

SUPPLEMENTARY MATERIAL

A new actinomycin Z analogue with an additional oxygen bridge between chromophore and β -depsipeptide from *Streptomyces* sp. KIB-H714

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Abstract

Actinomycin Z₆ (**1**), a new member of the actinomycin family, along with three congeners of the Z-type (Z₁, Z₃, Z₅) actinomycins, are produced from *Streptomyces* sp. KIB-H714. Their structures were authenticated by comprehensive spectroscopic data interpretation. Different from all the reported Z-type actinomycins, the β -ring of the new compound actinomycin Z₆ includes an additional ring linked between the actinoyl chromophore and β -peptidolactone. In Z₃ and Z₅, the L-threonine in β -depsipeptide is replaced by the unusual 4-chlorothreonine, an amino acid rarely found in actinomycin family. All isolates were evaluated for cytotoxicity against five human tumor cell lines and for inhibitory activity against *Candida albicans* and *Staphylococcus aureus*.

Keywords: actinomycin; *Streptomyces*; cytotoxicity; antibacterial activity; structure elucidation

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Figure S1. ^1H NMR (600 MHz, CDCl_3) spectrum of actinomycin Z_6 (**1**)

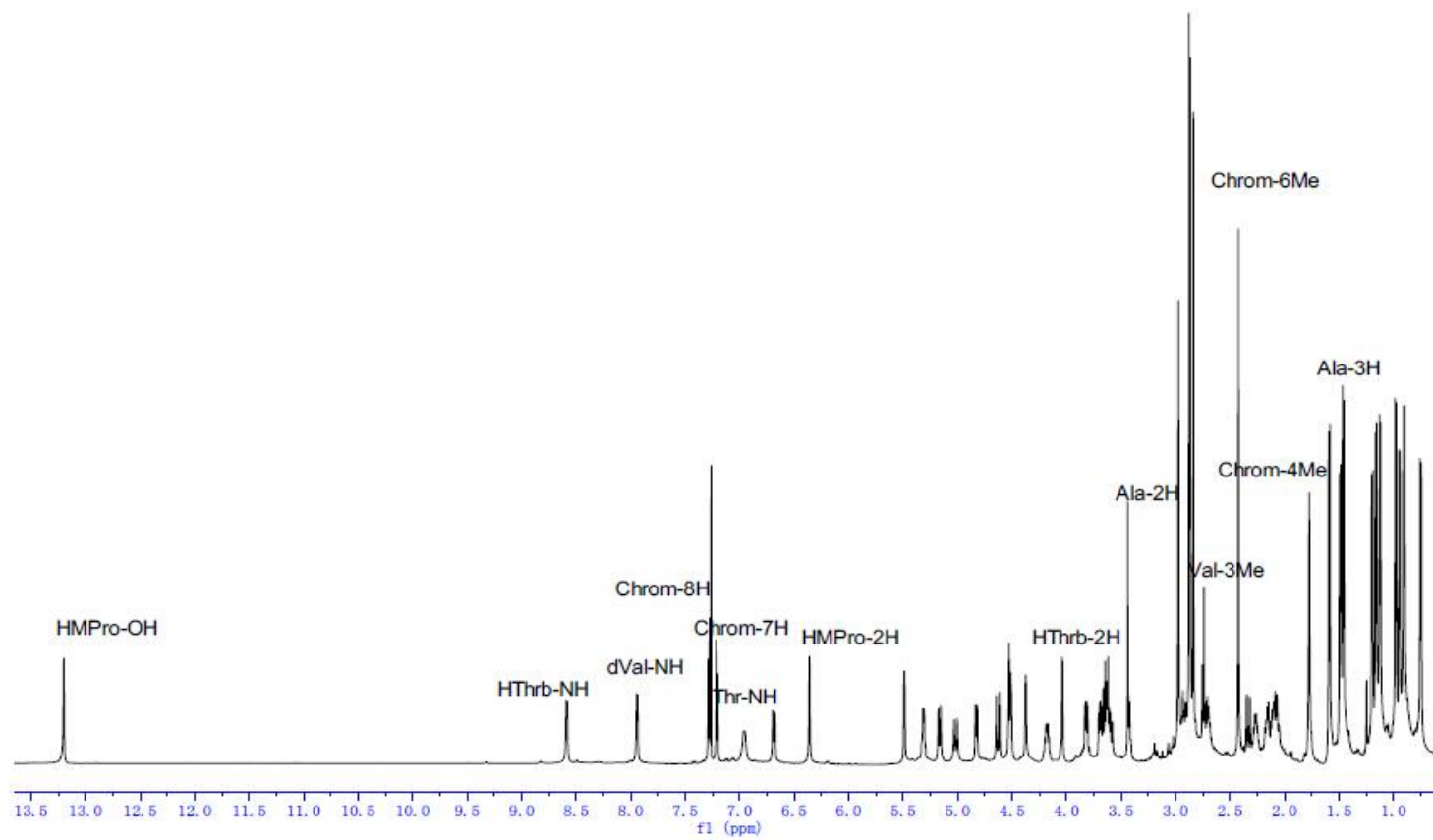


Figure S2. ^{13}C NMR (150 MHz, CDCl_3) spectrum of actinomycin Z_6 (**1**)

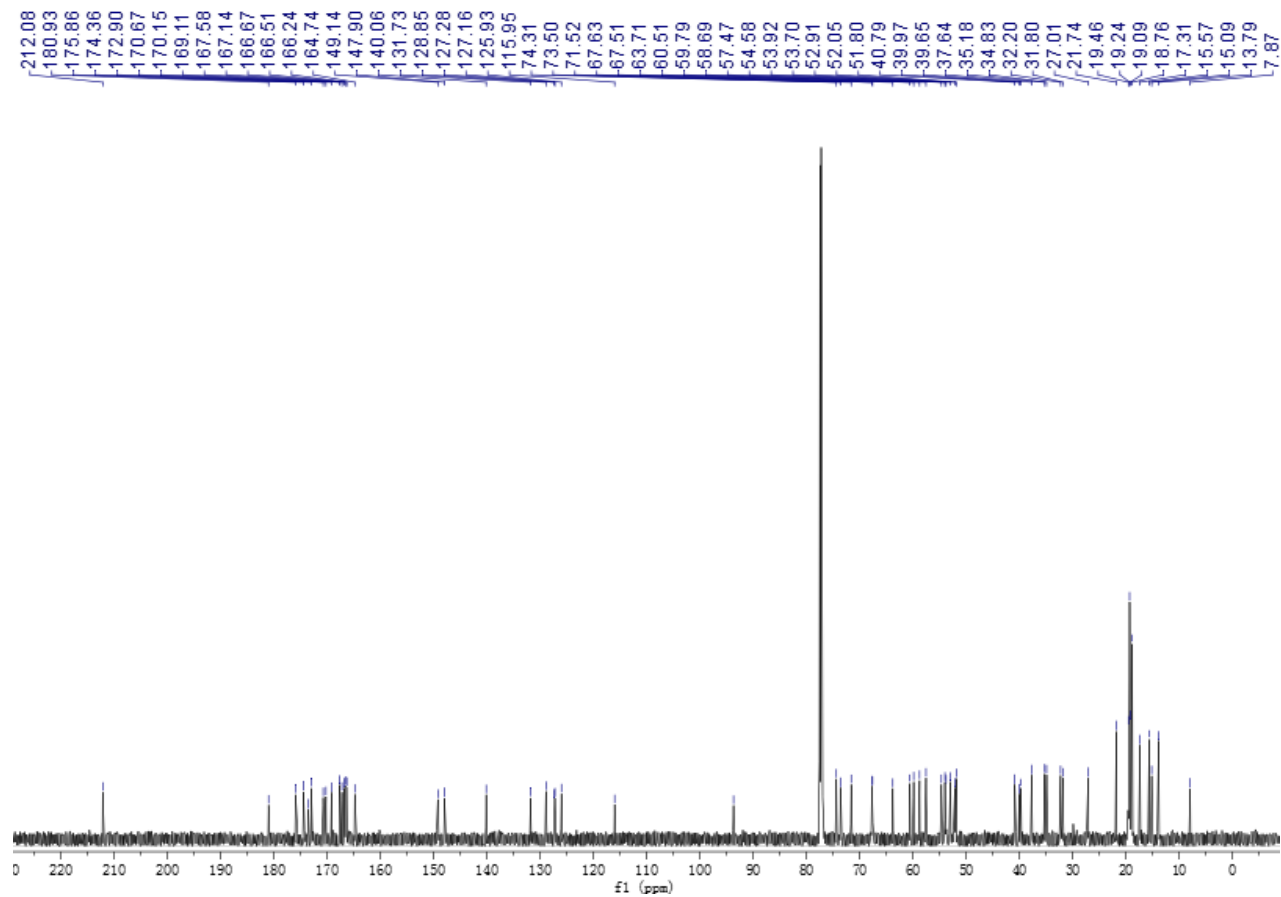


Figure S3. ^1H - ^1H COSY (600 MHz, CDCl_3) spectrum of actinomycin Z_6 (**1**)

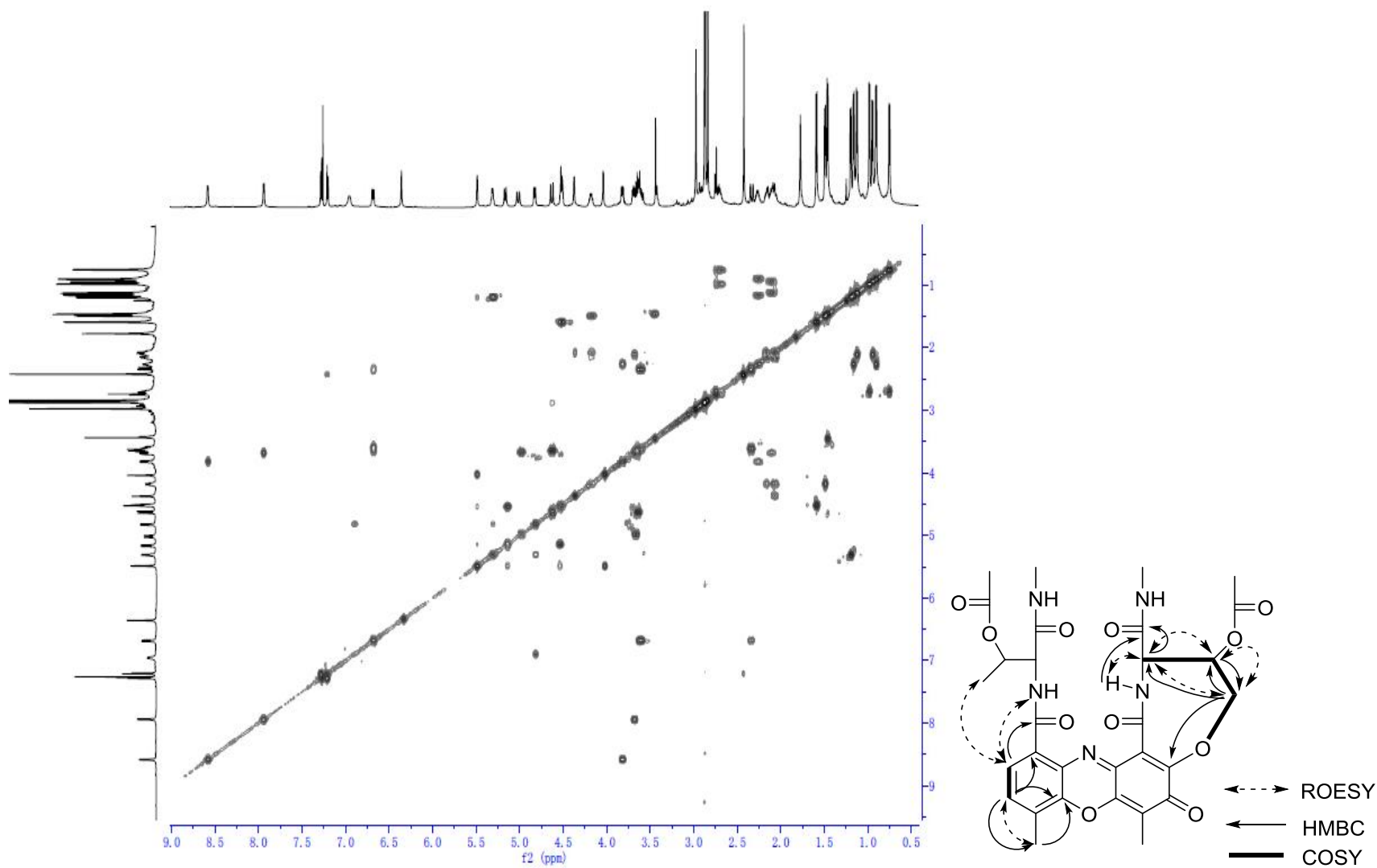


Figure S4. HSQC (600 MHz, CDCl₃) spectrum of actinomycin Z₆ (**1**)

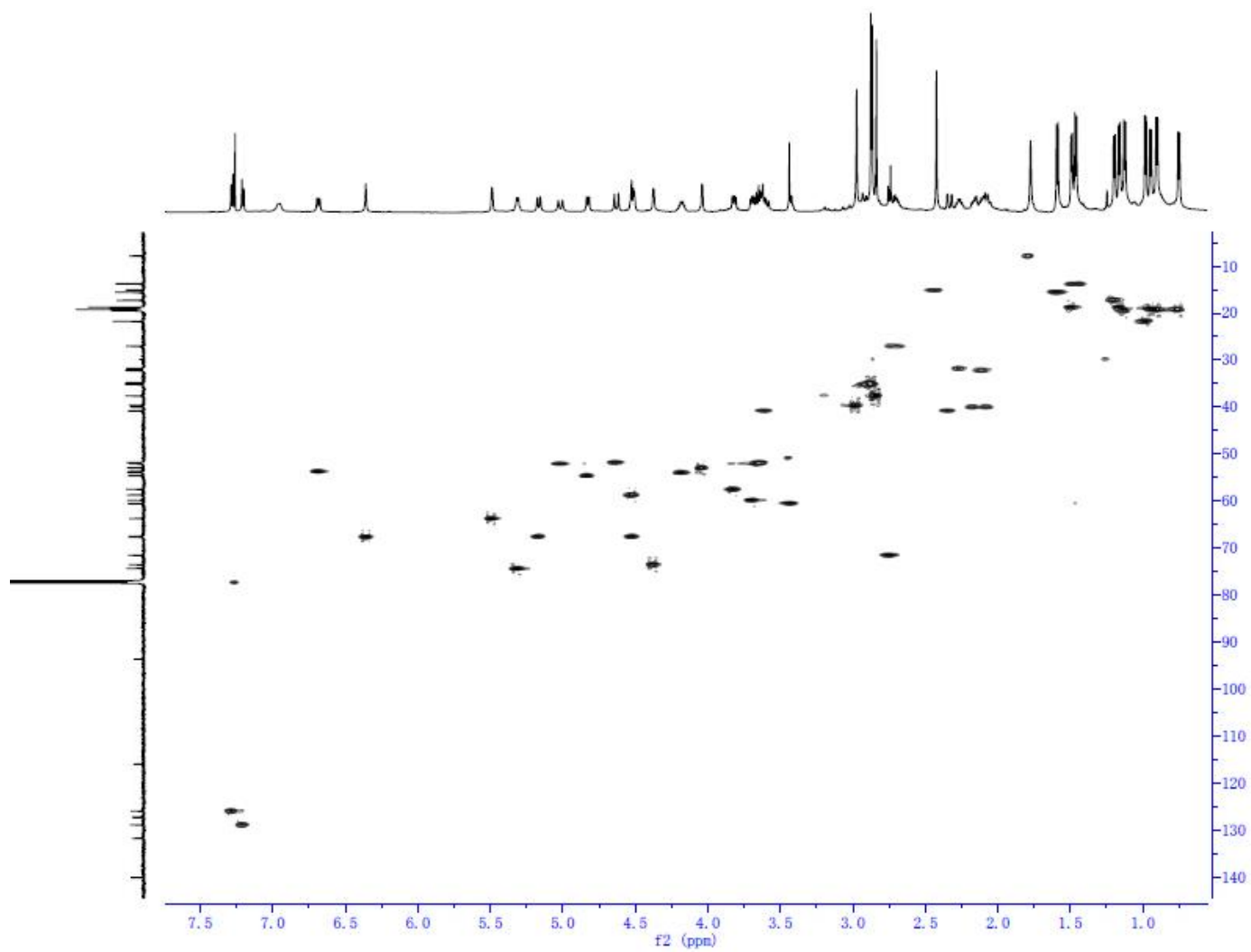


Figure S5. HMBC (600 MHz, CDCl₃) spectrum of actinomycin Z₆ (**1**)

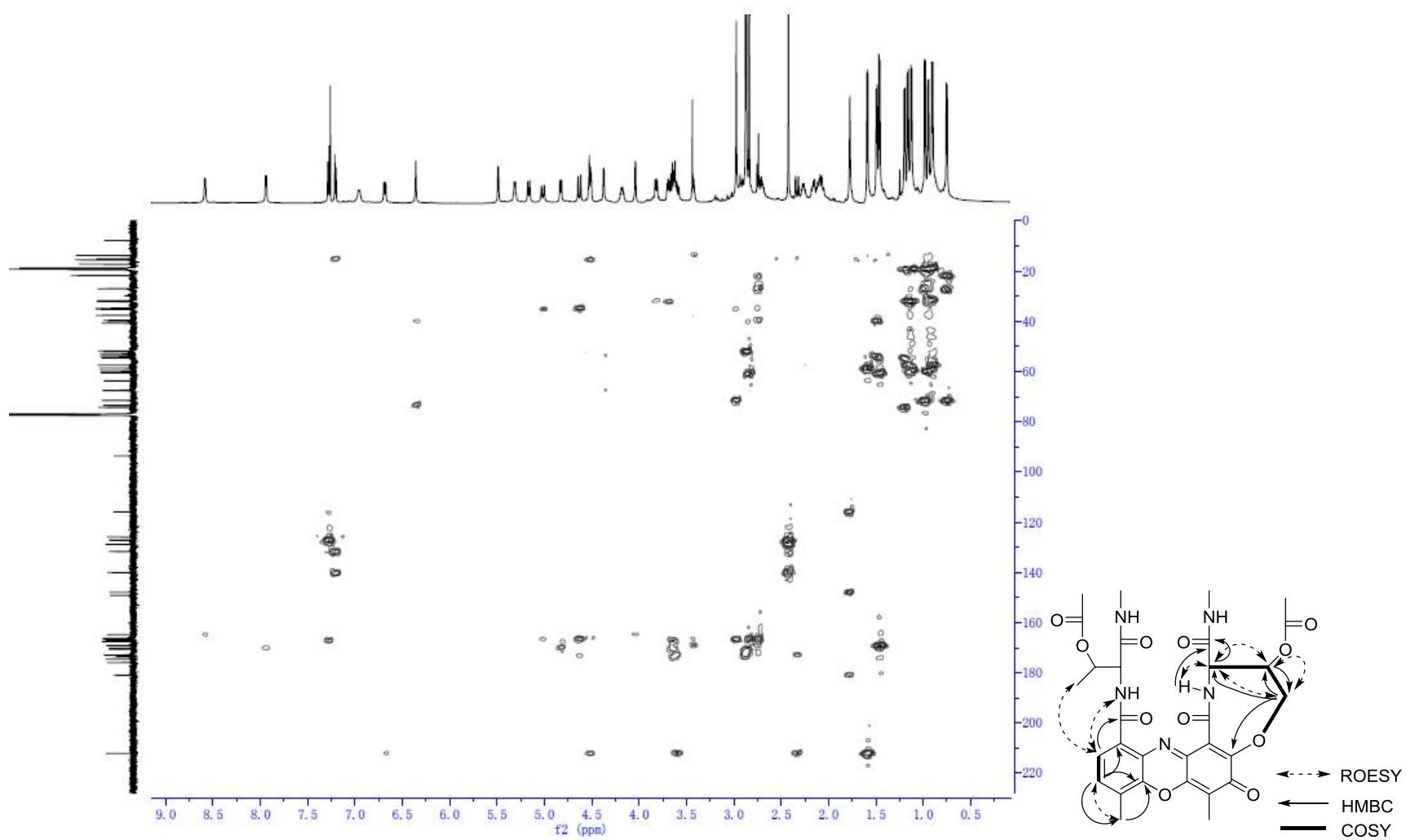


Figure S6. ROESY (600 MHz, CDCl_3) spectrum of actinomycin Z_6 (**1**)

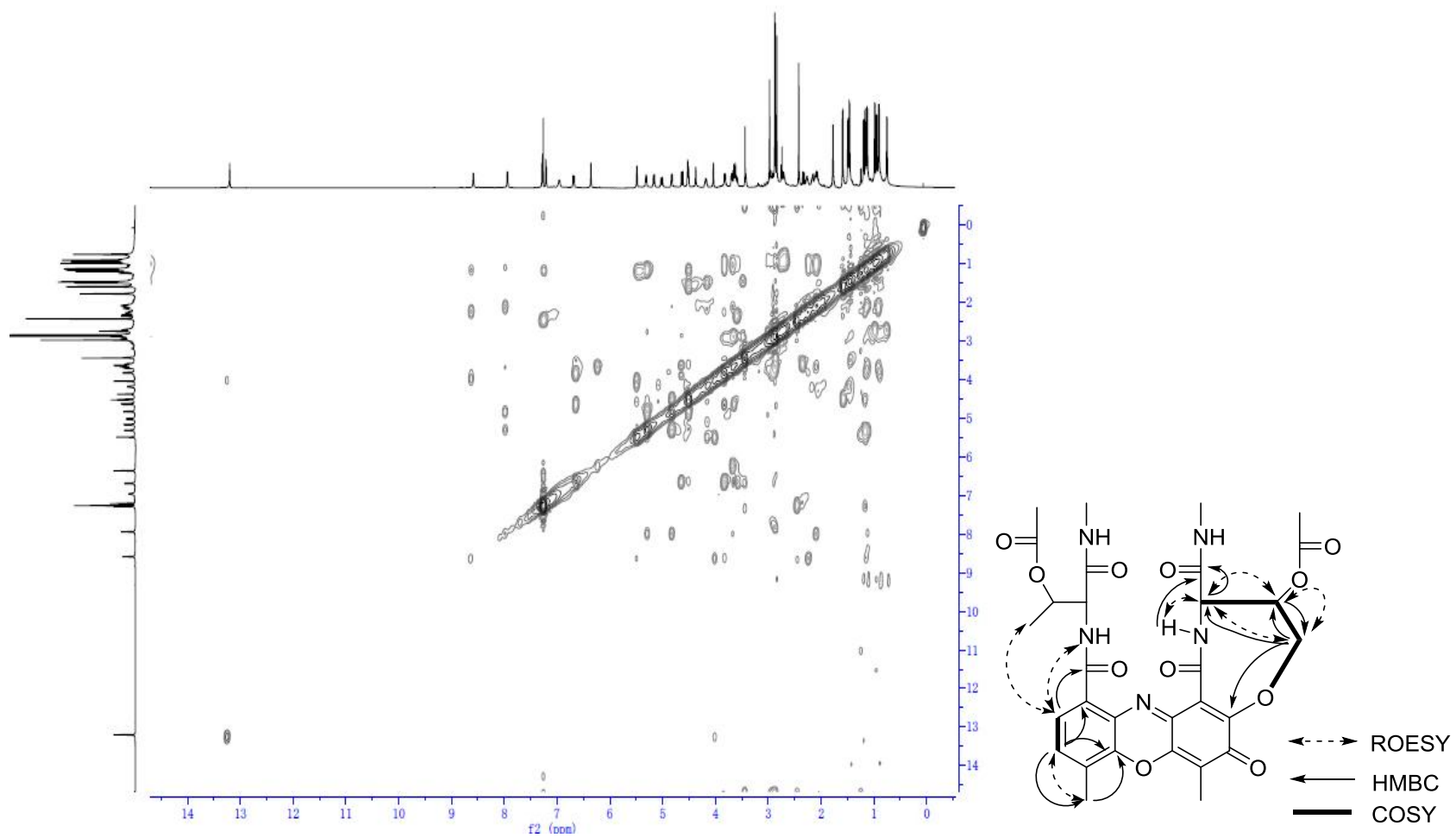


Figure S7. HRESIMS spectrum of actinomycin Z₆ (1)

Sample Name	DME-6	Instrument Name	Agilent G6230 TOF MS	User Name	KIB	IRM Calibration Status	Success
Data Filename	140603ESIA1.d	ACQ Method	ESI.m	Acquired Time	6/3/2014 12:21:43 PM		

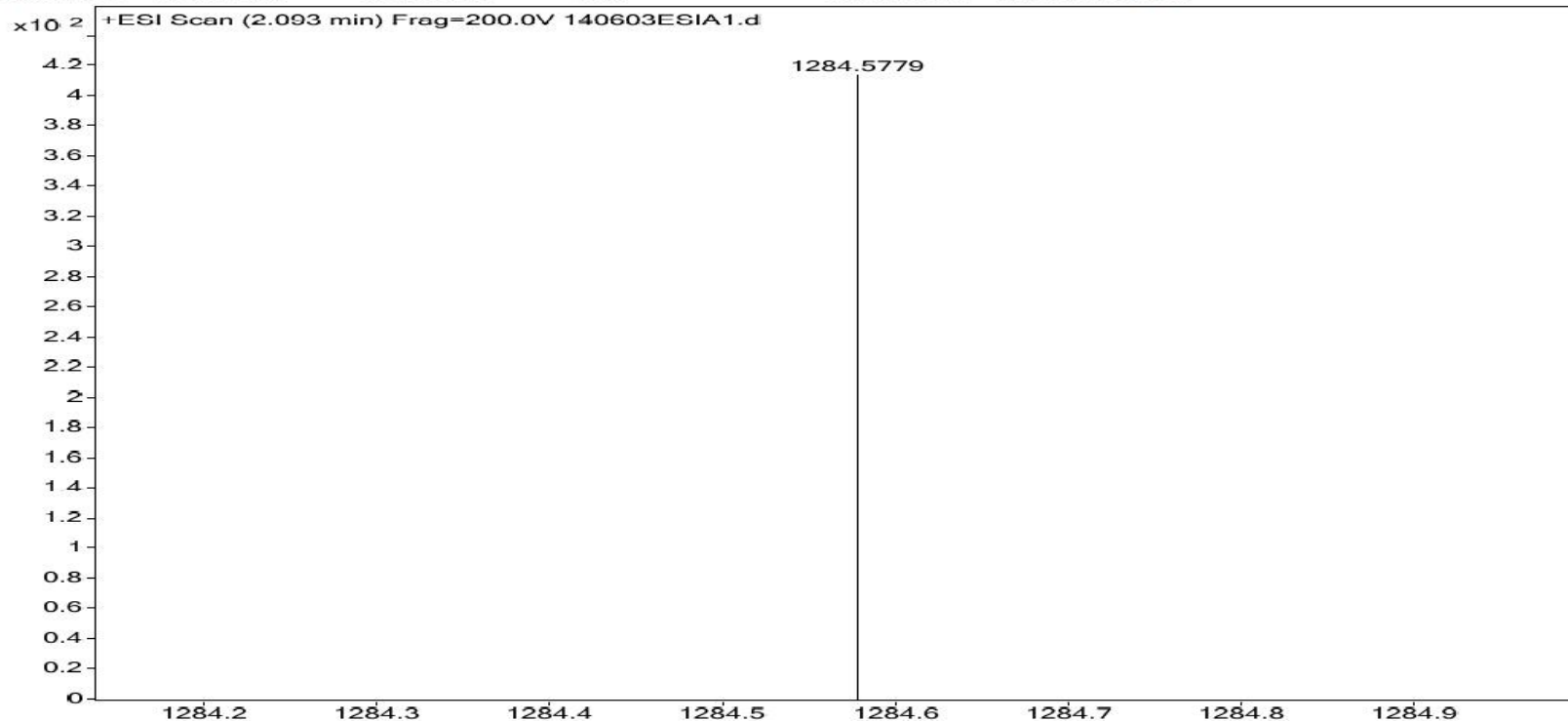
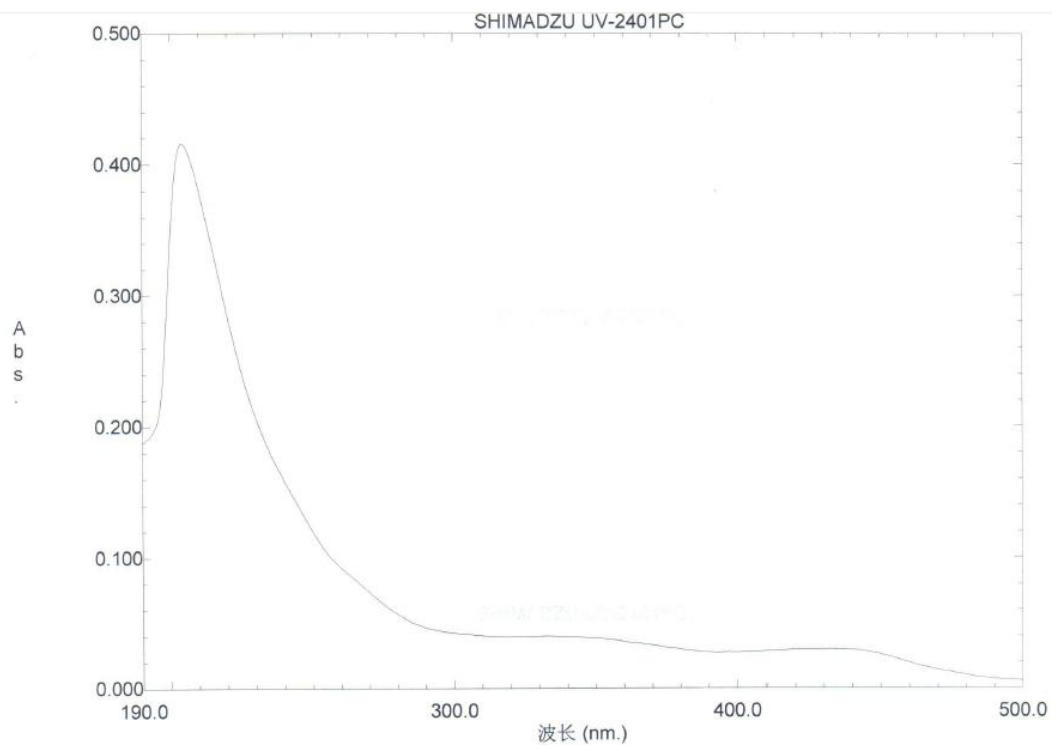


Figure S8. UV spectrum of actinomycin Z₆ (1)



文件名: DME-6

DME-6 ———

创建于: 14:16 14-07-11
数据: 原始

样品浓度: 0.0192毫克/毫升
溶剂: 甲醇

测量模式: Abs.
扫描速度: 中速
狭缝: 5.0
采样间隔: 0.5

否.	波长 (nm.)	Abs.
1	431.50	0.0298
2	333.00	0.0401
3	204.00	0.4157

Figure S9. ORD data of actinomycin Z₆ (**1**)

Optical rotation measurement

Model : P-1020 (A060460638)

No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	4 (1/3)	Sp.Rot	-24.1670	-0.0058 0.0000	24.8 10.00 Cell	Fri Jul 11 10:38:41 2014 0.00240g/mL MeOH DME-6	Na 589nm	2 sec 10 sec
No.2	4 (2/3)	Sp.Rot	-23.3330	-0.0056 0.0000	24.8 10.00 Cell	Fri Jul 11 10:38:54 2014 0.00240g/mL MeOH DME-6	Na 589nm	2 sec 10 sec
No.3	4 (3/3)	Sp.Rot	-25.0000	-0.0060 0.0000	24.8 10.00 Cell	Fri Jul 11 10:39:08 2014 0.00240g/mL MeOH DME-6	Na 589nm	2 sec 10 sec

-24.1667

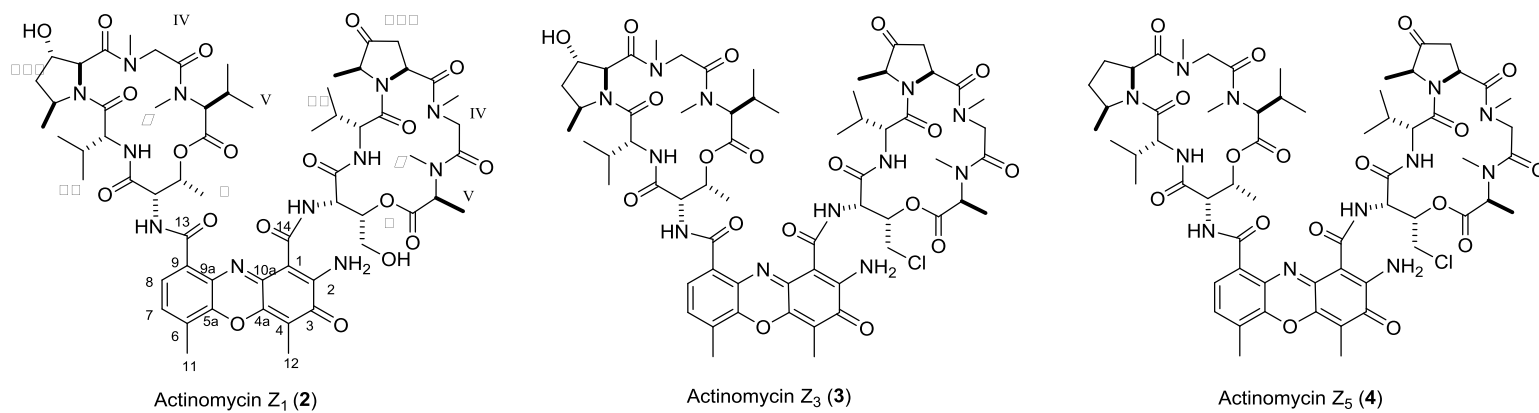


Figure S10. The structures of Actinomycins Z₁ (2), Z₃ (3) and Z₅ (4)

Table S1. The 1D and 2D NMR Data for Actinomycin Z₆ (**1**)

Pos.	δ_H	HSQC- δ_C	COSY- δ_H	Noesy- δ_H	HMBC- δ_C
H-NH	8.58 (d, $J = 5.5$ Hz, 1H)		4.03	4.03,5.48	164.7
D-NH	7.94 (d, $J = 6.2$ Hz, 1H)		3.68	2.19, 4.82,5.31	32.2, 170.1
H-8	7.28 (d, $J = 7.7$ Hz, 1H)	125.9	7.20	1.20,2.42	127.3,167.1
T-NH	7.26 (s, 1H)				125.9, 128.8
H-7	7.20 (d, $J = 7.8$ Hz, 1H)	128.8	2.42,7.28	1.16, 2.42, 6.96	15.3,131.8,140.1
D-NH	6.96 (d, $J = 6.8$ Hz, 1H)		4.82		
MO-2	6.68 (dd, $J = 2.0, 10.7$ Hz, 1H)	53.7	2.34,3.60,	4.62	212.0,174.4
HM-2	6.35 (br s, 1H)	67.6		3.66	39.9,73.5,170.7,175.8
H-3	5.48 (br s, 1H)	63.7	4.04	4.03,4.53,5.16	
H-3	5.31 (dd, $J = 6.2, 2.5$ Hz, 1H)	74.3	1.20,4.82	4.81,1.20,7.94	167.5
H-4	5.16 (d, $J = 12.1$ Hz, 1H)	67.5	4.53		
S-2	5.01 (d, $J = 17.3$ Hz, 1H)	52.1		4.17	35.1,166.7
T-2	4.82 (dd, $J = 2.0, 9.3$ Hz, 1H),	54.6	5.31,6.95	7.94,5.31	167.5,170.1
Sa-2	4.62 (d, $J = 17.1$ Hz, 1H),	51.8	2.87,3.64	3.60,6.68	34.7,166.5,172.9
MO-5	4.51 (m, 1H)	67.5	5.16	4.03,5.48	52.9, 63.7, 166.3
H-3	4.53 (m, 1H)	58.6	1.58	1.12,1.58,3.60,6.68	15.6, 212.1
HM-3	4.37 (d, $J = 2.3$ Hz, 1H)	73.6	2.08	1.49	67.6
HM-5	4.17 (m, 1H)	53.9	1.49,2.08,2.16	1.49,5.01,2.08	
HT-2	4.03 (d, $J = 3.1$ Hz, 1H)	52.9	5.48,8.58	5.48,8.58	164.7
D-2	3.82 (q, $J = 5.8$ Hz, 1H)	57.5	2.26	0.90,1.16,2.26	31.7
DV-2	3.68(q, $J = 6.4$ Hz, 1H)	59.8	2.11,7.94	1.12, 4.62, 0.95	32.2, 174.4
S-2	3.66 (m, 2H)	52.0	5.01	2.08	170.7
Sa-2	3.64 (m, 2H)	51.7	4.62	2.87,4.62	172.9
MO-3	3.60 m	40.8	2.34,6.68	0.95,2.34,6.68	172.9, 212.1
Me-2	3.44 (q, $J = 6.7$ Hz, 1H)	60.5	1.46	1.46,2.87,6.68	13.7, 37.6,169.1
NMe	2.97 (s, 3H)	39.7		2.75	71.3, 166.7
NMe	2.87 (s, 3H)	34.7		0.95,3.64	172.9
NMe	2.86 (s, 3H)	35.2			52.1
NMe	2.83 (s, 3H)	37.5		3.64,7.94	60.5, 166.5
M-2	2.75 (m, 1H)	71.5	0.75,0.98		27.0, 166.7,39.7
M-3	2.71 (m, 1H)	27.1	0.75,0.98	0.98	71.3
H-11	2.42 (s, 3H)	15.1	7.20	7.28	127.1, 128.8, 140.0
MO-3	2.34 (dd, $J = 2.1, 17.8$ Hz, 1H)	40.8	3.60,6.68	3.60	172.9, 212.1
D-3	2.26 (m,1H)	31.9	0.90,1.16,3.82	8.58	57.4
DV-3	2.11 (m,1H)	32.3	0.95,1.12, 3.69	0.95,3.69,7.94,1.59	59.8
HM-4	2.16 (m,1H)	40.0	4.17	0.90	
HM-4	2.08 (m,1H)	40.0	4.17,4.36	0.90,3.66	
H-12	1.76 (s, 3H)	7.9			115.9, 147.9, 180.9
MO-6	1.59 (d, $J = 6.9$ Hz, 3H)	15.6	4.51	4.51,2.11	58.6, 212.1
HM-6	1.49 (d, $J = 5.9$ Hz, 3H)	18.8	4.17	4.17, 2.08, 4.31	53.9
Me-3	1.46 (d, $J = 6.9$ Hz,3H)	13.9	3.44	3.44,1.12	60.4, 169.1
T-4	1.20 (d, $J = 6.2$ Hz, 3H)	17.3	5.31	4.53, 7.28	54.6, 74.3
D-5	1.16 (d, $J = 6.7$ Hz, 3H)	18.8	2.26	5.31,7.20,8.58,3.82	31.8, 57.4
DV-4	1.12 (d, $J = 6.7$ Hz, 3H)	19.5	2.11	2.11,3.69,7.94	59.8
Me-4	0.98 (d, $J = 6.2$ Hz,3H)	21.8	2.75	2.75	27.0, 71.4
DV-5	0.95 (d, $J = 6.7$ Hz, 3H)	19.1	2.11	2.87,2.11,3.60	32.2
D-4	0.90 (d, $J = 6.7$ Hz, 3H)	19.2	2.26	3.82	31.7
Me-5	0.75 (d, $J = 6.6$ Hz, 3H)	19.5	2.75	2.75	21.7, 27.0, 71.5

Table S2. Peptidic ¹H NMR Data for Actinomycins Z₁ (**2**), Z₃ (**3**) and Z₅ (**4**)

H	Z ₁	Z ₃	Z ₅				
site	α-ring	β-ring	α-ring	β-ring	α-ring	β-ring	
Thr/4-OH- Thr/4- Cl-Thr	2	4.68 m	4.91 d (4.6)	4.49 dd (6.5, 2.3)	5.25 m	4.71 m	4.61 m
	3	5.27 dd (6.1, 2.9)	5.12 m	5.21 m	5.19 m	5.23 s, br	5.24 m
	4	1.14 m	3.74 m	1.14 d (5.6)	3.99 q (6.6), 4.01m	1.15 m	4.11 m, 4.08 m
	NH	7.26 s, br	8.13 t (5.4)	6.94 d (6.2)	7.91 t (6.7)	7.52 m	7.82 d (5.7)
D-Val	2	3.44 dd (9.6, 5.3)	3.83 q (6.2)	3.42 m	3.82 q (6.3)	3.54 q (6.6)	3.75 q (5.9)
	3	2.15 m	2.18 m	2.16 m	2.12 m	2.14 m	2.23 m
	3-Me	0.93 d (6.7)	0.97 d (6.0)	0.92 d (6.7)	0.94 d (6.7)	1.14 d (6.3)	0.93 t (6.7)
		1.16 m	1.15 m	1.11 d (6.2)	1.14 d (5.6)	0.94 d (5.7)	0.74 d (5.7)
	NH	8.13 t (5.4)	8.38 d (5.0)	7.92 t (6.7)	8.27 d (5.8)	8.48 s, br	7.93 s, br
HMPPro/ MOPro	2	5.89 s, br	6.48 d (11.1)	5.95 s, br	6.54 d (11.7)	6.05 d (6.0)	6.53 d (6.8)
	3	4.21 d (2.8)	2.32 d (17.2)	4.13 dd (11.8, 4.9)	2.34 d (17.5)	2.00 m	2.25 m
	4	2.11/2.18 m	3.96 q (11.1)	2.14 m, 2.26 m	3.98 m	2.24 m	3.89 dd (11.6, 4.8)
	5	4.68 m	4.58 d (7.2)	4.73 m	4.54 m	4.34 m	4.61 m
	5-Me	1.51 d (5.8)	1.62 d (6.8)	1.15 d (6.1)	1.62 d (6.8)	1.50 d (6.5)	1.61 d (6.7)
Sar	2	3.60 m	3.65 m	3.65 m	3.67 d (5.8)	3.64 m	3.64 m
		4.72 m	4.58 d (7.2)	4.77 m	4.59 m	4.56 d (17.2)	4.60 m
	N-Me	2.86 s	2.86 s	2.90 s	2.88 s	2.87 s	2.88 s
MeVal/ MeAla	2	2.65 m	3.24 m	2.64 m	3.37 m	2.67 m	3.40 q (6.7)
	3	2.70 m	1.35 d (6.8)	2.62 m	1.40 d (6.8)	2.68 m	1.41 d (6.7)
	3-Me	0.74 d (6.4)		0.75 d (6.2)		0.74 d (5.7)	
		0.93 d (6.7)		0.94 d (6.7)		0.96 d (5.6)	
	N-Me	2.90 s	2.90 s	2.95 s	2.97 s	2.89 s	2.95 s

Table S3. Peptidic ^{13}C NMR Assignments for Actinomycins Z_1 (**2**), Z_3 (**3**) and Z_5 (**4**)

		Z_1		Z_3		Z_5	
site	C	α -ring	β -ring	α -ring	β -ring	α -ring	β -ring
Thr/4-OH-	1	168.93	169.12	168.24	168.90	167.92	169.18
	2	54.75	52.31	54.64	54.21	54.77	54.46
Thr/4-Cl-Thr	3	74.91	77.48	74.73	74.60	74.93	74.37
	4	17.09	59.28	17.24	43.78	17.27	44.24
D-Val	1	174.12	174.48	174.12	174.31	174.05	174.14
	2	57.55	58.88	59.37	57.42	59.27	57.57
	3	32.02	32.19	32.09	32.40	32.15	32.16
	3-Me	19.11/19.39	19.17/19.54	18.99/19.22	19.20/19.22	18.99/19.22	19.07/19.28
HMPro/ MOPro	1	170.75	172.71	171.09	172.76	173.22	172.93
	2	68.07	53.40	68.70	54.01	58.81	54.05
	3	75.30	41.35	75.79	41.37	29.68	41.41
	4	41.17	212.29	41.53	211.96	27.07	212.39
	5	54.16	59.47	54.17	58.74	55.63	58.59
Sar	5-Me	18.93	15.34	19.40	15.35	19.22	15.25
	1	166.21	166.14	166.32	166.36	166.26	166.21
	2	51.43	51.59	51.55	51.68	51.57	51.68
	N-Me	35.01	35.27	35.04	35.22	35.02	35.12
MeVal/ MeAla	1	166.40	167.84	167.80	168.69	167.28	168.80
	2	71.27	60.23	71.47	60.09	71.48	60.23
	3	27.55	13.63	27.20	13.68	27.07	13.73
	3-Me	19.3/21.68		19.33/21.72		19.07/21.73	
	N-Me	37.19	39.17/39.33	39.19	37.32	39.35	37.48

Table S4. Chromophoric ^1H and ^{13}C NMR Assignments for Actinomycins Z_1 (**2**), Z_3 (**3**) and Z_5 (**4**)

site	Z_1 (δ_C/δ_H)	Z_3 (δ_C/δ_H)	Z_5 (δ_C/δ_H)
1	99.10	100.08	101.5
2	148.57	147.86	148.15
3	178.57	178.75	179.21
4	113.66	114.11	113.44
4a	145.94	146.34	146.04
5a	140.44	140.67	140.48
6	127.58	127.85	127.67
7	130.59/7.30 d (7.8)	130.48/7.34 d (7.7)	131.06/7.34 d (7.7)
8	126.09/7.50 d (7.8)	125.85/7.58 d (7.7)	126.26/7.56 d (7.7)
9	133.14	132.70	130.31
9a	128.91	129.07	129.24
10a	145.33	145.38	145.25
11	15.16/2.51 s	15.17/2.53 s	15.18/2.04 s
12	7.54/1.95 s	7.99/2.20 s	7.65/2.53 s
13	166.39	166.40	166.44
14	169.11	168.98	168.83
NH ₂	7.20 s	7.24 s	7.25 s

Taxonomic identification of strain *Streptomyces* sp. KIB-H714

16S rDNA gene sequence

CAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCTTTCGGGGTGGATTAGTGGCGAACGGGTGAGTA
ACACGTGGGCAATCTGCCCTTCACTCTGGGACAAGCCCTGGAACGGGGTCTAATACCGGATAAACTCTGTCCCGCATGGGACGGGGTAAAAGCTCCGGCGG
TGAAGGATGAGCCCGCGCCTATCAGCTTGTGGTGGGGTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACGCTGGGACT
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CGCGAGGCGGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTAATCGCAGATCAGCATTGCT
GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTCTGA
AGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCC