Electronic Supplementary Information

Experimental and Computational Insights into the Conformations of Tunicyclin E, A New Cycloheptapeptide from *Psammosilene tunicoides*

Jun-Mian Tian,[a,f] Si-Sheng Ou-Yang,[b,f] Xu Zhang,[c] Ying-Tong Di,[d] Hua-Liang Jiang,[b,e] Hong-Lin Li,[e] Wei-Xing Dai,[a] Ke-Yu Chen,[a] Mai-Li Liu,[c] Xiao-Jiang Hao,[d] Yun-Heng Shen,[*,a] Cheng Luo,[*,b] Wei-Dong Zhang[*,a]

[a] *Department of Phytochemistry, School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, PR China.*
Fax: +86-21-81871244; Tel: +86-21-81871244;
E-mail: wdzhangy@hotmail.com; shenyunheng9217018@yahoo.com.cn

[b] *Drug Discovery and Design Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China,*
E-mail: cluo@mail.shcnc.ac.cn

[c] *Sate Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematic, Chinese Academy of Sciences, Wuhan 430071, China,*

[d] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China,*

[e] *School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China*

[f] *These authors contributed equally to this work*

Contents **Page number**

The structure elucidation of tunicyclin E (1)

Compound **1** was isolated as colorless crystal ($[\alpha]_D^{20}$ - 2, *c* 0.13, MeOH) with the molecular formula $C_{35}H_{50}N_8O_9$ as established by negative HR-ESI-MS (*m*/*z* $[M-H]^-$ 725.3625, cacld 725.3623). Two sets of proton resonances with a ratio of 3:1 in ^1H NMR spectrum implicated the presence of two conformations in solution (**1a** and **1b**). By 1D and 2D NMR experiments, two sets of NMR data (**1a** and **1b**) were unambiguously assigned. In **1a**, the correlation between the amide proton (δ_H 10.03, 1 H) and two α-H (δ_H 4.84, 1 H and 3.88, 1 H) in ^1H-^1H COSY and TOCSY spectra indicated that the presence of a Gly residue. The chemical shift of α-C of Gly residue was assigned as 43.80 ppm from the HSQC spectrum. The chemical shift of carbonyl carbon of Gly residue was assigned as 169.36 ppm based on the HMBC correlation of α-H (δ_H 4.84 and 3.88) and Gly-CO (δ_C 169.36). A spin system comprized with the amide proton (δ_H 9.36, 1 H) and other protons of two methyl (δ_H 1.05, 3 H and 1.02, 3 H) and two methine (δ_H 4.31, 1 H and 2.28, 1 H) in TOCSY spectrum indicated that **1a** also contained a Val residue. The chemical shifts of α-C, β-C, γ-C and γ'-C of Val residue were respectively assigned as 61.18, 30.05, 19.25,19.44 ppm, based on the HSQC correlations. Furthermore, the carbonyl carbon of Val residue was undoubtedly assigned to δ_C 172.74 based on HMBC correlations between the carbonyl carbon and β protons of Val residue (δ_H 2.28). The spin system comprized with the protons of α-H (δ_H 4.75, 1 H), β-H (δ_H 2.07, 1 H), β'-H (δ_H 1.92, 1 H), γ-H (δ_H 1.70, 1 H), γ'-H (δ_H 1.50, 1 H), δ-H (δ_H 3.62, 1 H) and δ'-H (δ_H 3.40, 1 H) in TOCSY spectrum, corresponding α-C (δ_C 61.25), β-C (δ_C 28.39), γ-C (δ_C 24.92) and δ-C (δ_C 47.31) determined by HSQC spectrum, indicated the presence of Pro residue in **1a**. The HMBC correlation between β-H (δ_H 2.07, 1 H), β'-H (δ_H 1.92, 1 H) of Pro and the carbonyl carbon (δ_C 172.74) determined the chemical shift of carbonyl carbon of Pro residue. Based on the TOCSY correlations between the amide proton (δ_H 8.15) and the protons of α-H (δ_H 4.90, 1 H), β-H (δ_H 1.95, 1 H), β'-H (δ_H 1.87, 1 H), γ-H (δ_H 1.78, 1 H), δ-H (δ_H 0.76, 3 H) and δ'-H (δ_H 0.73, 3 H), a Leu residue could be deduced in **1a**. The chemical shifts of carbons of Leu residue also were assigned to δ_C 53.40 (α-C), 40.64 (β-C), 24.79 (γ-C) 22.93 (δ-C) and 21.52 (δ'-C) from HSQC spectrum. The chemical shift of carbonyl carbon of Leu also was assigned to δ_C 174.49 by the

HMBC correlation between β-H (δ_H 1.95, 1 H), β'-H (δ_H 1.87, 1 H) of Leu and the carbonyl carbon (δ_C 174.49). The presence of the four protons (δ_H 7.92 d, J = 7.80, 1 H; 7.07 dd, J = 7.80, 7.14, 1 H; 7.16 dd, J = 7.98, 7.14, 1 H; and 7.49 d, J = 7.98, 1 H) and eight carbons (δ_C 124.50, 111.23, 128.02, 118.93, 119.09, 121.61, 111.83, 137.33) indicated the presence of a 3-substituted indolyl group. The HMBC correlations of proton resonance at δ_H 3.87 (2H, d, J = 7.14 Hz, H–β of Trp) with carbon resonances at δ_C 111.23, 124.50 and 128.02 identified the Trp residue. Furthermore, the carbonyl carbons of Trp was undoubtedly assigned to δ_C 172.60 based on HMBC correlations between carbonyl carbons and β protons of Trp residue. The presence of two hydroxymethylene signals in DEPT spectrum suggested that there two Ser residues in **1a** (δ_C 171.31, 56.41, and 62.14, and δ_C 170.38, 53.49, and 63.80). The two Ser residues could be confirmed by their spin system in TOCSY spectrum based on the correlations between the amide proton (δ_H 8.41, 1 H) and the protons of α-H (δ_H 4.97, 1 H), and β-H (δ_H 4.17, 2 H) and the correlations between the amide proton (δ_H 8.63, 1 H) and the protons of α-H (δ_H 5.36, 1 H), and β-H (δ_H 4.27, 2 H), β'-H (δ_H 4.08, 1 H). Thus, all the chemical shifts of the seven amino acid residues of **1a** were assigned (Table 1 in manuscript).

The HMBC correlation between Ser^2-NH (δ_H 8.41, 1 H) and Pro^1-CO (δ_C 171.98) indicated that the amide group of Ser^2 residue attached to the carbonyl group of Pro^1 residue *via* an amide bond. The linkage of Ser^2-Trp^3 was also confirmed by the HMBC correlation of Trp^3-NH/CO-Ser^2. Furthermore, based on the HMBC correlation between Leu^4-NH (δ_H 8.15, 1 H) and Trp^3-CO (δ_C 172.60), The Leu^4 residue was assigned to attach at the Trp^3 residue. The linkages of Val^5-Leu^4, Gly^6-Val^5 and Ser^7-Gly^6 were also determined by the following HMBC crosspeaks: Val^5-NH/CO-Leu^4, Gly^6-NH/CO-Val^5 and Ser^7-NH/CO-Gly^6 (Fig. 3 in manuscript). The plane structure of **1a** was deduced as cyclo(Pro^1-Ser^2-Trp^3-Leu^4-Val^5-Gly^6-Ser^7). Furthermore, the ROESY correlations of Pro^1-αH/NH-Ser^2, Pro^1-δH/NH-Ser^2, Ser^2-NH/NH-Trp^3, Trp^3-NH/NH-Leu^4, Leu^4-αH/NH-Val^5, Val^5-αH/NH-Gly^6, Gly^6-NH/NH-Ser^7, and Ser^7-αH/δH,δ'H-Pro^1 also supported the gross structure (Fig. 4 in manuscript). The presence of strong NOE correlations between α proton of Ser^7 and both of δ, δ' protons of Pro^1 suggested that the amide bond of Ser^7-Pro^1 was *trans*. The β and γ carbon chemical shifts of Pro^1 at 28.39

and 24.92 ppm further supported the presence of *trans* peptidyl-prolyl bond. The amino acid sequence of **1a** was established as cyclo(Pro[1]-Ser[2]-Trp[3]-Leu[4]-Val[5]-Gly[6]-Ser[7])

In **1b**, the same amino acid residues of Pro, Ser, Trp, Leu, Val, Gly, and Ser as those in **1a** were also deduced by 1D and 2D NMR experiments. The complete assignment for the ^{1}H and ^{13}C NMR data of **1b** (Table 2 in manuscript) was similar that of **1a**. In addition, based on the HMBC correlations of Ser[2]-NH (δ_H 8.83, 1 H) and Pro[1]-CO (δ_C 172.74), Trp[3]-NH (δ_H 9.73, 1 H) and Ser[2]-CO (δ_C 173.44), Leu[4]-NH (δ_H 8.45, 1 H) and Trp[3]-CO (δ_C 172.60), Val[5]-NH (δ_H 7.81, 1 H) and Leu[4]-CO (δ_C 173.67), Gly[6]-NH (δ_H 8.00, 1 H) and Val[5]-CO (δ_C 171.60), and Ser[7]-NH (δ_H 9.13, 1 H) and Gly[6]-CO (δ_C 170.63) (Fig. 3 in manuscript), the amino acid sequence of **1b** was then established to be identical with that of **1a**. The result was also supported by the ROESY correlations of Pro[1]-δH/NH-Ser[2], Trp[3]-NH/NH-Leu[4], Leu[4]-αH/NH-Val[5], Val[5]-NH/NH-Gly[6], Gly[6]-NH/NH-Ser[7], and Ser[7]-αH/αH-Pro[1] (Fig. 4 in manuscript). However, the β and γ carbon chemical shifts of Pro[1] in **1b** at δ_C 31.59 and 22.50 ppm respectively suggested the presence of *cis* peptidyl-prolyl bond in **1b**.[5] This was further confirmed by the strong NOE correlation between α proton of Pro[1] and α proton of Ser[7]. The result indicated that **1a** and **1b** were *cis*/*trans* peptidyl-prolyl bond isomers.

Identification the absolute configuration of amino acid residues of tunicyclin E (1)

50 μL of a 50 mM aqueous solution of L–configurations of Pro, Ser, Leu and Val were added 20 μL of 1 M sodium bicarbonate and then 100 μL of 25 mM L-FDLA (TCI, Japan) in acetone, respectively. The solutions were incubated at 37 °C for 60 min. Reactions were quenched by addition of 20 μL of 1 N HCl, respectively. Samples were diluted with 810 μL of acetonitrile, and 400 μL of the solutions were analyzed by HPLC-ESI-MS, respectively.

100 μL of 1 mg/mL **1** was hydrolyzed at 100 °C for 24 h by adding 200 μL of 10 N HCl. The solution was evaporated to dryness. Then, the residue was dissolved in 50 μL of water. The amino acid solution was added 20 μL of 1 M sodium bicarbonate and 50 μL of 25 mM L-FDLA in acetone. The solution was incubated at 37 °C for 60 min. Reaction

was quenched by addition of 20 μL of 1 N HCl. Sample was diluted with 810 μL of acetonitrile, and 400 μL of the solution was analyzed by HPLC-ESI-MS.

HPLC was performed on an Agilent 1100 system. Separations were carried out on a TSKgel ODS-100V column (150 × 4.6 mm i.d., 3 μm, TOSOH) maintained at 40 °C. Acetonitrile-0.01 M trifluoroacetic acid (TFA) was used as mobile phase under a linear gradient elution mode (acetonitrile, 20-100%, 80 min). The flow rate was 1 mL/min with detection at 340 nm by photodiode array detection and ESI-MS. The mass spectrometer used was a LC/MSD Trap XCT mass spectrometer (Agilent, USA). The auxiliary and sheath gas nitrogen pressure were set at 10 unit and 35 psi, respectively, and the capillary was heated to 350 °C. A mass range of *m/z* 100-1000 was covered with a scan time of 200 μs, and data were collected in negative ion mode.

The absolute configurations of Pro[1], Ser[2], Leu[4], Val[5] and Ser[7] residues of **1** were all identified as L configurations by comparison the retention times and *m/z* values of the chiral derivatives of the amino acid residues in acid hydrolysate of **1** with those of corresponding standard L-configuration amino acids (see Table 1).[2] Taken together with the relative configuration, established by X-ray crystallography, all amino acid residues had the L (*S*) configuration. Thus, **1** was determines as cyclo(L-Pro[1]-L-Ser[2]-L-Trp[3]-L-Leu[4]-L-Val[5]-L-Gly[6]-L-Ser[7]).

Table 1 Analysis of L-FDLA derivates of acid hydrolysate of **1** and those of standard L-configuration amino acids by HPLC-ESI-MS

Amino acid	t_{RL} (min)	t_R (min)	$[M-H]^-$ (*m/z*)	absolute configurations
Pro	24.54	24.54	408	L
Ser	20.34	20.37	398	L
Leu	31.98	31.97	424	L
Val	29.10	28.90	410	L

The crystal data of tunicyclin E (1)

Table 2 Crystal data and structure refinement for **1**

molecular formula	$C_{35}H_{62}N_8O_{15}$

molecular weight	834.93
crystal system	Orthorhombic
space group	P 2_1 2_1 2_1
Z, molecules / unit cell	4
unit cell dimensions	a = 10.798 (17) Å
	b = 15.09 (2) Å
	c = 27.46 (4) Å
volume	4473 (12) Å ^{3}
calculated density	1.240 g/cm^3
wavelength	0.71073 Å
Reflections collected / unique	17448 / 5080 [R(int) = 0.0791]
Final R indices [I>2σ(I)]	R_1 = 0.0810, wR_2 = 0.1884
Temperature	293(2) K

Table 3 Final atomic parameters (×10^4) and equivalent isotropic displacement parameters (Å^2×10^3) for **1**

atom	*x*	*y*	*z*	*U*(eq)	atom	*x*	*y*	*z*	*U*(eq)
N_1	5657 (1)	184 (1)	4490 (1)	52 (1)	C_4^{γ}	9178 (2)	4777 (1)	3495 (1)	122 (1)
C_1^{α}	4523 (1)	142 (1)	4779 (1)	57 (1)	C_4^{δ}	10570 (2)	4577 (2)	3436 (1)	190 (1)
C_1^{β}	4848 (2)	-567 (1)	5153(1)	90 (1)	$C_4^{\delta'}$	8893 (4)	5672 (2)	3284 (1)	197 (2)
C_1^{γ}	6170 (2)	-467 (2)	5229 (1)	136 (1)	C_4'	6479 (2)	3334 (1)	3008 (1)	63 (1)
C_1^{δ}	6727 (2)	-230 (1)	4742 (1)	77 (1)	O_4'	6252 (1)	2611 (1)	3210 (1)	75 (1)
C_1'	4185 (1)	996 (1)	5036 (1)	47 (1)	N_5	6272 (1)	3462 (1)	2534 (1)	77 (1)
O_1'	3344 (1)	992 (1)	5335 (1)	65 (1)	C_5^{α}	5759 (2)	2738 (1)	2238 (1)	72 (1)
N_2	4829 (1)	1730 (1)	4924 (1)	45 (1)	C_5^{β}	5581 (2)	3070 (2)	1709 (1)	102 (1)
C_2^{α}	4603 (1)	2569 (1)	5178 (1)	46 (1)	C_5^{γ}	4747 (2)	2453 (2)	1434 (1)	145 (1)
C_2^{β}	5576 (1)	2757 (1)	5567 (1)	57 (1)	$C_4^{\gamma'}$	6768 (2)	3217 (2)	1440 (1)	124 (1)
O_2^{γ}	6736 (1)	2963 (1)	5383 (1)	71 (1)	C_5'	6589 (2)	1926 (1)	2283 (1)	72 (1)
C_2'	4373 (1)	3310 (1)	4817 (1)	45 (1)	O_5'	7740 (1)	1972 (1)	2277 (1)	98 (1)
O_2'	3351 (1)	3425 (1)	4645 (1)	98 (1)	N_6	6013 (1)	1137 (1)	2315 (1)	79 (1)
N_3	5319 (1)	3841 (1)	4699 (1)	46 (1)	C_6^{α}	6647 (2)	314 (1)	2376 (1)	88 (1)
C_2^{α}	5161 (1)	4633 (1)	4404 (1)	45 (1)	C_6'	6914 (2)	33 (1)	2897 (1)	78 (1)
C_2^{β}	5920 (1)	5401 (1)	4616 (1)	50 (1)	O_6'	7382 (2)	-684 (1)	2986 (1)	134 (1)
$N_3^{1'}$	5265 (1)	5832 (1)	5905 (1)	95 (1)	N_7	6640 (1)	632 (1)	3239 (1)	66 (1)
$C_3^{2'}$	5879 (2)	5402 (1)	5554 (1)	82 (1)	C_7^{α}	6875 (1)	493 (1)	3750 (1)	58 (1)
$C_3^{3'}$	5493 (1)	5705 (1)	5111 (1)	55 (1)	C_7^{β}	7599 (1)	1266 (1)	3963 (1)	64 (1)
$C_3^{3a'}$	4583 (1)	6364 (1)	5198 (1)	55 (1)	O_7^{γ}	6865 (1)	1984 (1)	4113 (1)	74 (1)
$C_3^{4'}$	3861 (1)	6893 (1)	4908 (1)	66 (1)	C_7'	5624 (1)	412 (1)	4013 (1)	56 (1)
$C_3^{5'}$	3029 (2)	7483 (1)	5128 (1)	104 (1)	O_7'	4648 (1)	595 (1)	3809 (1)	77 (1)
$C_3^{6'}$	2928 (2)	7524 (1)	5619 (1)	118 (1)			Solvent		

$C_3^{7'}$	3642 (2)	7018 (1)	5920 (1)	105 (1)	O_W^1	3548 (2)	285 (1)	2882 (1)	143 (1)
$C_3^{7a'}$	4455 (2)	6443 (1)	5703 (1)	73 (1)	O_W^2	9667 (2)	3281 (1)	2083 (1)	206 (1)
C_3'	5493 (2)	4497 (1)	3874 (1)	63 (1)	O_W^3	5889 (2)	4962 (2)	6902 (1)	238 (1)
O_3'	4795 (1)	4739 (1)	3559 (1)	127 (1)	O_W^4	8043 (2)	-2141 (1)	2476 (1)	222 (1)
N_4	6616 (1)	4155 (1)	3774 (1)	59 (1)	O_W^5	9762 (2)	1623 (2)	2935 (1)	217 (1)
C_4^{α}	7081 (2)	4107 (1)	3273 (1)	67 (1)	O_W^6	3148 (3)	2666 (2)	3493 (1)	220 (2)
C_4^{β}	8483 (2)	4021 (1)	3268 (1)	84 (1)	O_W^7	3468 (6)	1386 (2)	2320 (2)	281 (2)

[a]*U*(eq) is defined as one third of the trace of the orthogonalized Uij tensor

Determination of the key interproton distances of 1a and 1b in solution by off-resonance experiment

For determination of proton-proton distance, an off-resonance ROESY experiment was performed by employing ROESYPHPR.2 pulse sequence to suppress the TOCSY type magnetization transfer: π = 200 μs, - π = 200 μs, total mixing time 200 ms, zero filling in F2 to 8 K and in F1 to 2 K.

The quantitative measured the interproton-distance using the equation:

$$r_{ij} = r_{\mathrm{ref}} \left(\frac{a_{\mathrm{ref}} c_{\mathrm{ref}}}{a_{ij} c_{ij}} \right)^{1/6}$$

The correction factor, $c_{ij,}$ was given by

$$c_{ij} = \frac{1}{(\sin^2\theta_i \sin^2\theta_j)}$$

with

$$\tan\,\theta_i = \frac{\gamma B_1}{(\omega_i - \omega_0)}.$$

a_{ij} was the volume intergration of ROESY cross peak of nucleus *i* and *j*, B_1 was the field strength of locking field (γB_1 = 2500 Hz), and (ω_i-ω_0) was the resonance offset of nucleus *i*. The interprotpon-distance were calibrated with respect to the distance of the

indole NH proton to the 7'H proton (r_{ref} = 282 pm). All the unambiguous interproton-distance of **1a**, **1b** were obtained and shown in Table 4.

Table 4 The key interproton distances (in Å) obtained from the ROESY spectrum of **1a**, **1b** and those from crystal structure of **1**

1a		**1b**		**Crystal**	
Involved protons	distance	Involved protons	distance	Involved protons	distance
Pro^1 Hα – Ser^2 NH	2.81	Pro^1 Hα – Ser^7 Hα	2.20	Pro^1 Hα – Ser^2 NH	3.10
Pro^1 Hδ – Ser^2 NH	3.05	Pro^1 Hδ – Ser^2 NH	3.03	Pro^1 Hδ – Ser^2 NH	3.29
Pro^1 Hδ – Ser^7 Hα	2.47	Ser^2 Hα – Trp^3 NH	2.27	Pro^1 Hδ – Ser^7 Hα	2.72
Pro^1 Hδ – Ser^7 Hβ	2.15	Ser^2 Hβ – Val^5 NH	3.12	Pro^1 Hδ – Ser^7 Hβ	2.10
Pro^1 Hδ' – Ser^7 Hα	2.08	Ser^2 Hβ – Gly^6 NH	3.14	Pro^1 Hδ' – Ser^7 Hα	2.40
Pro^1 Hδ' – Ser^7 Hβ	3.00	Trp^3 NH – Leu^4 NH	2.39	Pro^1 Hδ' – Ser^7 Hβ	3.09
Ser^2 NH – Trp^3 NH	2.99	Trp^3 Hα – Leu^4 NH	2.82	Ser^2 NH – Trp^3 NH	3.24
Ser^2 NH – Ser^7 Hβ	3.30	Trp^3 Hα – Gly^6 NH	3.47	Ser^2 NH – Ser^7 Hβ	3.39
Ser^2 Hα – Trp^3 NH	2.73	Trp^3 Hβ' – Leu^4 NH	3.07	Ser^2 Hα – Trp^3 NH	3.40
Trp^3 NH – Leu^4 NH	2.69	Leu^4 NH – Val^5 NH	2.32	Trp^3 NH – Leu^4 NH	2.56
Trp^3 Hα – Leu^4 NH	3.14	Leu^4 Hα – Val^5 NH	3.03	Trp^3 Hα – Leu^4 NH	3.49
Leu^4 Hα – Val^5 NH	2.56	Val^5 NH – Gly^6 NH	2.34	Leu^4 Hα – Val^5 NH	2.29
Val^5 Hα – Gly^6 NH	2.23	Gly^6 NH – Val^5 Hα	2.93	Val^5 Hα – Gly^6 NH	2.41
Val^5 Hα – Ser^7 NH	3.73	Gly^6 NH – Ser^7 NH	3.19	Val^5 Hα – Ser^7 NH	3.48
Gly^6 NH – Ser^7 NH	2.28			Gly^6 NH – Ser^7 NH	2.49
Ser^7 Hα – Ser^7 Hβ	2.28			Ser^7 Hα – Ser^7 Hβ	2.22

Determination of the thermodynamics and kinetics parameters of *cis*/*trans* isomerization of 1 by inversion-magnetization transfer experiment

To clearly determine the thermodynamics and kinetics parameters, the pair of *cis*/*trans* proton resonance of Ser^7-NH was subjected. The proton resonance of Ser^7-NH in **1a** (*trans*) was selectively inverted with the pulse sequence: $90°_x - \tau - 90°_x - t - 90°_{\pm x, \pm y}$ – acquisition, where the τ is a fixed delay equal to $1/(2|\upsilon_{cis} - \upsilon_{trans}|)$ and *t* is a variable delay during which transfer of magnetization takes place by interchange between the *cis* and *trans* isomers. The intensity of proton resonance of Ser^7-NH in **1b** was measured as a function of *t*. *t* values were varied from 0.001 to 24 s. Analysis of the time-depended

intensity of the *cis*/*trans* pair of Ser^{7}-NH to obtain rate constants was similar to that developed by Alger et al. (J. R. Alger, J. H. Prestegard, *J. Magn. Reson.* **1977**, 27, 137).

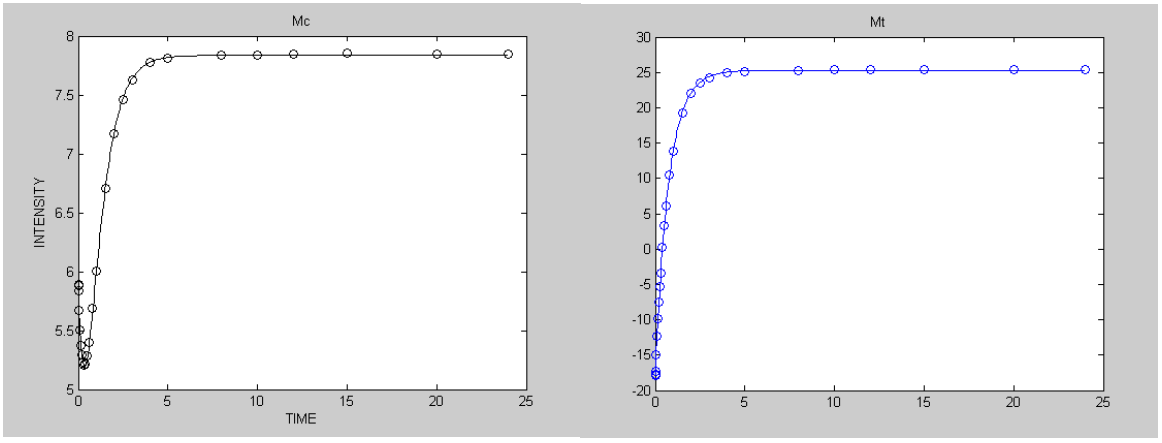


Fig. 1 Inversion-magnetization transfer of **1** at 300 K

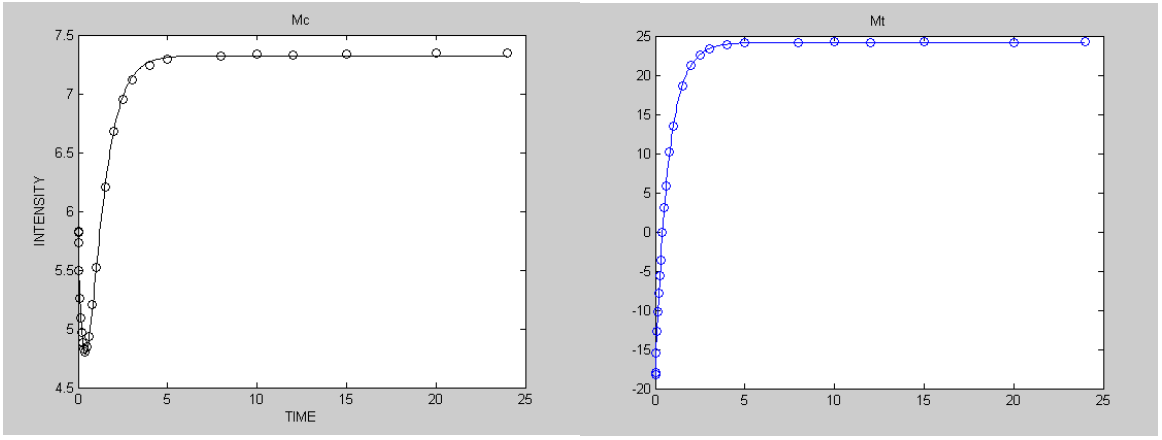


Fig. 2 Inversion-magnetization transfer of **1** at 305 K

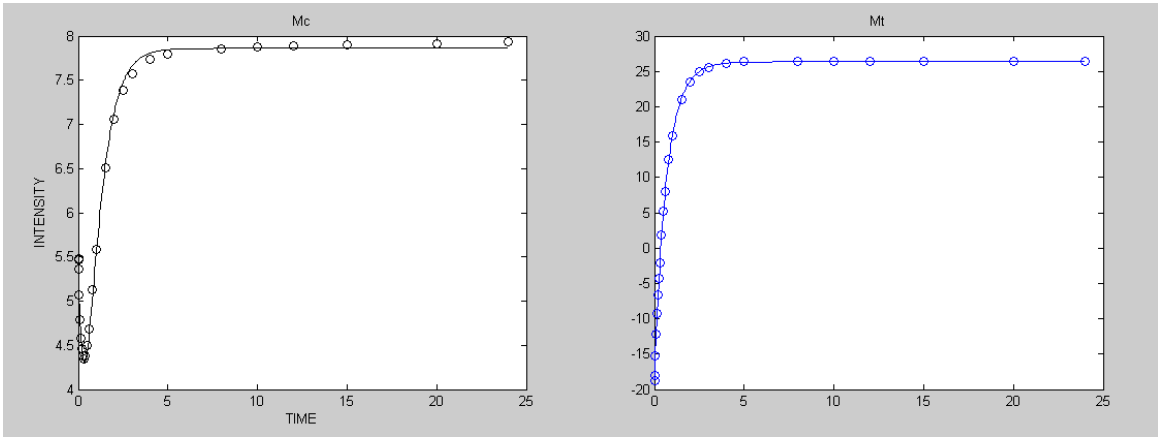


Fig. 3 Inversion-magnetization transfer of **1** at 310 K

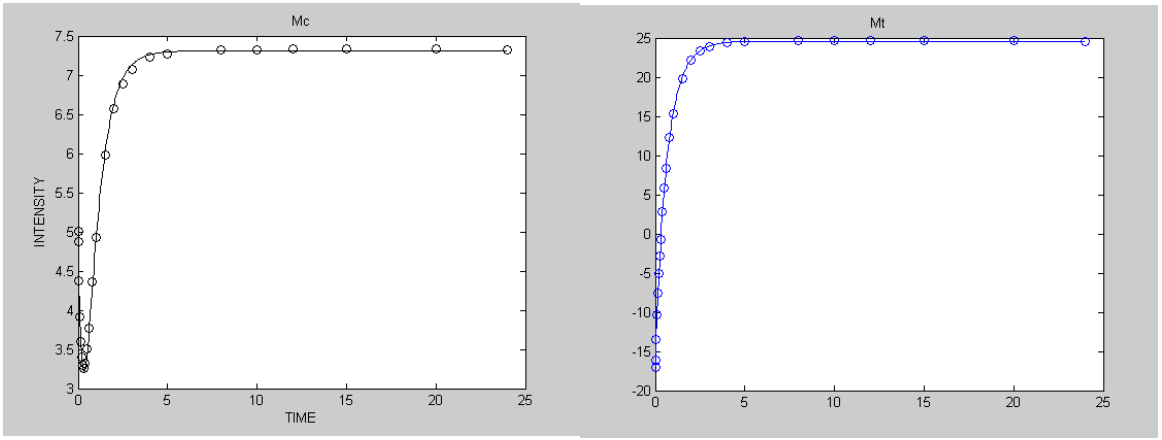


Fig. 4 Inversion-magnetization transfer of **1** at 315 K

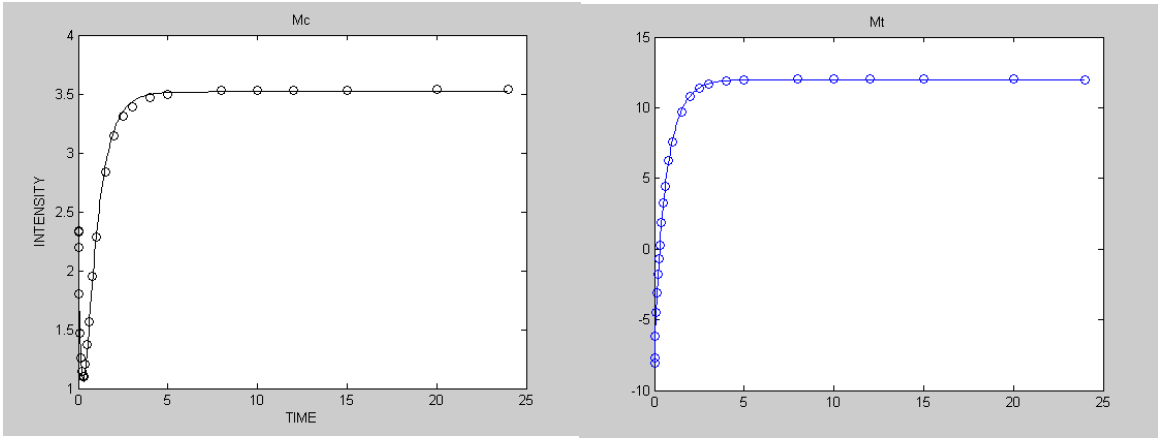


Fig. 5 Inversion-magnetization transfer of **1** at 320 K

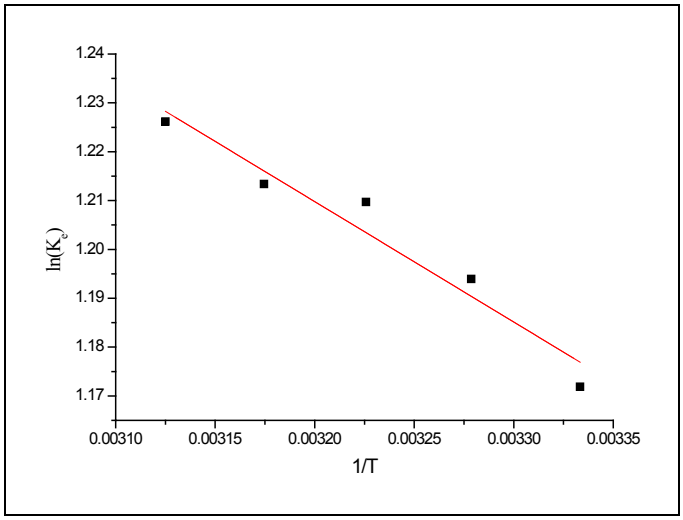


Fig. 6 Van't Hoff plot for the *cis*/*trans* isomerization of **1**

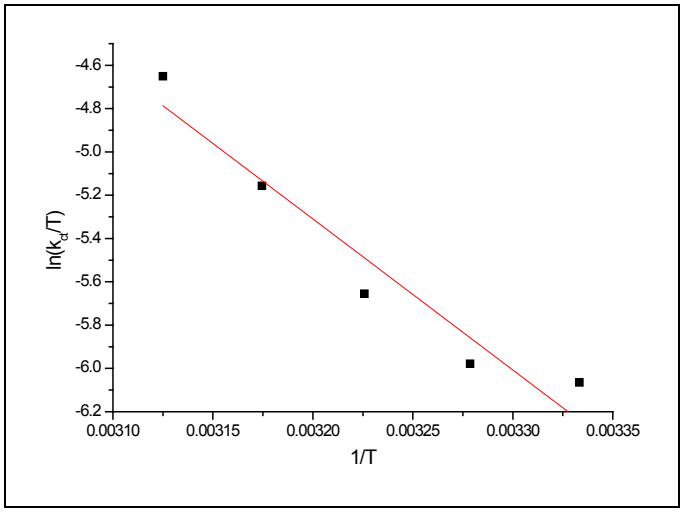


Fig. 7 Erying plot for *cis*-to-*trans* isomerization of **1**

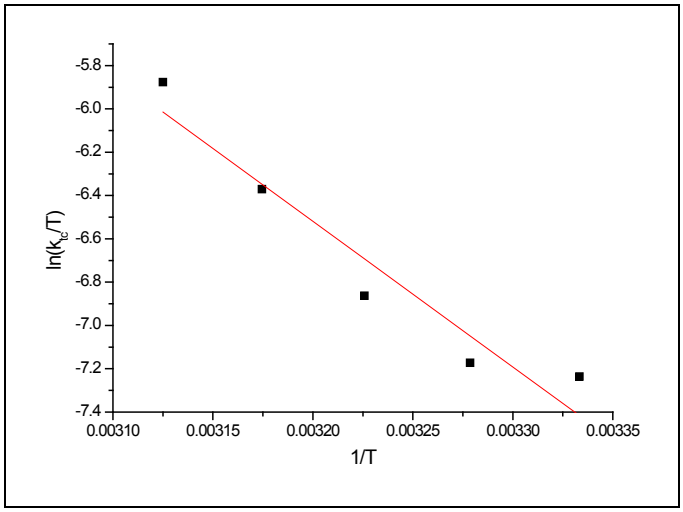
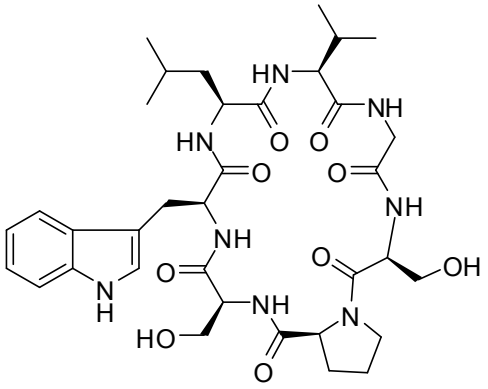
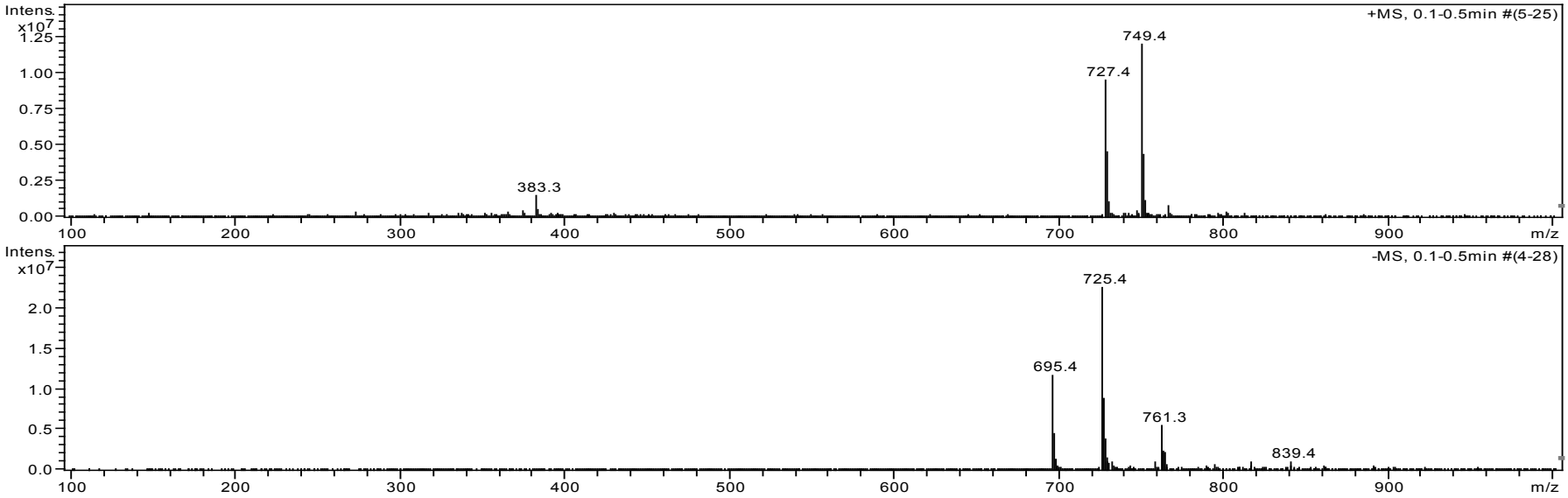


Fig. S8 Erying plot for *trans*-to-*cis* isomerization of **1**

ESI-MS spectrum of tunicyclin E (**1**)





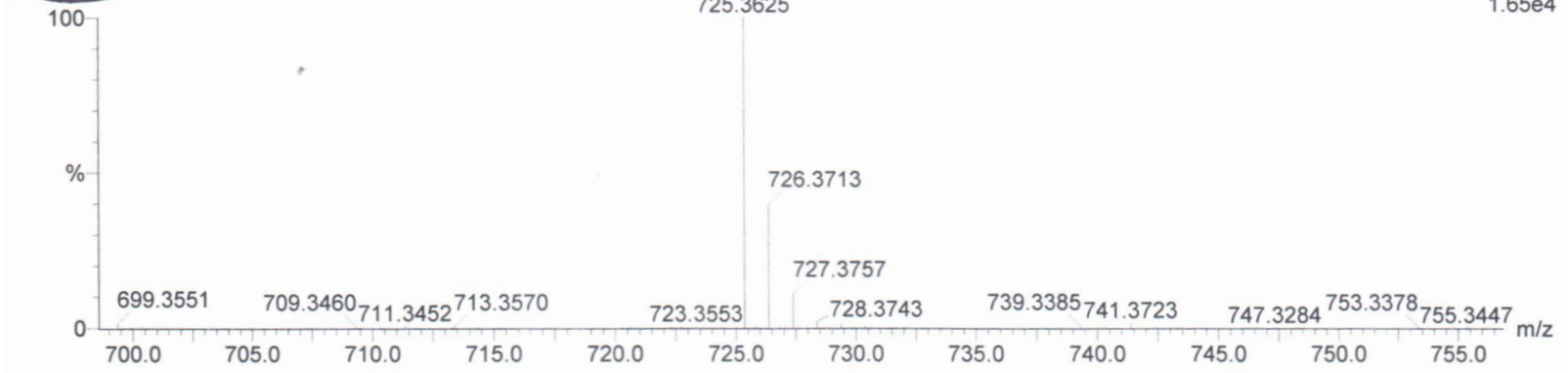
HR-ESI-MS spectrum of tunicyclin E (**1**)

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: None

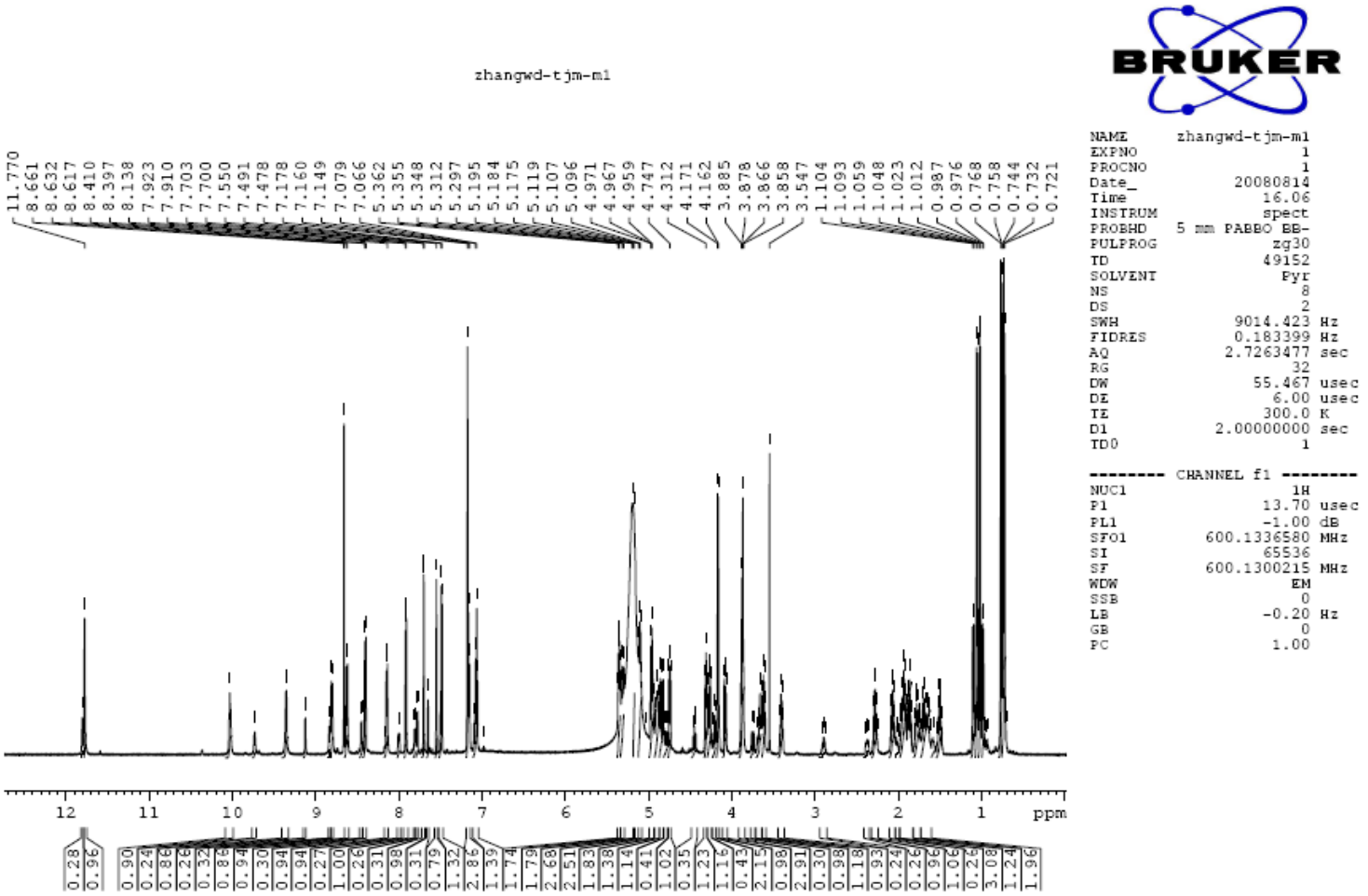
Monoisotopic Mass, Even Electron Ions
2 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 34-35 H: 47-50 N: 7-8 O: 9-10 Na: 0-1

JTS-M1 13 (0.143) AM (Cen,4, 80.00, Ar,5000.0,568.28,0.47,LS 20); Sm (SG, 2x3.00); Cm (6:16) 1: TOF MS ES-
1.65e4

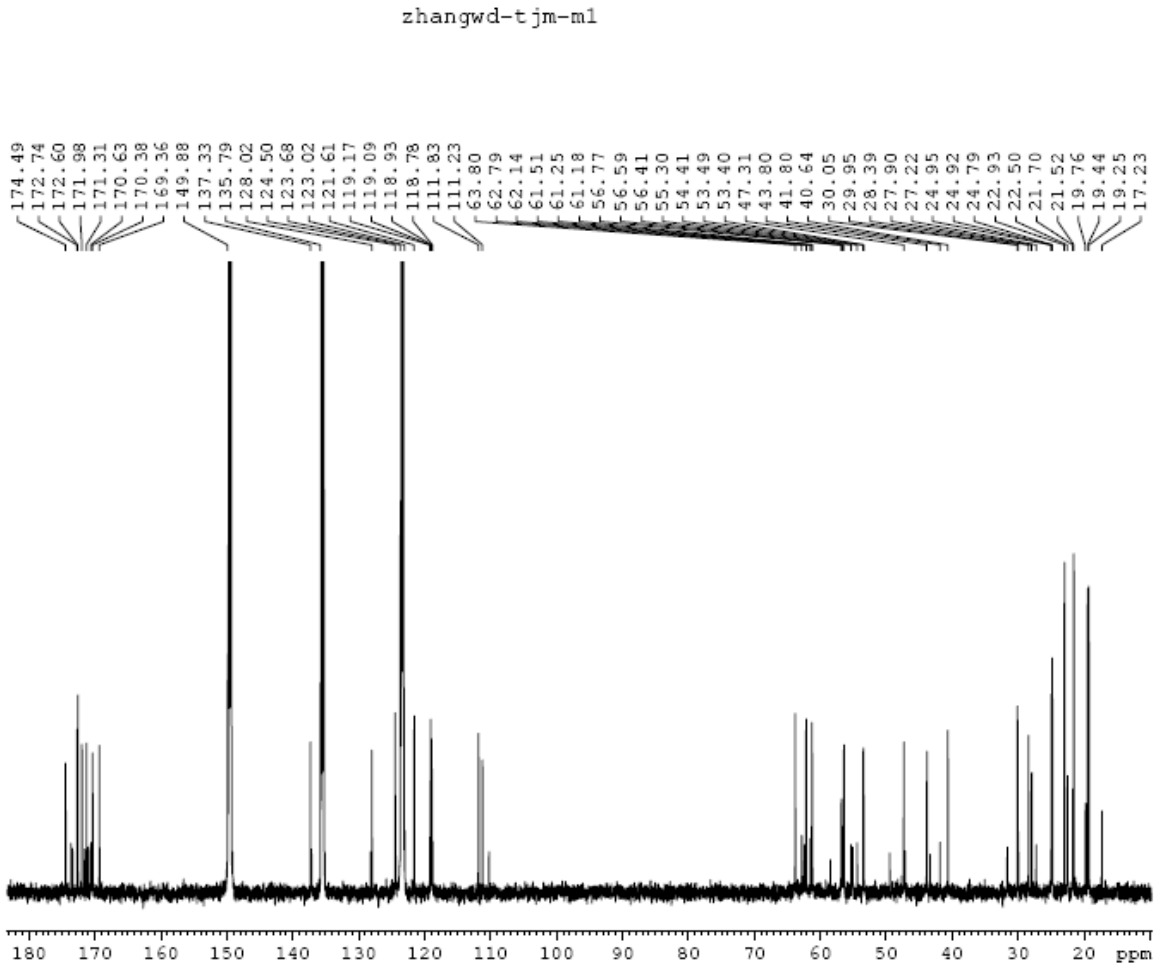


Minimum:				-1.5		
Maximum:		5.0	50.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
725.3625	725.3623	0.2	0.3	15.5	16.8	C35 H49 N8 O9

^{1}H NMR spectrum of tunicyclin E (**1**) (in C_5D_5N)

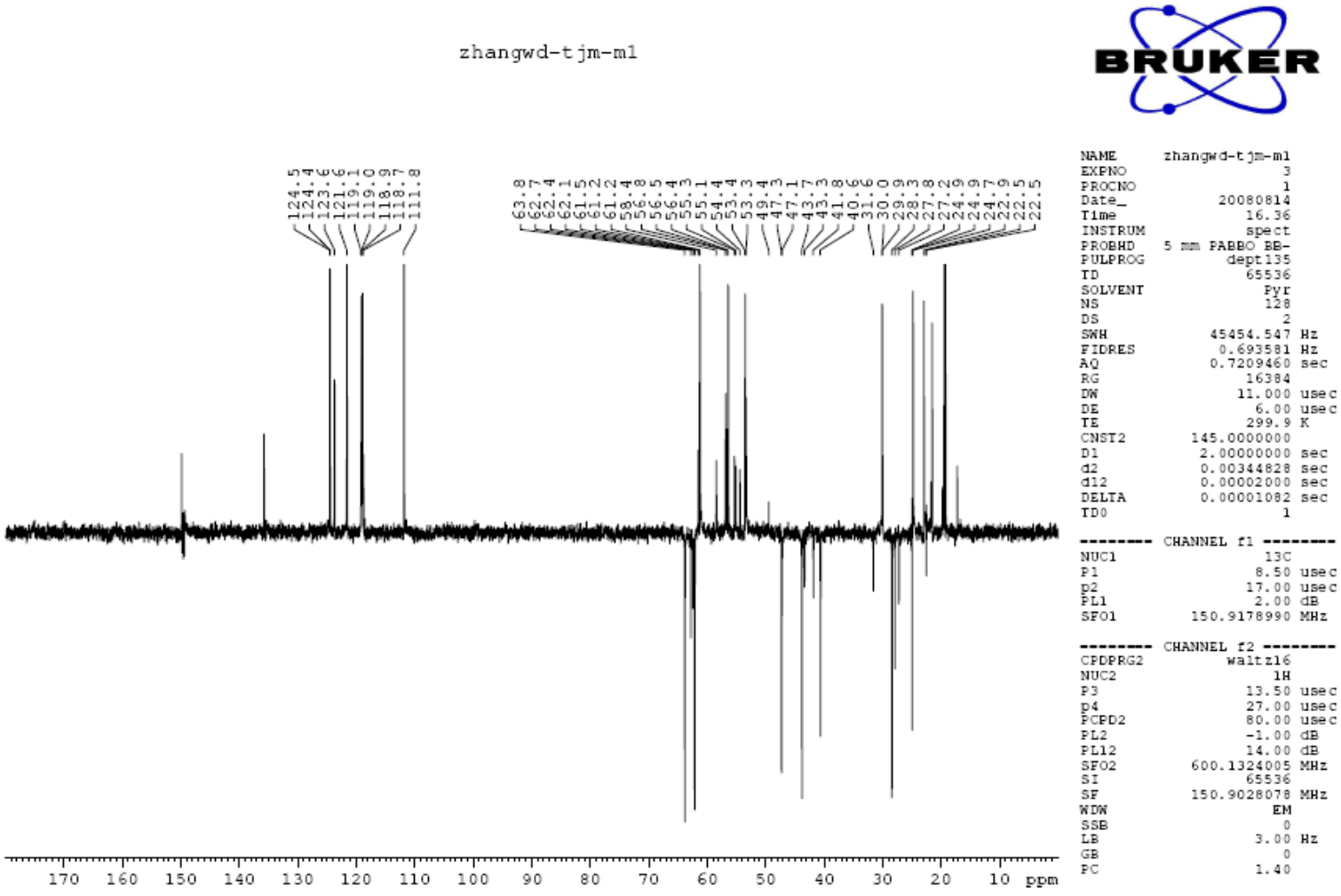


^{13}C NMR spectrum of tunicyclin E (**1**) (in C_5D_5N)



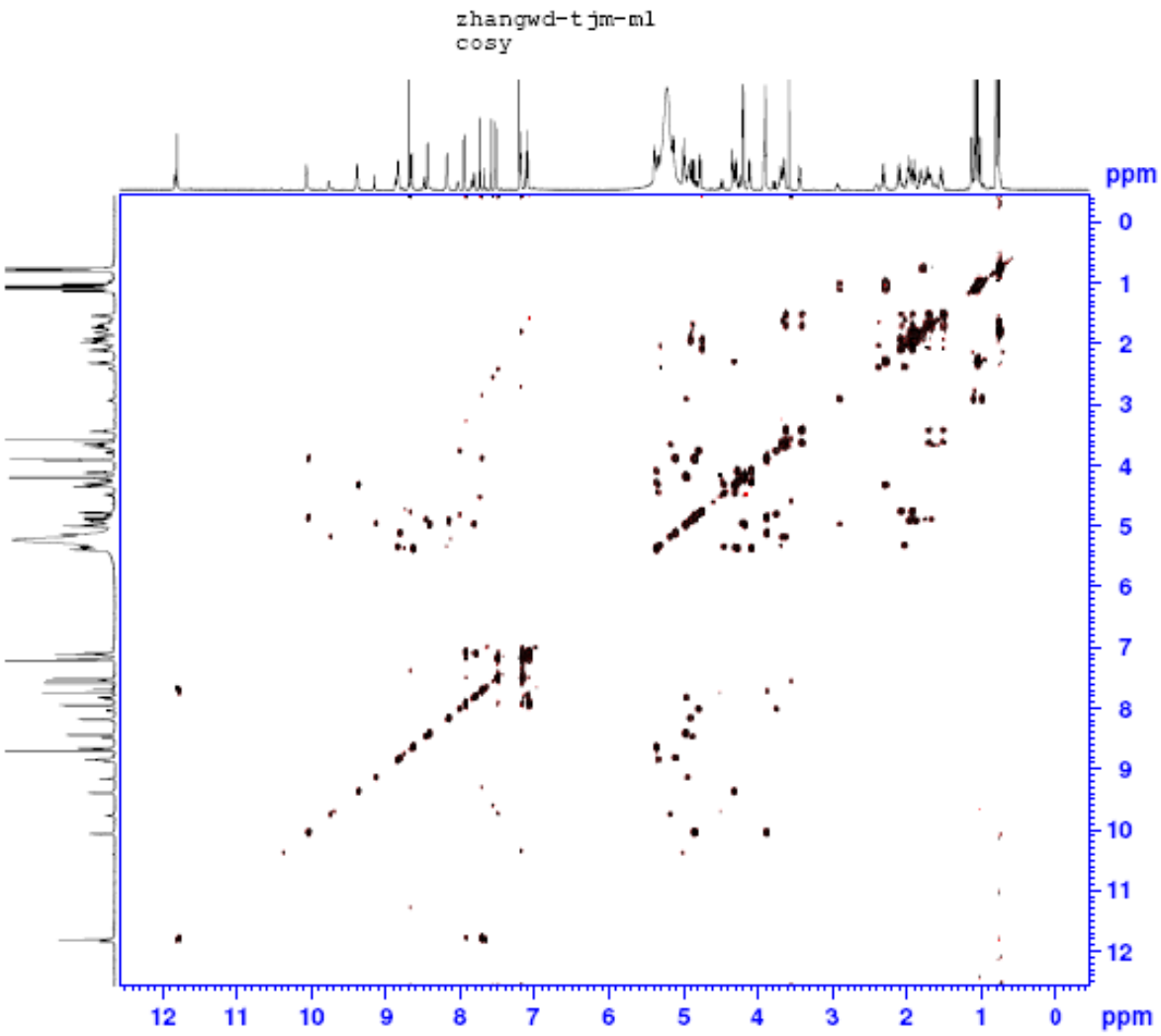
NAME	zhangwd-tjm-m1
EXPNO	2
PROCNO	1
Date_	20080814
Time	16.29
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgpg30
TD	65536
SOLVENT	Pyr
NS	446
DS	0
SWH	45454.547 Hz
FIDRES	0.693581 Hz
AQ	0.7209460 sec
RG	32768
DW	11.000 usec
DE	6.00 usec
TE	300.1 K
D1	2.00000000 sec
d11	0.03000000 sec
DELTA	1.89999998 sec
TD0	1
--------	CHANNEL f1 --------
NUC1	13C
P1	8.50 usec
PL1	2.00 dB
SFO1	150.9178990 MHz
--------	CHANNEL f2 --------
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	-1.00 dB
PL12	14.00 dB
PL13	14.00 dB
SFO2	600.1324005 MHz
SI	65536
SF	150.9028078 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40

DEPT spectrum of tunicyclin E (**1**) (in C_5D_5N)



NAME	zhangwd-tjm-m1	
EXPNO	3	
PROCNO	1	
Date_	20080814	
Time	16.36	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	dept135	
TD	65536	
SOLVENT	Pyr	
NS	128	
DS	2	
SWH	45454.547	Hz
FIDRES	0.693581	Hz
AQ	0.7209460	sec
RG	16384	
DW	11.000	usec
DE	6.00	usec
TE	299.9	K
CNST2	145.0000000	
D1	2.00000000	sec
d2	0.00344828	sec
d12	0.00002000	sec
DELTA	0.00001082	sec
TD0	1	
--------	CHANNEL f1 --------	
NUC1	13C	
P1	8.50	usec
p2	17.00	usec
PL1	2.00	dB
SFO1	150.9178990	MHz
--------	CHANNEL f2 --------	
CPDPRG2	waltz16	
NUC2	1H	
P3	13.50	usec
p4	27.00	usec
PCPD2	80.00	usec
PL2	-1.00	dB
PL12	14.00	dB
SFO2	600.1324005	MHz
SI	65536	
SF	150.9028078	MHz
WDW	EM	
SSB	0	
LB	3.00	Hz
GB	0	
PC	1.40	

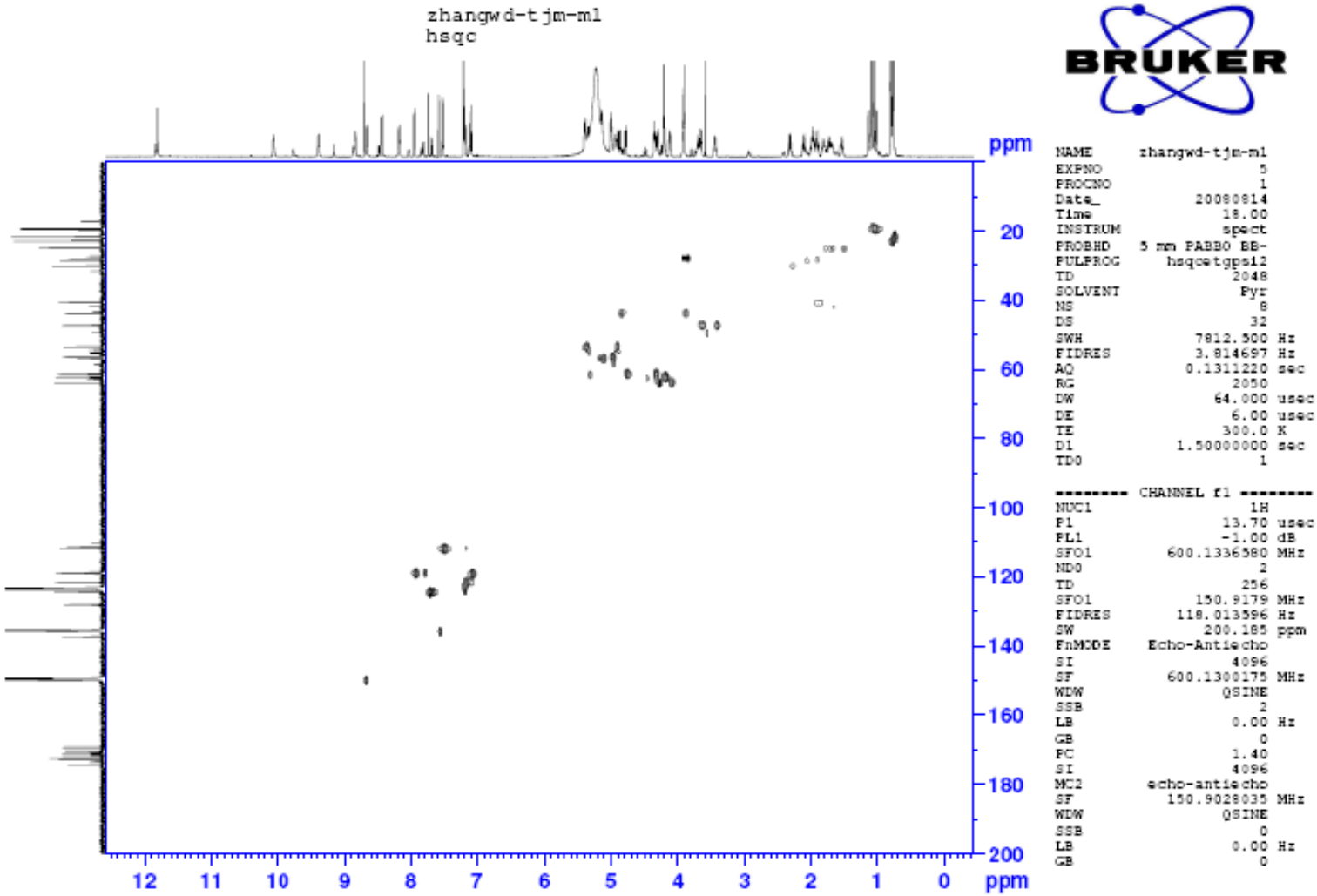
COSY spectrum of tunicyclin E (**1**) (in C_5D_5N)



BRUKER

NAME	zhangwd-tjm-m1	
EXPNO	4	
PROCNO	1	
Date_	20080814	
Time	16.40	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	cosygpmfph	
TD	4096	
SOLVENT	Pyr	
NS	4	
DS	16	
SWH	7812.500	Hz
FIDRES	1.907349	Hz
AQ	0.2621940	sec
RG	2050	
DW	64.000	usec
DE	6.00	usec
TE	300.0	K
D1	2.00000000	sec
TD0	1	
========	CHANNEL f1	========
NUC1	1H	
P1	13.70	usec
PL1	-1.00	dB
SFO1	600.1336580	MHz
ND0	1	
TD	512	
SFO1	600.1337	MHz
FIDRES	15.258789	Hz
SW	13.018	ppm
FnMODE	States-TPPI	
SI	4096	
SF	600.1300218	MHz
WDW	QSINE	
SSB	0	
LB	0.00	Hz
GB	0	
PC	1.40	
SI	2048	
MC2	States-TPPI	
SF	600.1300183	MHz
WDW	QSINE	
SSB	0	
LB	0.00	Hz
GB	0	

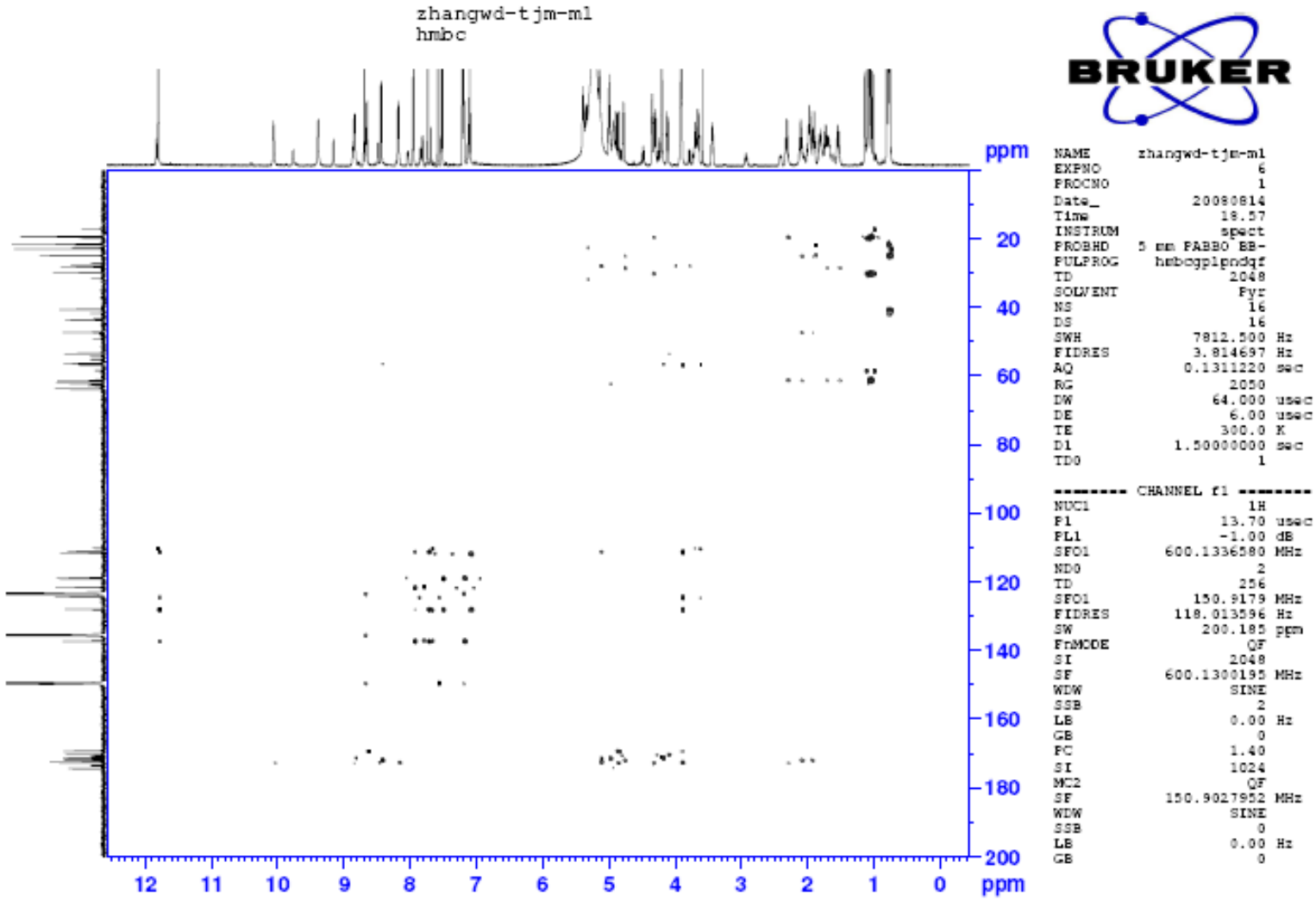
HSQC spectrum of tunicyclin E (**1**) (in C_5D_5N)



BRUKER

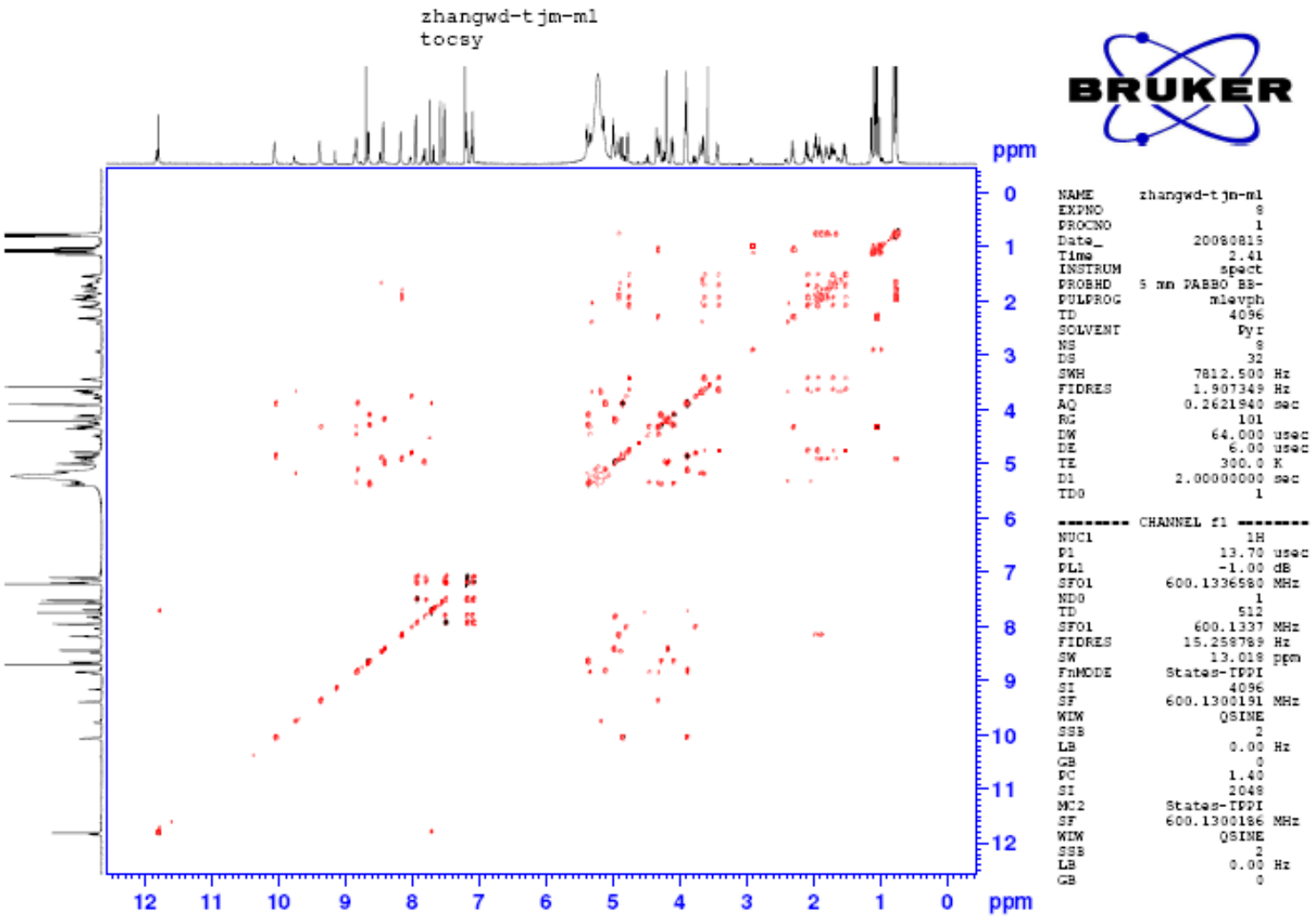
NAME	zhangwd-tjm-ml	
EXPNO	5	
PROCNO	1	
Date_	20080814	
Time	18.00	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	hsqcetgpsi2	
TD	2048	
SOLVENT	Pyr	
NS	8	
DS	32	
SWH	7812.500	Hz
FIDRES	3.814697	Hz
AQ	0.1311220	sec
RG	2050	
DW	64.000	usec
DE	6.00	usec
TE	300.0	K
D1	1.50000000	sec
TD0	1	
========	CHANNEL f1 ========	
NUC1	1H	
P1	13.70	usec
PL1	-1.00	dB
SFO1	600.1336580	MHz
ND0	2	
TD	256	
SFO1	150.9179	MHz
FIDRES	118.013596	Hz
SW	200.185	ppm
FnMODE	Echo-Antiecho	
SI	4096	
SF	600.1300175	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
PC	1.40	
SI	4096	
MC2	echo-antiecho	
SF	150.9028035	MHz
WDW	QSINE	
SSB	0	
LB	0.00	Hz
GB	0	

HMBC spectrum of tunicyclin E (**1**) (in C_5D_5N)



NAME	zhangwd-tjm-ml	
EXPNO	6	
PROCNO	1	
Date_	20080814	
Time	18.57	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	hmbcgplpndqf	
TD	2048	
SOLVENT	Pyr	
NS	16	
DS	16	
SWH	7812.500	Hz
FIDRES	3.814697	Hz
AQ	0.1311220	sec
RG	2050	
DW	64.000	usec
DE	6.00	usec
TE	300.0	K
D1	1.50000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	1H	
P1	13.70	usec
PL1	-1.00	dB
SFO1	600.1336580	MHz
ND0	2	
TD	256	
SFO1	150.9179	MHz
FIDRES	118.013596	Hz
SW	200.185	ppm
FnMODE	QF	
SI	2048	
SF	600.1300195	MHz
WDW	SINE	
SSB	2	
LB	0.00	Hz
GB	0	
PC	1.40	
SI	1024	
MC2	QF	
SF	150.9027952	MHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	

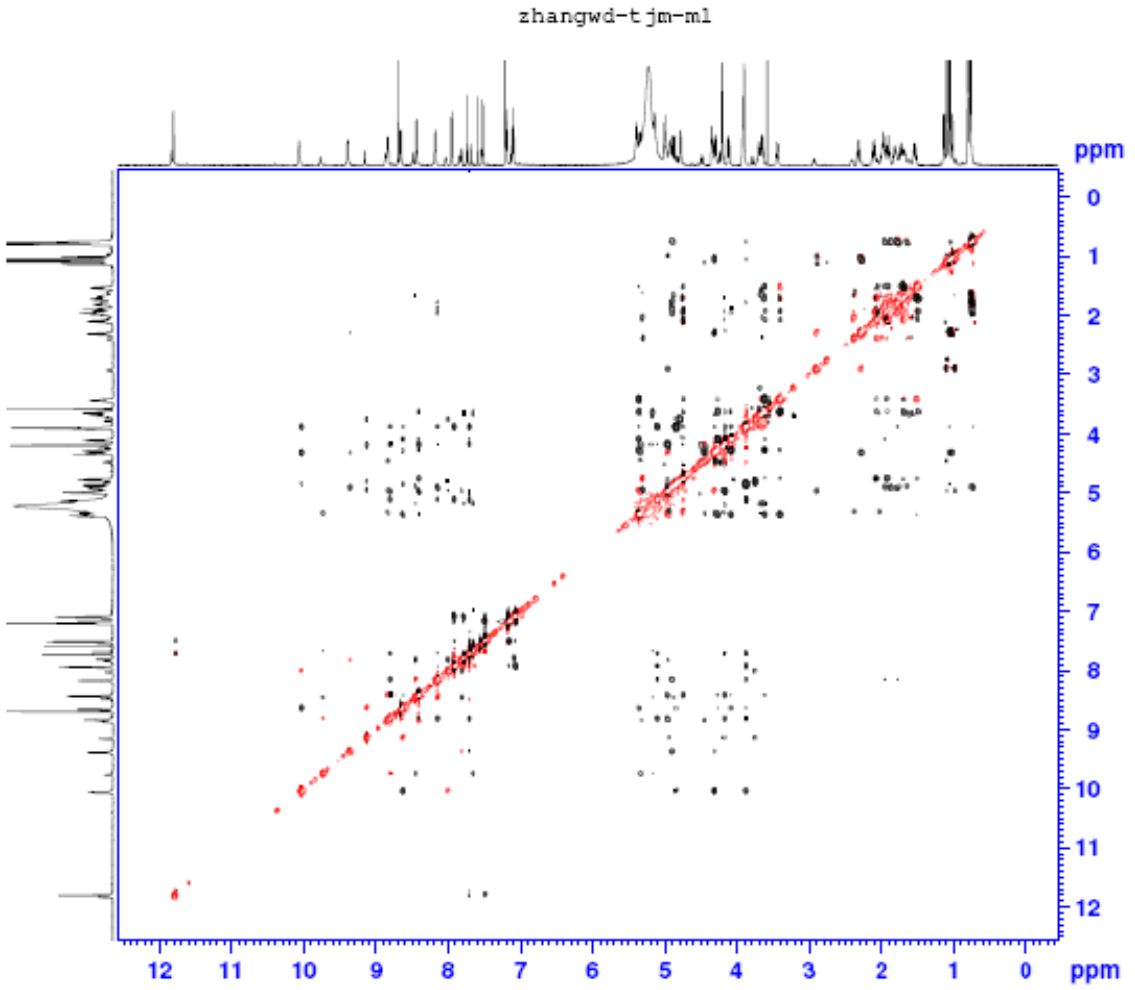
TOCSY spectrum of tunicyclin E (**1**) (in C_5D_5N)



BRUKER

NAME	zhangwd-tjm-ml
EXPNO	8
PROCNO	1
Date_	20080815
Time	2.41
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	mlevph
TD	4096
SOLVENT	Pyr
NS	8
DS	32
SWH	7812.500 Hz
FIDRES	1.907349 Hz
AQ	0.2621940 sec
RG	101
DW	64.000 usec
DE	6.00 usec
TE	300.0 K
D1	2.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	13.70 usec
PL1	-1.00 dB
SFO1	600.1336580 MHz
ND0	1
TD	512
SFO1	600.1337 MHz
FIDRES	15.258789 Hz
SW	13.018 ppm
FnMODE	States-TPPI
SI	4096
SF	600.1300191 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.40
SI	2048
MC2	States-TPPI
SF	600.1300186 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

ROESY spectrum of tunicyclin E (**1**) (in C_5D_5N)



BRUKER

NAME	zhangwd-tjm-m1	
EXPNO	7	
PROCNO	1	
Date_	20080814	
Time	20.55	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	roesyphpp.2	
TD	4096	
SOLVENT	Pyr	
NS	16	
DS	32	
SWH	7812.500	Hz
FIDRES	1.907349	Hz
AQ	0.2621940	sec
RG	203	
DW	64.000	usec
DE	6.00	usec
TE	300.0	K
D1	2.00000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	1H	
P1	13.70	usec
PL1	-1.00	dB
SFO1	600.1336580	MHz
ND0	1	
TD	512	
SFO1	600.1337	MHz
FIDRES	15.258789	Hz
SW	13.018	ppm
FnMODE	States-TPPI	
SI	4096	
SF	600.1300215	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
PC	1.00	
SI	2048	
MC2	States-TPPI	
SF	600.1300215	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	