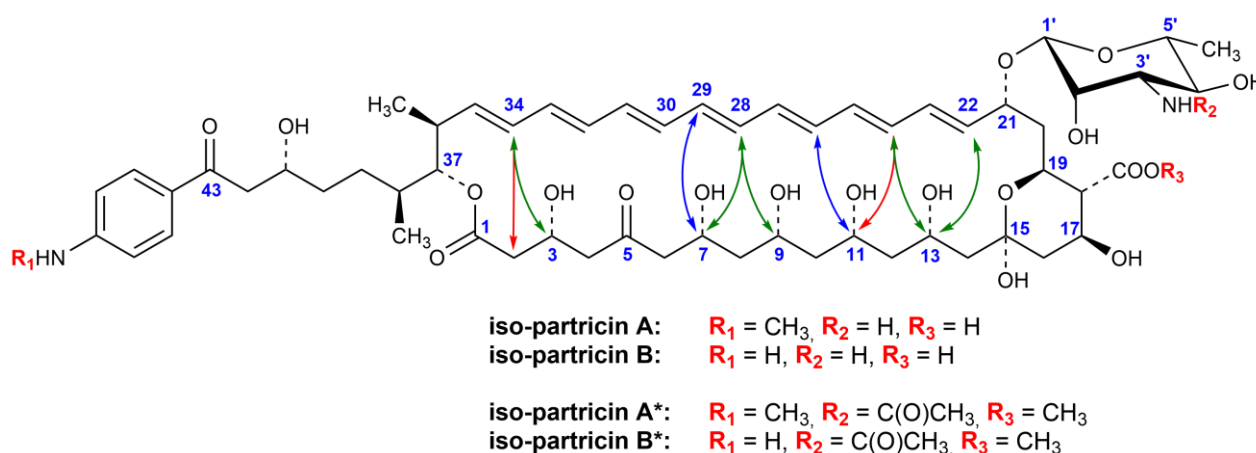


Figure 3. Structure of iso-partricins A and B, along with their methyl esters of N-acetyl derivatives. Dipolar couplings between the protons of the chromophores and the polyol chains are depicted as bidirectional arrows. Colors: blue—ROEs observed only for the native partricins [8,10]; green—ROEs observed for both native and isomeric partricins; red—ROEs observed only for the *all-trans* iso-partricins.

No other constitutional and/or stereochemical changes in iso-partricin A* and iso-partricin B* were found in comparison to the native compounds. Thus, we have proven that 28Z→28E and 30Z→30E switches are the only chemical changes of partricins' structures, occurring as a result of moderate UV–VIS irradiation.

More detailed information on the ¹H and ¹³C resonances of iso-partricin A* and iso-partricin B* is given in Table A1.

3. Discussion

The work presented in this manuscript, along with the previously conducted studies on candicidin D and its *all-trans* isomer [17], have proven that the native aromatic heptaene macrolide antifungal antibiotics are in fact susceptible to a chromophore-straightening photoisomerization process, regardless of the positions of the Z double bonds within their original chromophores. The resulting transformations are irreversible in the given experimental conditions, yet it must be noted that the yield of the whole process is below 100%, since the welcomed structural changes are in fact competed by the antibiotics' degradation. The latter fact presumably creates a space for further optimization of the production of the AHs' isoforms.

The *all-trans* aromatic heptaenes (especially the iso-partricins, exhibiting high resemblance of their polyol chains to the one of AmB) might be, therefore, considered as aromatic analogues of amphotericin B—the only polyene macrolide antibiotic used clinically in treatment of systemic fungal infections. Our preliminary results on in vitro biological activity of iso-candicidin D, iso-partricin A and iso-partricin B have strongly suggested that fungicidal activity of the *all-trans* isoforms remains comparable to the native molecules, thus still exceeding the one of AmB by almost two orders of magnitude. Meanwhile, the hemolytic activity of iso-AHs has been substantially reduced in comparison to the native *cis-trans* forms. The calculated selective toxicity indexes (STIs), which relate to the EH₅₀ to MIC ratio, were equal to > 20 and 19.28 for iso-partricin A and iso-partricin B, respectively. Initial assessments have therefore demonstrated that both iso-partricins were more selective than AmB (STI = 13.84), mainly due to the lowered hemolytic activity. For more details on biological activity of iso-partricins, along with computational studies on their interactions with membrane sterols, please consult [27].

In the end, the in vitro selective toxicity index of AHs seems to benefit a lot from the straightening of their heptaenic chromophores, which encourages us to have high hopes, regarding following studies on these compounds and their further development.

4. Materials and Methods

4.1. Partricin Complex

The crude partricin complex was obtained by extraction with n-butanol from fermentative broth of *Streptomyces aureofaciens* NRRL 3878 in the Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology (Gdańsk, Poland). The volume of resulting solution was reduced by evaporation under reduced pressure and centrifuged. The precipitate was washed several times with acetone and dry ethyl ether and dried under reduced pressure. The crude antibiotic complexes were then purified by using the procedure described in patent no. 83,710 [28].

4.2. Synthesis of Methyl Ester of 3'-N-Acetylpartricin Complex (Partricin*)

Derivatization of the purified partricin complex was performed with the general procedure previously elaborated in our laboratory and described in References [5,21,22].

4.3. Photochemical Cis–Trans Isomerization of Partricin*

The partricin* complex was dissolved in a 95% methanol/5% water solvent system to a concentration of 1 mg/mL. While gently stirring, one liter of this solution (~2 cm depth) was then irradiated with two long-wavelength UV lamps ($\lambda = 365$ nm, 8 W) for ca. 1 h. The reaction vessel was placed in the dark, at room temperature. The progress of the photochemical isomerization reaction was monitored by using UV–VIS spectroscopy and RP-HPLC analysis.

The RP-HPLC analysis conditions were as follows: column, Luna 100 C18 (2) (150 × 4.6 mm, 5 µm); mobile phase composition, 38% acetonitrile/62% ammonium acetate buffer (5.5 mmol, pH = 4.5), *v/v*; flow rate, 1 mL/min; detection at 378 nm; room temperature.

4.4. Isolation of the Methyl Esters of 3'-N-Acetyl-Iso-Partricin A and B (Iso-Partricin A* and Iso-Partricin B*)

The isolation of the iso-partricin A* and iso-partricin B* from the partricin* complex was performed by means of semi-preparative HPLC on a Merck–Hitachi apparatus L-6200A, equipped with Merck–Hitachi L-4250 UV–VIS detector. The separation conditions were as follows: column LiChrosorb Si60 (250 mm × 10 mm, 7 µm), mobile phase composition: chloroform/methanol/water (5:0.4:0.035, *v/v/v*); flow rate 6.25 mL/min; detection at 407 nm, room temperature. A sample of 10 mg/mL (dissolved in the mobile phase) in a volume of 0.625 mL was injected. The retention time was 18 min and 29 min for iso-partricin A* and iso-partricin B*, respectively. The semi-preparative HPLC separation was performed several times, yielding 7 mg of the iso-partricin A* and 8 mg of the iso-partricin B*.

4.5. NMR Experiments

The NMR spectra were recorded with a Bruker Avance III HD 700 MHz spectrometer equipped with QCI CryoProbe in solvent system pyridine-*d*₅-methanol-*d*₄, 9:1 (*v/v*) at 25 °C with a sample concentration of 15 mg/mL. Chemical shifts were reported in δ (ppm) units, using ¹H residual resonance from pyridine-*d*₅ (7.19 ppm) as internal standard. The 1D ¹H NMR spectra were collected with digital resolution of 0.5 Hz. The ¹H 90° pulse length was 7.0 µs.

Two-dimensional ¹H spectra were measured in the phase-sensitive mode with a spectral width of 7704 Hz.

The DQF-COSY spectra were acquired in a 4096 × 512 matrix with 32 accumulations per increment and were processed in a 4K × 2K matrix.

The TOCSY spectra were acquired with a mix time of 60 ms in a 2048 × 512 matrix with 32 accumulations per increment in a 2K × 1K matrix.

The ROESY spectra were acquired with a mix time of 300 ms in a 2048 × 512 matrix with 64 accumulations per increment in a 2K × 1K matrix.

HSQC and HMBC experiments were performed with pulse field gradients.

The edited HSQC spectra were acquired in the phase-sensitive mode with ¹J_(CH) set to 140 Hz. The spectral windows for ¹H and ¹³C axes were 7716 and 29,177 Hz, respectively. The data were collected with 64 accumulations per increment in a 2048 × 256 matrix and processed in a 2K × 1K matrix.

The HMBC spectra were acquired in absolute value mode with ⁿJ_(CH) set to 9 Hz. The spectral windows for ¹H and ¹³C axes were 7716 and 40,515 Hz, respectively. The data were collected with 192 accumulations per increment in a 2048 × 256 matrix and processed in a 2K × 1K matrix.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/antibiotics10091102/s1>. Figure S1: The ¹H NMR spectrum of iso-partricin A. Figure S2: The ¹H NMR spectrum of iso-partricin B. Figure S3: The DQF-COSY spectrum of iso-partricin A. Figure S4: The DQF-COSY spectrum of iso-partricin B. Figure S5: The TOCSY spectrum of iso-partricin A. Figure S6: The TOCSY spectrum of iso-partricin B. Figure S7: The ROESY spectrum of iso-partricin A. Figure S8: The ROESY spectrum of iso-partricin B. Figure S9: Edited ¹H-¹³C HSQC spectrum of iso-partricin A. Figure S10: The ¹H-¹³C HMBC spectrum of iso-partricin A. Figure S11: Edited ¹H-¹³C HSQC spectrum of iso-partricin B. Figure S12: The ¹H-¹³C HMBC spectrum of iso-partricin B. Figure S13: HPLC-DAD-ESIMS chromatogram of isolated iso-partricin A. Figure S14: HPLC-DAD-ESIMS chromatogram of isolated iso-partricin B.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. ^1H and ^{13}C NMR spectroscopic data for iso-partricin A* and B* (700 MHz, $\text{C}_6\text{D}_5\text{N}/\text{CD}_3\text{OD}$ (9:1, v/v)). In every column, the letter A stands for spectroscopic data for iso-partricin A*, and the letter B stands for the analogous value for iso-partricin B*, whereas no letter given means that the presented data are identical for both antibiotics.

Iso-Partricin A* and B*					
Position	δ_{C} , Type	δ_{H}	$\text{J}_{\text{H,H}}$ (Hz)	ROE Contacts	
<i>Aglycone</i>					
1	170.85, C	–	–	–	–
2ab ¹	43.48, CH ₂	2.796	? (3) ¹	3, 4a, 4b	
3	64.18, CH	4.863	? (2ab) ¹ , 8.8 (4a), 4.3 (4b)	2ab, 4a, 4b, 34, Me38	
4a	51.05, CH ₂	2.849	8.8 (3), 16.8 (4b)	2ab, 3, 4b	
4b	51.05, CH ₂	2.990	4.3 (3), 16.8 (4b)	2ab, 3, 4a	
5	208.54, C	–	–	–	
6a	51.55, CH ₂	2.632	16.4 (6b), 1.9 (7)	4a, 6b, 7	
6b	51.55, CH ₂	2.874	16.4 (6a), 10.2 (7)	4b, 6a, 7	
7	67.79, CH	4.591	1.9 (6a), 10.2 (6b), 2.0 (8a), 9.7 (8b)	6a, 6b, 8a, 8b, 9, 28	
8a	43.77, CH ₂	1.529	2.0 (7), 13.8 (8b), 1.9 (9)	6a, 7, 8b, 9, 10a	
8b	43.77, CH ₂	1.732	9.7 (7), 13.8 (8b), 10.0 (9)	6b, 7, 8a, 10b	
9	72.79, CH	4.269	1.9 (8a), 10.0 (8b), 2.2 (10a), 9.8 (10b)	7, 8a, 10a, 11, 28	
10a	44.49, CH ₂	1.465	2.2 (9), 14.1 (10b), 1.9 (11)	8a, 9, 10b, 11, 12a	
10b	44.49, CH ₂	1.670	9.8 (9), 14.1 (10a), 10.3 (11)	8b, 10a, 11	
11	73.02, CH	4.283	1.9 (10a), 10.3 (10b), 2.2 (12a), 9.4 (12b)	9, 10a, 10b, 12a, 13, 24	
12a	44.31, CH ₂	1.411	2.2 (11), 13.2 (12b), 1.8 (13)	10a, 11, 12b, 13, 14a	
12b	44.31, CH ₂	1.685	9.4 (11), 13.2 (12a), 10.1 (13)	12a, 14b	
13	69.11, CH	4.746	1.8 (12a), 10.1 (12b), 2.0 (14a), 9.4 (14b)	11, 12a, 14a, 22, 24	
14a	46.71, CH ₂	1.755	2.0 (13), 14.6 (14b)	12a, 13, 14b	
14b	46.71, CH ₂	1.953	9.4 (13), 14.6 (14a)	12b, 14a, 16b	
15	98.11, C	–	–	–	
16a	45.28, CH ₂	1.743	12.4 (16b), 10.3 (17)	16b, 18	
16b	45.28, CH ₂	2.543	12.4 (16a), 4.5 (17)	14b, 16a, 17	
17	66.34, CH	5.006	10.3 (16a), 4.5 (16b), 10.2 (18)	16b, 18, 19	
18	58.39, CH	2.853	10.2 (17), 10.1 (19)	16a, 17, 19, 20a, 21	
19	66.21, CH	5.049	10.1 (18), 10.5 (20a)	17, 18, 20a, 20b, 22, 1', 2'	
20a	37.86, CH ₂	2.013	10.5 (19), 15.6 (20b)	18, 19, 20b, 21	
20b	37.86, CH ₂	2.431	15.6 (20a), 9.6 (21)	19, 20a, 21, 1'	
21	75.91, CH	4.924	9.6 (20b), 9.2 (22)	18, 20a, 20b, 22, 1'	
22	136.89, CH	6.403	9.2 (21), 15.3 (23)	13, 19, 21, 24	
23	132.93, CH	6.449	15.3 (22), 11.1 (24)	25	
24	133.90, CH	6.674	11.1 (23), 15.4 (25)	11, 13, 22, 26	
25	130.00, CH	6.380	15.4 (24), 10.9 (26)	23, 27	
26	133.89, CH	6.476	10.9 (25), 15.1 (27)	24, 28	
27	132.42, CH	6.299	15.1 (26), 11.0 (28)	27, 29	
28	133.90, CH	6.649	11.0 (27), 15.6 (29)	7, 9, 26, 30	
29	133.88, CH	6.630	15.6 (28), 10.9 (30)	27, 31	
30	133.25, CH	6.496	10.9 (29), 15.4 (31)	28, 32	
31	133.91, CH	6.673	15.4 (30), 11.3 (32)	29, 33	

Table A1. Cont.

Iso-Partricin A* and B*					
Position	δ_C , Type		δ_H	$J_{H,H}$ (Hz)	ROE Contacts
32	133.01,	CH	6.405	11.3 (31), 15.1 (33)	30, 34
33	133.84,	CH	6.624	15.1 (32), 11.0 (34)	31, 35
34	132.49,	CH	6.301	11.0 (33), 15.5 (35)	2ab, 3, 32, 36
35	136.99,	CH	5.598	15.5 (34), 9.3 (36)	33, 36, 37, Me36
36	40.14,	CH	2.542	9.3 (35), 9.2 (37), 6.8 (Me36)	34, 35, 37, 39a, 39b, Me36, Me38
37	78.63,	CH	5.080	9.2 (36), 2.9 (38)	35, 36, 38, 39a, Me36, Me38
38	33.81,	CH	1.927	2.9 (37), ? (39a, 39b) ² , 6.7 (Me38)	37, 39a, 39b, 40ab, 41, Me36, Me38
39a	30.84,	CH ₂	A: 1.719 B: 1.714	? (38, 39b) ² , ? (40ab) ¹	36, 37, 38, 39b, 40ab, 41, Me38
39b			A: 1.761 B: 1.757	? (38, 39a) ² , ? (40ab) ¹	36, 37, 38, 39a, 40ab, 41, Me38
40ab ¹	A: 35.64, B: 35.66,	CH ₂	A: 1.895 B: 1.877	? (39a, 39b, 41) ¹	38, 39a, 39b, 41, 42a, 42b, Me38
41	A: 68.35, B: 68.28,	CH	A: 4.609 B: 4.592	? (40ab)1, 3.5 (42a), 9.1 (42b)	38, 39a, 39b, 40ab, 42a, 42b, 45/45'
42a	A: 46.11, B: 46.04,	CH ₂	A: 3.233 B: 3.211	3.5 (41), 15.2 (42b)	40ab, 41, 42b, 45/45'
42b			A: 3.435 B: 3.410	9.1 (41), 15.2 (42a)	40ab, 41, 42a, 45/45'
43	A: 197.76, B: 197.67,	C	–	–	–
Me36	16.48,	CH ₃	0.972	6.8 (36)	35, 36, 37, 38
Me38	12.91,	CH ₃	1.040	6.7 (38)	36, 37, 38, 39a, 39b
COOMe	173.91,	C	–	–	–
COOMe	51.66,	CH ₃	3.818	–	2', 3'
NHMe	A: 29.36, B: –,	CH ₃	A: 2.811 B: –	–	46/46'
<i>Aromatic moiety</i>					
45/45'	A: 131.05, B: 131.23,	CH	8.161	8.6 (46/46')	41, 42a, 42b, 46/46'
46/46'	A: 110.94, B: 110.24,	CH	6.757	8.6 (45/45')	45/45', NHMe
C*CO	A: 154.38, B: 154.25,	C	–	–	–
C*NH	A: 126.12, B: 126.64,	C	–	–	–
<i>Mycosamine moiety</i>					
1'	98.16,	CH	4.974	1.9 (2')	2', 3', 5', 19, 20b, 21
2'	70.79,	CH	4.444	1.9 (1'), 3.5 (3')	1', 3', 19, COOMe
3'	56.09,	CH	4.677	3.5 (2'), 9.4 (4'), 4.5 (NHCOMe)	1', 2', 5', COOMe, NHCOMe
4'	74.62,	CH	4.034	9.4 (3'), 9.5 (5')	6', NHCOMe
5'	74.69,	CH	3.790	9.5 (4'), 6.3 (6')	1', 3', 6'
6'	18.41,	CH ₃	1.591	6.3 (5')	4', 5'
NHCOMe	22.83,	CH ₃	2.113	–	3', NHCOMe
NHCOMe	170.90,	C	–	–	–
NHCOMe	–	–	8.850	4.5 (3')	4', NHCOMe

¹ These protons were perfectly superimposed; hence, the values of the coupling constants involving protons 2a, 2b, 40a and 40b could not be measured. ² These coupling constants could not be measured due to severe signal overlaps and higher order effects.

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