Total Assignment of the ^{1}H- and ^{13}C-NMR Spectra for TZT-1027 and Related Compounds

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The total assignment of the ^{1}H- and ^{13}C-NMR spectra for TZT-1027 was carried out using various NMR methods (1D, 2D NMR). It was found that TZT-1027 exists in two different conformations resulting from the *cis–trans* isomerization of the amide bond at N-11 and C-12 in DMSO-d_6. The ^{1}H- and ^{13}C-NMR spectra of compound 1 and 2 comprised of the partial structure of TZT-1027 were also assigned to be TZT-1027. These assignments showed that compound 1 is in good agreement with TZT-1027 with regard to formation of the conformers.

Key words TZT-1027; antitumor agent; NMR assignment; *cis–trans* isomerization

TZT-1027[1] derived from research on the structural requirements for the antitumor activity of dolastatin 10,[2] a pentapeptide isolated from the marine mollusk *Dolabella auricularia*, is a potent antimicrotubule agent. A number of analogues of dolastatin 10 have been synthesized.[3] To our knowledge, the detailed assignments of the NMR spectra have only been reported for dolastatin 10[4,5] and its 6*R*-isomer.[6]

TZT-1027 possesses a unique chemical structure and a broad spectrum of antitumor activity different from those of other clinically available drugs.[7] TZT-1027 inhibits the polymerization of microtubule protein and purified tubulin. The identification of tubulin interaction sites with ligands is very important from the standpoint of revealing the mechanism of tubulin polymerization.[8] Assignment of the ^{1}H- and ^{13}C-NMR spectra of TZT-1027 would not only be useful in explaining its physicochemical properties, but also helpful for analyzing the conformation of TZT-1027 in solutions. However, the NMR spectra of TZT-1027 are highly complicated because of the chemical shift equivalence of methine and methyl groups of this drug. In addition, it is expected that the conformers of TZT-1027 are formed due to *cis–trans* isomerization around the amide bonds. In this work, the complete ^{1}H- and ^{13}C-NMR signals of TZT-1027 and related compounds were assigned by analysis of the 1D and 2D NMR spectra [distortionless enhancement by polarization transfer (DEPT), double quantum filtered correlation spectroscopy (DQF-COSY), phase sensitive ^{13}C–^{1}H correlation spectroscopy (C–H COSY), heteronuclear multiple bond coherence (HMBC), and rotating frame nuclear Overhauser and exchange spectroscopy (ROESY)] in DMSO-d_6. It was found that TZT-1027 exists in two different conformations resulting from the *cis–trans* isomerization of the amide bond at N-11 and C-12 in DMSO-d_6.

Results and Discussion

The NMR spectra of TZT-1027 (Fig. 1) dissolved in DMSO-d_6 were recorded at 303 K (Table 1). Under these conditions, two distinct signal species were observed (intensity rate: conformer I/conformer II=1/1). This ratio of conformers was altered by change of a solvent such as $CDCl_3$ or CD_3OD. The ^{1}H- and ^{13}C-NMR signals of TZT-1027 were assigned as follows. The ^{13}C-NMR signals and the multiplicity were identified by the ^{13}C-NMR and DEPT spectra. The chemical shifts of the corresponding protons were directly assigned from the C–H COSY spectrum. The ^{13}C-NMR spectrum of TZT-1027 showed two quaternary aromatic carbons [δ 140.0 (C-1′, conformer I), δ 139.8 (C-1′, conformer II)]. In the HMBC spectrum, the C-1′ of conformer I at δ 140.0 was correlated with the proton signals at δ 2.71, 3.21, 3.33, and 7.25. The low field proton signal at δ 7.25 was assigned to H-3′, 5′ (aromatic protons). The signals at δ 3.21 and 3.33 were assigned to H-2 methylene protons based on the cross peaks between H-2 and C-4 (carbonyl carbon at δ 173.6) observed in HMBC. The proton signal at δ 2.71 was assigned to H-1 methylene protons based on the cross peaks between H-1 and H-2 observed in DQF-COSY. In this manner, the sequential assignment of two conformers was achieved by careful analysis of the DQF-COSY, HMBC, and ROESY spectra. The ^{1}H- and ^{13}C-NMR chemical shifts were reported in Tables 1 and 2. The ^{1}H-NMR spectrum (Table 1) of TZT-1027 showed the methine protons of H-6 and H-7

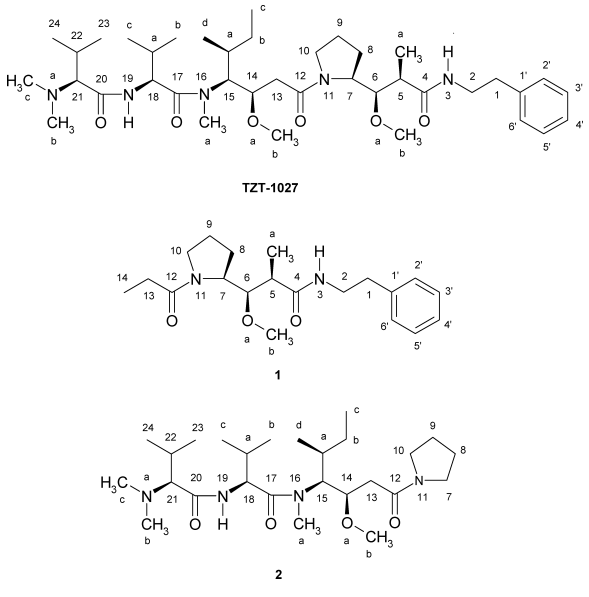

Fig. 1. Structures of TZT-1027, Compounds **1** and **2**

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Table 1. ^{1}H-NMR Spectra of TZT-1027, Compounds **1** and **2**

Position	TZT-1027		Compound **1**		Compound **2**
	Conformer I	Conformer II	Conformer I	Conformer II	
1	2.71	2.74	2.71	2.71	
2	3.21, 3.33	3.21, 3.44	3.22, 3.31	3.21, 3.46	
3	7.82	8.04	7.81	8.02	
4	None	None	None	None	
5	2.18	2.19	2.19	2.19	
5a	1.04	1.07	1.04	1.05	
6	3.82	3.41	3.77	3.37	
6b	3.26	3.29	3.25	3.27	
7	3.82	3.67	3.84	3.50	3.28
8	1.63, 1.79	1.59, 1.83	1.64, 1.79	1.69, 1.85	1.76
9	1.61, 1.87	1.55, 1.82	1.65, 1.88	1.58, 1.84	1.85
10	3.28, 3.49	3.11, 3.58	3.26, 3.45	3.08, 3.55	3.40
12	None	None	None	None	None
13	2.30, 2.43	2.43	2.22	2.10, 2.22	2.33, 2.38
14	4.00	3.99	0.98	0.99	3.95
14b	3.18	3.17			3.21
15	4.64	4.72			4.61
15a	1.75	1.82			1.75
15b	0.89, 1.29	0.89, 1.29			0.88, 1.31
15c	0.74	0.75			0.74
15d	0.88	0.89			0.86
16a	2.99	3.14			2.99
17	None	None			None
18	4.54	4.58			4.53
18a	1.94	1.96			1.94
18b, 18c	0.89, 0.86, 0.85, 0.90				0.90, 0.92
19	8.00	8.00			7.99
20	None	None			None
21	2.64	2.64			2.63
21b, 21c	2.19, 2.21				2.20
22	1.90	1.90			1.89
23, 24	0.72, 0.87				0.71, 0.87
1′	None	None	None	None	
2′, 6′	7.19	7.20	7.20	7.20	
3′, 5′	7.25	7.25	7.27	7.26	
4′	7.16	7.16	7.17	7.17	

ppm from TMS, in DMSO-d_6, 303 K.

shifted to a high field. These shifts may be attributed to the shielding effect of the aromatic ring of phenethylamine moiety. We plan to analyze the three-dimensional structure of TZT-1027 in solutions by various NMR methods and molecular simulations to specify its conformation including the arrangement of phenethylamine moiety.

In order to exhibit the formation of the conformers, four amide bonds of TZT-1027 were determined from the ROESY spectrum. The correlation observed in the ROESY spectrum made it possible to estimate the through-space interaction between the following pairs of protons: H-3–H-5, H-16a–H-18, and H-19–H-21 in conformer I and conformer II. These results have shown that three amide bonds (N-3–C-4, N-16–C-17, N-19–C-20) exist as the *trans* form in conformer I and conformer II (Fig. 2). Around the amide bond at N-11 and C-12, the cross peak was observed between H-13 and H-10 of conformer I in the ROESY spectrum (Fig. 3). This observation was characterized as the *trans* amide bond at N-11 and C-12. In conformer II, on the other hand, the cross peaks were observed between H-13 and H-6, and between H-13 and H-7. These observations were characterized as the *cis* amide bond at N-11 and C-12.

To confirm that the *cis–trans* isomerization of the amide

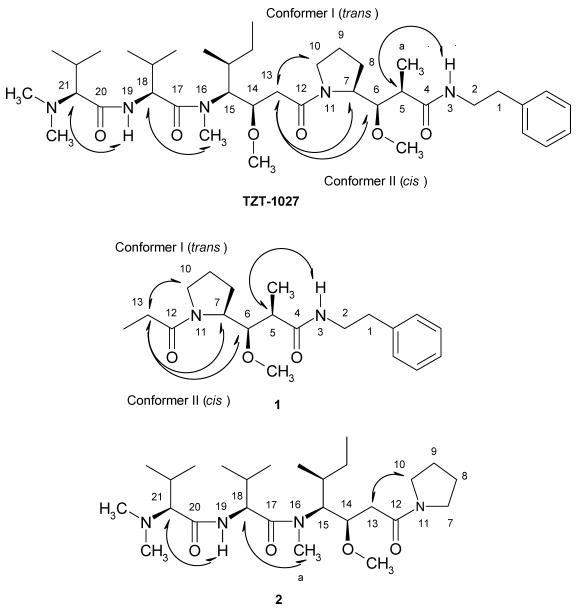

Fig. 2. ROESY Correlations of TZT-1027, Compounds **1** and **2**

Table 2. ^{13}C-NMR Spectra of TZT-1027, Compounds **1** and **2**

Position	TZT-1027		Compound **1**		Compound **2**
	Conformer I	Conformer II	Conformer I	Conformer II	
1	35.5	35.4	35.6	35.5	
2	40.4	40.0	40.5	40.0	
3	None	None	None	None	
4	173.6	174.0	173.7	174.0	
5	44.3	43.9	44.2	44.1	
5a	15.4	16.0	15.4	15.8	
6	82.4	86.2	82.6	86.2	
6b	60.8	61.5	61.0	61.6	
7	59.3	59.1	59.1	59.0	45.9
8	25.1	26.1	25.2	26.2	24.5
9	24.9	23.7	25.0	23.7	26.1
10	47.7	46.7	47.4	46.5	46.5
12	169.5	169.7	171.7	171.9	169.1
13	37.8	36.0	28.1	26.6	37.2
14	78.3	78.5	9.6	10.3	78.1
14b	57.6	57.8			57.7
15	56.5	55.7			56.7
15a	32.6	32.2			32.7
15b	25.9	25.9			25.9
15c	11.0	10.8			11.1
15d	16.2	16.0			16.3
16a	32.0	32.0			32.1
17	173.6	173.6			173.6
18	54.4	54.4			54.4
18a	30.4	30.4			30.4
18b, 18c	19.3, 19.4, 19.5, 19.6				19.4, 19.6
19	None	None			None
20	170.5	170.5			170.5
21	73.5	73.5			73.5
21b, 21c	41.9, 42.0				41.9
22	27.4	27.4			27.4
23, 24	19.6, 20.2				19.7, 20.2
1′	140.0	139.8	140.0	140.0	
2′, 6′	129.1	129.1	129.2	129.2	
3′, 5′	128.7	128.7	128.8	128.7	
4′	126.5	126.5	126.5	126.5	

ppm from TMS, in DMSO-d_6, 303 K.

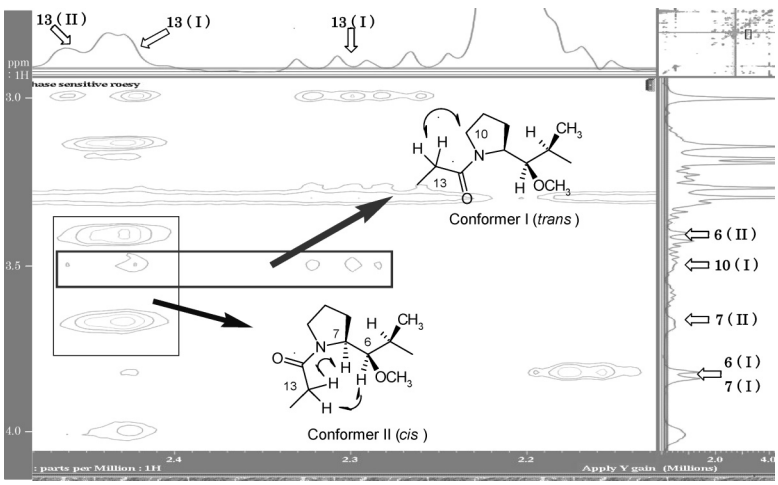

Fig. 3. ROESY Spectrum of TZT-1027 in DMSO-d_6, at 303 K

bond at N-11 and C-12 takes part in the formation of the conformers, compounds **1** and **2** were designed and prepared. Compound **1** is a molecule composed of C-1 to C-14 of TZT-1027. The ^{1}H- and ^{13}C-NMR spectra showed the existence of two conformers in DMSO-d_6 (conformer I/conformer II=2/3). In the case of conformer I of compound **1**, the cross peak H-13–H-10 that characterizes the *trans* amide bond was observed (Fig. 2), while in conformer II of compound **1**, the cross peaks H-13–H-6 and H-13–H-7 that characterize the *cis* amide bond were observed. These conformers of com-

pound **1** correspond to the *trans* and *cis* conformers for the amide bond at N-11 and C-12 of TZT-1027. Thus, the difference in the conformer ratio of TZT-1027 and compound **1** is influenced by the steric interaction of the acyl groups.

Compound **2** is a molecule that is composed of C-7 to C-24 of TZT-1027. The amine part of the amide bond at N-11 and C-12 is composed of a symmetrical cyclic amine, pyrrolidine. In the ROESY spectrum of compound **2**, the proton signal at H-13 correlated with the H-10 methylene protons (Fig. 2). This observation suggests that the amide bond at N-11 and C-12 has a partial double bond character. Therefore, the proton and carbon signals of four methylenes (C-7, C-8, C-9, C-10) in the pyrrolidine ring were respectively assigned to one another. However, since the amine part of the amide bond at N-11 and C-12 is a symmetrical cyclic pyrrolidine ring, the ^{1}H- and ^{13}C-NMR spectra of compound **2** were assigned as a single conformer in DMSO-d_6. The ^{1}H- and ^{13}C-NMR chemical shifts of compound **1** and **2** were similar to those of TZT-1027.

The existence of conformers can be explained in terms of the equilibrium between *cis* and *trans* amide bond at N-11 and C-12. Using various NMR methods (1D, 2D NMR), the ^{1}H- and ^{13}C-NMR signals of two distinct conformers of TZT-1027 were completely assigned. Based on the ROESY NMR experiment, it was concluded that these conformers were due to the partial double bond character of the amide bond at N-11 and C-12. The assignments described here should allow further conformational analysis of TZT-1027 in solutions using various NMR and other methods.

Experimental

General Experimental Procedures All NMR measurements were performed on a JEOL ECP-400 spectrometer system at 303 K. The ^{1}H-, ^{13}C-NMR, DEPT, DQF-COSY, C–H COSY, HMBC, and ROESY spectra were collected in DMSO-d_6 with tetramethylsilane (TMS) as an internal standard.

Compounds **1** and **2** were prepared by the method described by Miyazaki *et al.*[1)]

Compound **1**: Colorless viscous oil, $[\alpha]_D^{28}$ −55.3° (c=1.02, $CHCl_3$), EI-MS m/z: 346 (M^+). ^{1}H- and ^{13}C-NMR are shown in Tables 1 and 2, respectively.

Compound **2**: Colorless amorphous powder, $[\alpha]_D^{29}$ −34.3° (c=1.07, $CHCl_3$), EI-MS m/z: 482 (M^+). ^{1}H- and ^{13}C-NMR are shown in Tables 1 and 2, respectively.

References

1) Miyazaki K., Kobayashi M., Natsume T., Gondo M., Mikami T., Sakakibara K., Tsukagoshi S., *Chem. Pharm. Bull.*, **43**, 1706—1718 (1995).
2) Pettit G. R., Kamano Y., Herald C. L., Tuinman A. A., Boettner F. E., Kizu H., Schmidt J. M., Baczynskayj L., Tomer K. B., Bontems R. J., *J. Am. Chem. Soc.*, **109**, 6883—6885 (1987).
3) Poncet J., *Curr. Pharm. Design*, **5**, 139—162 (1999).
4) Alattia T., Roux F., Poncet J., Cave A., Jouin P., *Tetrahedron*, **51**, 2593—2604 (1995).
5) Benedetti E., Carlomagno T., Fraternali F., Hamada Y., Hayashi K., Paolillo L., Shioiri T., *Biopolymers*, **36**, 525—538 (1995).
6) Roux F., Mangras I., Poncet J., Niel G., Jouin P., *Tetrahedron*, **50**, 5345—5360 (1994).
7) Kobayashi M., Natsume T., Tamaoki S., Watanabe J., Asano H., Mikami T., Miyasaka K., Miyazaki K., Gondo M., Sakakibara K., Tsukagoshi S., *Jpn. J. Cancer Res.*, **88**, 316—327 (1997).
8) Natsume T., Watanabe J., Tamaoki S., Fujio N., Miyasaka K., Kobayashi M., *Jpn. J. Cancer Res.*, **91**, 737—747 (2000).