Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information

First Y-Type Actinomycins from *Streptomyces* with Divergent Structure-Activity Relationships for Antibacterial and Cytotoxic Properties

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General Experimental Procedures

NMR spectra were recorded on Varian Inova 600 and Varian Inova 500 spectrometers at 298 K. Chemical shifts were determined relative to the solvent as internal standard ($CDCl_3$ δ_H 7.25, δ_C 77.0; CD_3OD δ_H 3.30, δ_C 49.0). Optical rotation values were measured with a Perkin-Elmer 241 polarimeter. UV and CD spectra were obtained in methanol on a Varian Cary 3E spectrometer and Jasco J-500 spectrometer respectively. Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer as KBr pellets. ESIMS spectra were obtained on Finnigan LC-Q and high resolution ESIMS spectra on Bruker Apex-Q III (field strength 7 Tesla). TLC was done with silica gel 60 F_{254} plates (Merck, 0.2 mm). Amino acid analysis was carried out on an Agilent 1100 HPLC system coupled with a Micromass LCT mass spectrometer in positive and negative ESI mode, using a Water Symmetry C18 column (3.5 µm, 150 × 2.1 mm) and a linear gradient from 5–40% MeCN (0.1% HCOOH, flow rate 0.5 mL/min) in 40 min.

Chiral Amino Acid Analysis

The absolute configuration of the valine, threonine, and sarcosine residues was determined according to Marfey's method.[1] 1.0 mg of **1** was hydrolyzed in 6 N HCl (500 µL) for 20 h at RT. After drying under argon flow the residue was resolved in 200 µL of acetone/H_2O (1:1). FDAA solution (1-fluoro-2,4-dinitrophenyl-5-L-alanine-amide, 100 µL, 1% in acetone) and Na_2CO_3 (20 µL, 1M) were added and the mixture incubated for 1 h at 40 °C. After cooling to rt, 10 µL of 2 N HCl were added and the solution directly applied to HPLC-MS as described above.

Figure S1: Isolation scheme for actinomycins Y_1 – Y_5 (1 – 5) from 4 L fermentation broth

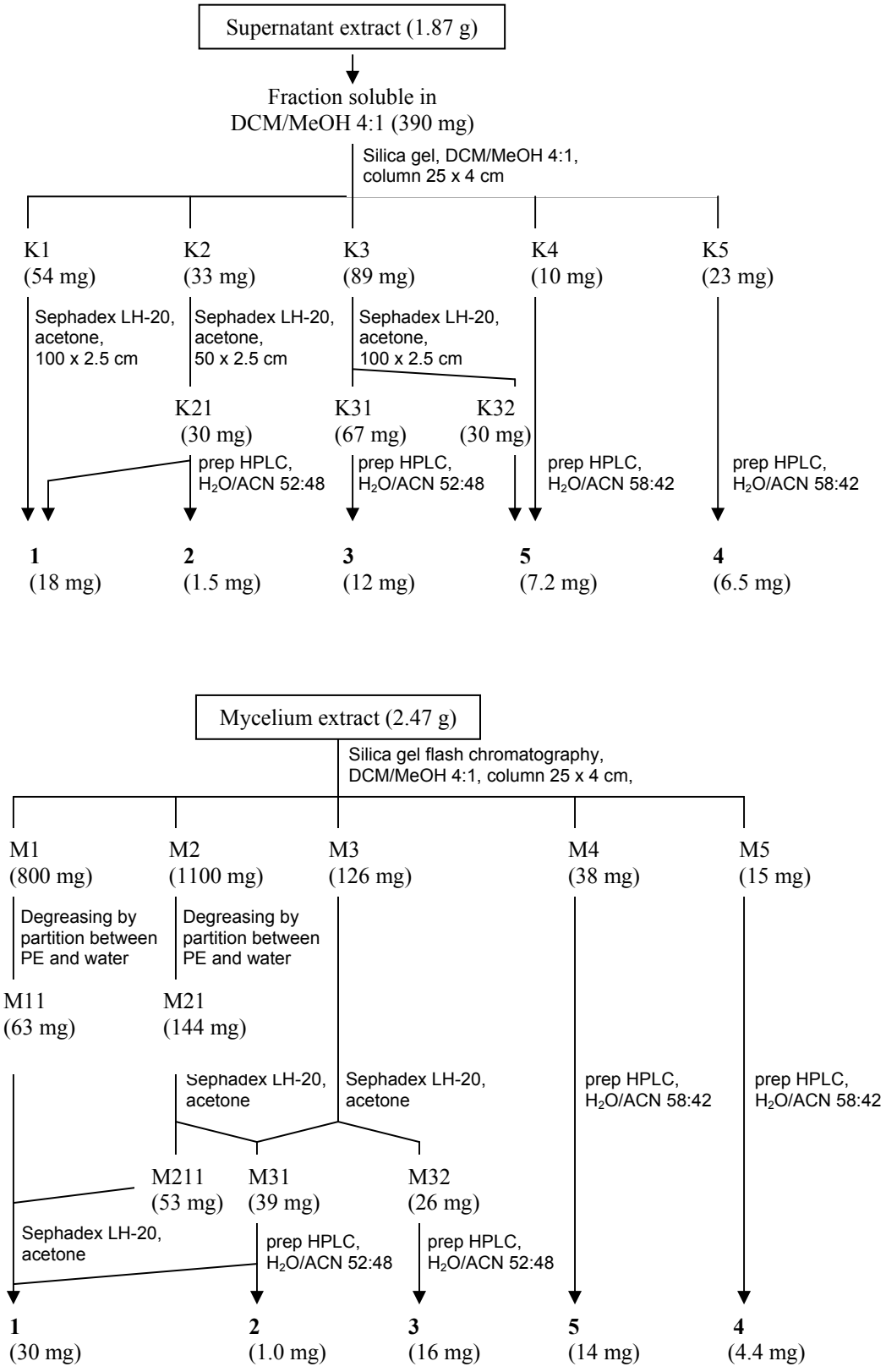


Figure S2: Selected details of important spectroscopic data

Comparison of important HMBC correlations in the β-rings of actinomycins Y_1 (**1**) and Y_3 (**3**).

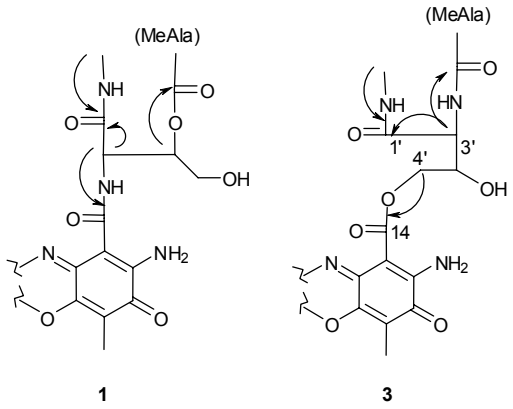


Figure S3: Structural formulae of actinomycins D, G_2, G_3 and G_5

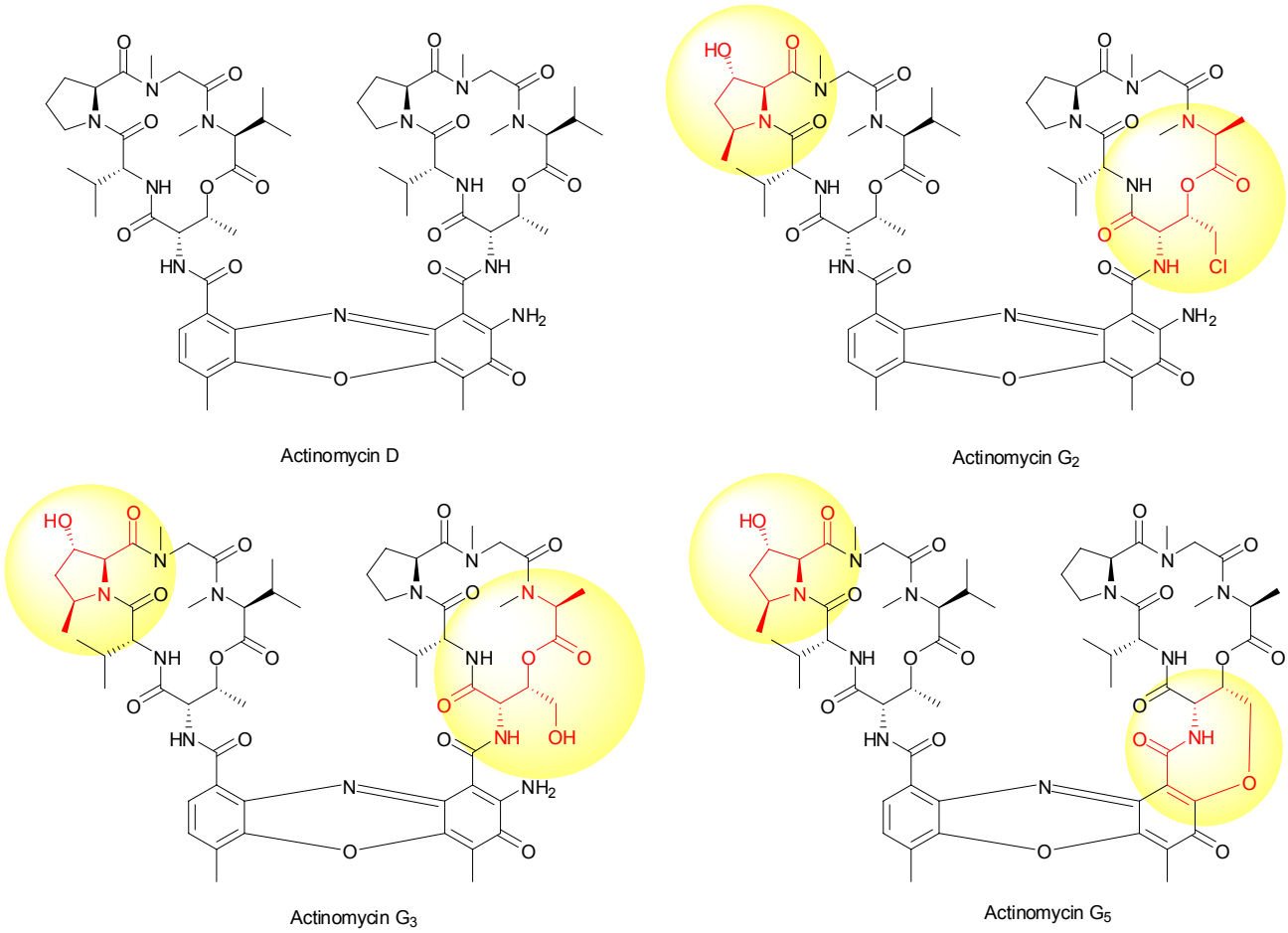


Table S1: HM02 cell cycle investigation with actinomycin Y_1

Cell cycle investigation with HM02 cells (% cells in the specified cell phase ± standard deviation; concentration of test compounds 5 ng/mL).

Cell phase	Sub G1 (apoptose)	G0/G1	S	G2/M
Control	3.6 ± 1.3	54.5 ± 3.5	19.7 ± 0.8	21.5 ± 5
Actinomycin D	6.0 ± 3.4	54.0 ± 6.5	10.0 ± 1.6	29.2 ± 8.5
Actinomycin Y_1 (**1**)	1.7 ± 0.3	52.5 ± 1.5	13.9 ± 1.0	31.0 ± 2.0

Table S2: Antiparasitic bioactivity of Y-type actinomycins

Antiparasitic bioactivity of actinomycins Y_1 (**1**) and Y_3 (**3**) compared with reference drugs. IC-50 values are given in µg/mL. No antiparasitic activity was found for compound **6**.

Parasite	*T. b. rhod.*	*T. cruzi*	*L. donovani*	*P. falciparum*
Reference drug *)	0.003	0.305	0.343	0.062
Actinomycin Y_1 (**1**)	0.001	dnp **)	0.097	0.0001
Actinomycin Y_3 (**3**)	4.96	>30	>30	1.28

*) Melarsoprol (*T. b. rhodesiense*), benznidazole (*T. cruzi*), miltefosine (*L. donovani*), and chloroquine (*P. falciparum*), respectively.
**) determination not possible due to cytotoxicity on macrophage cells.

Table S3: Overview on naturally occurring actinomycins

	***α*-ring**	***β*-ring**
(1) Actinomycin D as core structure		
Actinomycin D	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-Pro-Sar-MeVal
(2) *N*-demethyl actinomycins[2, 3]		
Actinomycin D_0	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-Pro-**Gly**-MeVal
N,N'-Didemethyl-actinomycin D	Thr-D-Val-Pro-**Gly**-MeVal	Thr-D-Val-Pro-**Gly**-MeVal
(2) C-type actinomycins[4-6]		
Actinomycin C_2	Thr-D-Val-Pro-Sar-MeVal	Thr-**D-*a*Ile**-Pro-Sar-MeVal
Actinomycin C_{2a}	Thr-**D-*a*Ile**-Pro-Sar-MeVal	Thr-D-Val-Pro-Sar-MeVal
Actinomycin C_3	Thr-**D-*a*Ile**-Pro-Sar-MeVal	Thr-**D-*a*Ile**-Pro-Sar-MeVal
(3) F-type actinomycins[7-9]		
Actinomycin F_8	Thr-D-Val-**Sar**-Sar-MeVal	Thr-D-Val-**Sar**-Sar-MeVal
Actinomycin F_9 [a)]	Thr-D-Val-**Sar**-Sar-MeVal Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-Pro-Sar-MeVal Thr-D-Val-**Sar**-Sar-MeVal
(4) X-type actinomycins[10-13]		
Actinomycin $X_{0\alpha}$	Thr-D-Val-**Sar**-Sar-MeVal	Thr-D-Val-**Hyp**-Sar-MeVal
Actinomycin $X_{0\beta}$	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-**Hyp**-Sar-MeVal
Actinomycin $X_{0\delta}$	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-***a*Hyp**-Sar-MeVal
Actinomycin X_{1a}	Thr-D-Val-**Sar**-Sar-MeVal	Thr-D-Val-**OPro**-Sar-MeVal
Actinomycin X_2	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-**OPro**-Sar-MeVal
(5) Z-type actinomycins[14]		
Actinomycin Z_1	Thr-D-Val-**HMPro**-Sar-MeVal	**HThr**-D-Val-**MOPro**-Sar-**MeAla**
Actinomycin Z_2	Thr-D-Val-**HMPro**-Sar-MeVal	Thr-D-Val-**MOPro**-Sar-**MeAla**
Actinomycin Z_3	Thr-D-Val-**HMPro**-Sar-MeVal	**ClThr**-D-Val-**MOPro**-Sar-**MeAla**
Actinomycin Z_4	Thr-D-Val-**MPro**-Sar-MeVal	Thr-D-Val-**MOPro**-Sar-**MeAla**
Actinomycin Z_5	Thr-D-Val-**MPro**-Sar-MeVal	**ClThr**-D-Val-**MOPro**-Sar-**MeAla**
Actinomycin ZP[15,b)]	Thr-D-Val-**MPro**-Sar-MeVal	Thr-D-Val-**MPro**-Sar-MeVal
(6) G-type actinomycins[16, 17]		
Actinomycin G_1	Thr-D-Val-Pro-Sar-MeVal	**HThr**-D-Val-**HMPro**-Sar-**MeAla**
Actinomycin G_2	Thr-D-Val-**HMPro**-Sar-MeVal	**ClThr**-D-Val-Pro-Sar-**MeAla**
Actinomycin G_3	Thr-D-Val-**HMPro**-Sar-MeVal	**HThr**-D-Val-Pro-Sar-**MeAla**
Actinomycin G_4	Thr-D-Val-**HMPro**-Sar-MeVal	Thr-D-Val-Pro-Sar-**MeAla**
Actinomycin G_5	Thr-D-Val-**HMPro**-Sar-MeVal	**cHThr**-D-Val-Pro-Sar-**MeAla**
Actinomycin G_6	Thr-D-Val-**HMPro**-Sar-MeVal	**rHThr**-D-Val-Pro-Sar-**MeAla**
(7) novel Y-type actinomycins		
Actinomycin Y_1 (**1**)	Thr-D-Val-**HMPro**-Sar-MeVal	**ClThr**-D-Val-**OPro**-Sar-**MeAla**
Actinomycin Y_2 (**2**)	Thr-D-Val-**HMPro**-Sar-MeVal	**ClThr**-D-Val-**Hyp**-Sar-**MeAla**
Actinomycin Y_3 (**3**)	Thr-D-Val-**HMPro**-Sar-MeVal	**rHThr**-D-Val-**OPro**-Sar-**MeAla**
Actinomycin Y_4 (**4**)	Thr-D-Val-**HMPro**-Sar-MeVal	**rHThr**-D-Val-**Hyp**-Sar-**MeAla**
Actinomycin Y_5 (**5**)	Thr-D-Val-**HMPro**-Sar-MeVal	**cThr**-D-Val-**OPro**-Sar-**MeAla**

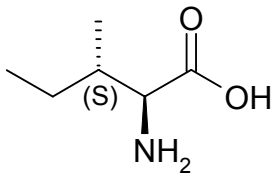
Differences to actinomycin D are shown in **bold letters**. All amino acids are L-configurated except when indicated otherwise. Abbreviations: MeVal = *N*-methylvaline, MeAla = *N*-methyl-L-alanine, *a*Ile = *allo*-isoleucine, Sar = sarcosine, MPro = *cis*-5-methylproline, HMPro = *trans*-3-hydroxy-*cis*-5-methylproline, Hyp = *trans*-4-hydroxyproline, OPro = 4-oxoproline, MOPro = *cis*-5-methyl-4-oxoproline, *a*Hyp = *cis*-4-hydroxyproline, HThr = 4-hydroxythreonine, ClThr = 4-chlorothreonine, cThr/cHThr = cyclic Thr or HThr, i.e. forming an additional ring closure to the chromophore, rHThr = rearrangement of the HThr (β-ring) connectivities.

a) Actinomycin F_9 consists of two isomers, which are also known as actinomycin III and IIIA =$X_{0\gamma}$ (sarcosine in *β*- or *α*-ring, respectively)[18]. F_0 to F_7 were obtained by precursor-directed biosynthesis, exhibiting an additional variation at position 2' with *a*Ile.

b) Actinomycin ZP was isolated as the monomeric chromo-pentapeptidolactone protactin and was converted semisynthetically to actinomycin ZP by ferricyanide oxidation.[15]

Table S4: Overview on actinomycins derived by precursor-directed biosynthesis

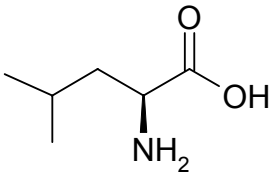
Feeding of L-isoleucine:[19, 20]

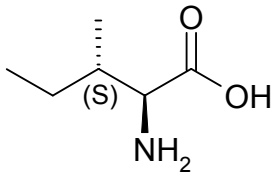


	α-ring	β-ring
Actinomycin E_1	Thr-***D*-*a*Ile**-Pro-Sar-MeVal	Thr-***D*-*a*Ile**-Pro-Sar-**MeIle**
Actinomycin E_2	Thr-***D*-*a*Ile**-Pro-Sar-**MeIle**	Thr-***D*-*a*Ile**-Pro-Sar-**MeIle**

(MeIle = *N*-methyl-L-isoleucine, D-*a*Ile = D-*allo*-isoleucine)

Feeding of L-leucine and L-isoleucine:

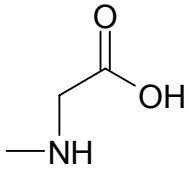




Actinomycin A_{u5}[21]	Thr-***D*-Leu**-Pro-Sar-MeVal	Thr-***D*-Leu**-Pro-Sar-MeVal
Actinomycin A_{u6a}	Thr-***D*-*a*Ile**-Pro-Sar-MeVal	Thr-***D*-Leu**-Pro-Sar-MeVal
Actinomycin A_{u6b}[22]	Thr-***D*-Leu**-Pro-Sar-MeVal	Thr-***D*-*a*Ile**-Pro-Sar-MeVal
Actinomycin A_{u7a}	Thr-***D*-Leu**-Pro-Sar-MeVal	Thr-*D*-Val-Pro-Sar-MeVal
Actinomycin A_{u7b}[23].	Thr-*D*-Val-Pro-Sar-MeVal	Thr-***D*-Leu**-Pro-Sar-MeVal

Remark: Actinomycin Au_{7b} was also found to occur naturally and named actinomycin C_{3a}, but results were not published. [24].

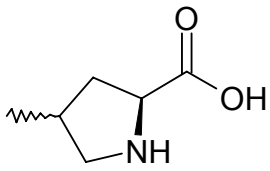
Feeding of sarcosine:[8]



Actinomycin F_1	Thr-*D*-Val-**Sar**-Sar-MeVal	Thr-***D*-*a*Ile-Sar**-Sar-MeVal
Actinomycin F_2	Thr-*D*-Val-Pro-Sar-MeVal	Thr-***D*-*a*Ile-Sar**-Sar-MeVal
Actinomycin F_3	Thr-***D*-*a*Ile-Sar**-Sar-MeVal	Thr-***D*-*a*Ile-Sar**-Sar-MeVal
Actinomycin F_4	Thr-***D*-*a*Ile**-Pro-Sar-MeVal	Thr-***D*-*a*Ile-Sar**-Sar-MeVal

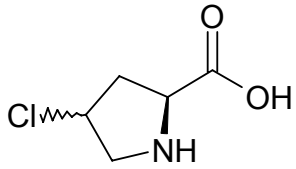
Remark: The chemical structures of F_0, F_5, F_6 and F_7 are either not known or not published.

Feeding of *(4R)*- and *(4S)*-4-methyl- L -proline:[25]



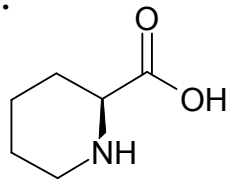
cis-4-methyl-L-proline:		
Actinomycin K_{1c}	Thr-*D*-Val-Pro-Sar-MeVal	Thr-*D*-Val-**MPro**-Sar-MeVal
Actinomycin K_{2c}	Thr-*D*-Val-**MPro**-Sar-MeVal	Thr-*D*-Val-**MPro**-Sar-MeVal
trans-4-methyl-L-proline:		
Actinomycin K_{1t}	Thr-*D*-Val-Pro-Sar-MeVal	Thr-*D*-Val-**MPro**-Sar-MeVal
Actinomycin K_{2t}	Thr-*D*-Val-**MPro**-Sar-MeVal	Thr-*D*-Val-**MPro**-Sar-MeVal

Feeding of *(4R)*- and *(4S)*-4-chloro-L-proline:[26]

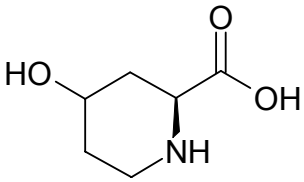


<u>*cis*-4-chloro-L-proline:</u>		
no trivial name (*)	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-**ClPro**-Sar-MeVal
no trivial name	Thr-D-Val-**ClPro**-Sar-MeVal	Thr-D-Val-**ClPro**-Sar-MeVal
<u>*trans*-4-chloro-L-proline:</u>		
no trivial name (*)	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-**ClPro**-Sar-MeVal

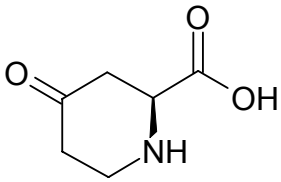
Feeding of pipecolid acid:[27]



Pipecolid acid (Pip)



4-Hydroxy- (HPip)

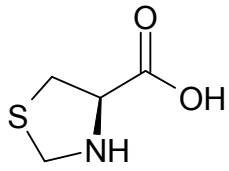


4-Oxo- (OPip)

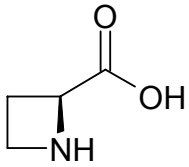
Actinomycin Pip 1_α (*)	Thr-*D*-Val-**Pip**-Sar-MeVal	Thr-*D*-Val-**OPip**-Sar-MeVal
Actinomycin Pip 1_β (*)	Thr-*D*-Val-**Pip**-Sar-MeVal	Thr-*D*-Val-Pro-Sar-MeVal
Actinomycin Pip 1_γ (*)	Thr-*D*-Val-**Pip**-Sar-MeVal	Thr-*D*-Val-**HPip**-Sar-MeVal
Actinomycin Pip 1_δ (*)	Thr-*D*-Val-Pro-Sar-MeVal	Thr-*D*-Val-**OPip**-Sar-MeVal
Actinomycin Pip 1_ε (*)	Thr-*D*-Val-Pro-Sar-MeVal	Thr-*D*-Val-**HPip**-Sar-MeVal
Actinomycin Pip 2 (*)	Thr-*D*-Val-**Pip**-Sar-MeVal	Thr-*D*-Val-**Pip**-Sar-MeVal

Feeding of thiazolidin-4-carboxylic acid:[28]

Incorporation of thiazolidin-4-carboxylic acid into two novel actinomycins was proven by ^{35}S-isotopic labelling experiments. The structure of the compounds was not elucidated. Presumably and analogous to the azetomycins, thiazolidine-4-carboxylic acid is expected to provoke both single and double incorporation.



Feeding of azetidin-2-carboxylic acid:[29, 30]



Azetomycin I (*)	Thr-D-Val-Pro-Sar-MeVal	Thr-D-Val-**AzC**-Sar-MeVal
Azetomycin II	Thr-D-Val-**AzC**-Sar-MeVal	Thr-D-Val-**AzC**-Sar-MeVal

(*) the marked compounds were not investigated wether incorporation was in the α- or β-ring. Presumably, the compounds were mixtures of both possibilies.

Table S5. NMR data of actinomycin Y_2 (**2**) ^{1}H: 600 MHz, ^{13}C: see footnote*, CD_3OD

Pentapeptidolactones

***α*-ring**	pos.	δ_C*	δ_H	*J* [Hz]	***β*-ring**	pos.	δ_C*	δ_H	*J* [Hz]
Thr	1	170.3	–	–	ClThr	1	169.7	–	–
	2	56.4	4.63	d, 2.9		2	54.0	5.36	d
	3	75.4	5.27	qd, 6.3, 3.0		3	72.3	5.15	m
	4	17.1	1.31	d, 6.3		4	43.0	3.85	m
								3.90	m
D-Val	1	175.3	–	–	D-Val	1	171.5	–	–
	2	58.7	3.76	d, 9.5		2	60.4	3.52	d, 10.1
	3	33.0	2.09	m		3	32.8	2.11	m
	4	19.4	1.13	d, 6.7		4	19.6	0.88	d, 6.9
	5	19.4	0.90	d, 6.9		5	19.3	1.11	d, 6.7
HMPro	1	169.6	–	–	Hyp	1	173.3	–	–
	2	69.2	6.24	s		2	58.0	6.12	d, 9.3
	3	76.6	4.11	m		3	39.6	2.05	m
	4	41.7	2.01	m				2.86	m
			2.29	m		4	68.8	4.69	m
	5	55.1	4.99	m		5	54.4	3.68	dd, 11.8, 7.8
	6	19.0	1.49	d, 6.0				3.87	dd, 11.6, 4.9
Sar	1	168.4	–	–	Sar	1	168.7	–	–
	2	52.6	4.04	d, 17.7		2	52.3	3.94	d, 17.9
			4.72	d, 18.0				4.79	d, 17.7
	*N*Me	35.2	2.86	s		*N*Me	35.2	2.84	s
MeVal	1	170.0	–	–	MeAla	1	171.2	–	–
	2	71.9	3.00	d, 9.3		2	60.6	3.61	q, 6.8
	3	28.3	2.58	m		3	13.3	1.30	d, 6.9
	4	21.4	0.98	d, 6.6		*N*Me	36.6	2.94	s
	5	19.1	0.79	d, 6.9					
	*N*Me	39.9	3.00	s					

Chromophore

δ_H 2.22 (s, 3H, 12-H_3), 2.56 (s, 3H, 11-H_3), 7.43 (d, *J* = 7.7 Hz, 1H, 7-H), 7.49 (d, *J* = 7.6 Hz, 1H, 8-H).

δ_C 7.4 (CH_3, C-12), 14.6 (CH_3, C-11), 113.7 (C, C-4), 126.1 (CH, C-8), 128.2 (C, C-6), 130.7 (C, C-9a), 131.6 (CH, C-7), 133.2 (C, C-9), 141.2 (C, C-5a), 146.2 (C, C-4a), 168.8 (C, C-13), 169.1 (C, C-14), 179.8 (C, C-3). C-1, C-2, and C-10a were not obtainable from HSQC/HMBC data.*

* Due to only low amount, ^{13}C chemical shifts were obtained from HSQC and HMBC experiments instead of recording a 1D ^{13}C NMR spectrum.

Table S6. NMR data of actinomycin Y_3 (**3**) ^1H: 600 MHz, ^{13}C: 150.8 MHz, CD_3OD

Pentapeptidolactones

***α*-ring**	pos.	δ_C	δ_H	*J* [Hz]	***β*-ring**	pos.	δ_C	δ_H	*J* [Hz]
Thr	1	171.3	–	–	HThr	1	171.5	–	–
	2	57.8	5.09	d, 2.5		2	55.5	4.68	d, 2.5
	3	74.1	5.36	qd, 6.5, 2.5		3	68.9	4.57	ddd, 8.0, 6.0, 2.5
	4	17.7	1.42	d, 6.0		4	66.9	4.14	dd, 11.0, 8.0
								4.33	dd, 11.0, 6.0
D-Val	1	174.4	–	–	D-Val	1	172.4	–	–
	2	59.8	3.55	d, 9.8		2	59.1	4.27	d, 9.5
	3	32.5	2.03	d hept, 10.0, 6.5		3	30.1	2.14	d hept, 10.0, 6.5
	4	19.8	1.05	d, 6.5		4	19.8	0.92	d, 6.5
	5	19.7	0.84	d, 6.5		5	18.8	0.91	d, 6.5
HMPro	1	173.6	–	–	OPro	1	174.6	–	–
	2	67.4	6.21	s		2	54.4	5.54	dd, 9.0, 1.0
	3	75.9	4.24	m		3	40.1	2.44	m
	4	40.9	1.88	ddd, 12.5, 7.5, 5.0				4.01	m
			1.97	ddd, 12.5, 7.0, 4.0		4	209.9	–	–
	5	54.6	4.32	m		5	54.4	4.03	d, 18.0
	6	19.3	1.33	d, 6.0				4.28	d, 18.0
Sar	1	168.8	–	–	Sar	1	172.9	–	–
	2	53.1	4.03	d, 18.0		2	51.8	3.39	d, 15.0
			5.02	d, 18.0				4.71	d, 15.0
	*N*Me	35.8	2.88	s		*N*Me	38.9	3.38	s
MeVal	1	170.1	–	–	MeAla	1	174.2	–	–
	2	71.9	3.16	d, 9.5		2	55.9	4.95	q, 7.0
	3	28.2	2.64	d hept, 9.5, 6.5		3	15.2	1.42	d, 7.5
	4	22.0	1.05	d, 6.5		*N*Me	31.9	3.11	s
	5	19.4	0.84	d, 6.5					
	*N*Me	39.3	3.04	s					

Chromophore

δ_H 2.15 (s, 3H, 12-H_3), 2.51 (s, 3H, 11-H_3), 7.39 (d, *J* = 7.5 Hz, 1H, 7-H), 7.78 (d, *J* = 7.5 Hz, 1H, 8-H).

δ_C 7.9 (CH_3, C-12), 15.1 (CH_3, C-11), 98.4 (C, C-1), 114.5 (C, C-4), 126.8 (CH, C-8), 130.2 (C, C-9), 130.3 (C, C-6), 131.1 (CH, C-7), 132.0 (C, C-9a), 141.9 (C, C-5a), 147.1 (C, C-10a), 147.4 (C, C-4a), 150.4 (C, C-2), 167.9 (C, C-14), 168.7 (C, C-13), 179.7 (C, C-3).

Table S7. NMR data of actinomycin Y_4 (**4**) ^{1}H: 600 MHz, ^{13}C: 150.8 MHz, CD_3OD

Pentapeptidolactones

***α*-ring**	pos.	δ_C	δ_H	J [Hz]	***β*-ring**	pos.	δ_C	δ_H	J [Hz]
Thr	1	171.3	–	–	HThr	1	171.7	–	–
	2	58.1	4.99	d, 2.0		2	54.8	4.72	d, 3.5
	3	74.0	5.35	qd, 7.0, 2.0		3	70.2	4.33	m
	4	17.9	1.46	d, 7.0		4	67.0	4.25	dd, 11.0, 8.5
								4.51	m
D-Val	1	174.3	–	–	D-Val	1	172.3	–	–
	2	59.9	3.54	d, 10.0		2	59.1	4.36	d, 11.0
	3	32.5	2.04	m		3	30.4	2.09	m
	4	19.8	1.04	d, 6.5		4	19.7	0.92	d, 6.5
	5	19.5	0.84	d, 6.5		5	19.1	0.93	d, 6.5
HMPro	1	173.5	–	–	Hyp	1	175.4	–	–
	2	67.5	6.23	s		2	56.7	5.05	t, 8.0
	3	75.8	4.20	m		3	37.5	2.00	m
	4	40.9	1.88	m				2.24	m
			1.95	m		4	71.6	4.51	m
	5	54.6	4.31	m		5	57.3	3.62	d, 11.0
	6	19.2	1.33	d, 6.5				3.98	dd, 11.0, 4.0
Sar	1	168.8	–	–	Sar	1	172.3	–	–
	2	53.1	4.00	d, 18.0		2	51.8	3.36	m
			4.99	d, 18.0				4.57	d, 14.5
	*N*Me	35.8	2.86	s		*N*Me	38.9	3.34	s
MeVal	1	170.0	–	–	MeAla	1	173.7	–	–
	2	71.9	3.18	m		2	54.3	5.32	q, 7.0
	3	28.2	2.66	m		3	14.6	1.41	d, 7.0
	4	22.2	1.09	d, 6.5		*N*Me	32.2	3.11	s
	5	19.6	0.85	d, 6.5					
	*N*Me	39.3	3.01	s					

Chromophore

δ_H 2.17 (s, 3H, 12-H_3), 2.51 (s, 3H, 11-H_3), 7.40 (d, J = 7.5 Hz, 1H, 7-H), 7.81 (d, J = 7.5 Hz, 1H, 8-H).

δ_C 7.9 (CH_3, C-12), 14.6 (CH_3, C-11), 98.6 (C, C-1), 114.6 (C, C-4), 127.0 (CH, C-8), 129.8 (C, C-9), 130.4 (C, C-6), 131.1 (CH, C-7), 132.0 (C, C-9a), 141.9 (C, C-5a), 147.3 (C, C-4a), 147.3 (C, C-10a), 149.9 (C, C-2), 167.3 (C, C-14), 168.6 (C, C-13), 179.7 (C, C-3).

Table S8. NMR data of actinomycin Y_5 (**5**) ^{1}H: 600 MHz, ^{13}C: 150.8 MHz, CD_3OD

Pentapeptidolactone					**Cyclopentapeptide**				
***α*-ring**	pos.	δ_C	δ_H	*J* [Hz]	***β*-ring**	pos.	δ_C	δ_H	*J* [Hz]
Thr	1	170.5	–	–	HThr	1	169.0	–	–
	2	56.4	4.83	m		2	54.7	5.22	m
	3	74.5	5.33	qd, 6.0, 2.3		3	70.1	5.04	m
	4	16.3	1.26	d, 6.0		4	45.3	2.78	m
								3.10	m
D-Val	1	174.0	–	–	D-Val	1	172.4	–	–
	2	60.4	3.71	m		2	57.8	4.05	d, 10.7
	3	33.0	2.04	m		3	32.4	2.22	m
	4	19.4	1.09	d, 6.8		4	19.6	0.89	d, 6.9
	5	19.2	0.91	d, 6.9		5	18.8	1.18	d, 6.9
HMPro	1	172.4	–	–	OPro	1	174.9	–	–
	2	69.1	6.30	s		2	57.1	5.64	m
	3	74.7	4.27	m		3	40.3	2.33	m
	4	40.6	1.92	m				3.92	m
			1.96	m		4	209.5	–	–
	5	55.2	4.24	m		5	53.8	3.89	d, 19.2
	6	18.6	1.45	d, 6.0				4.43	d, 19.2
Sar	1	168.1	–	–	Sar	1	169.5	–	–
	2	52.6	4.05	d, 18.0		2	52.5	4.00	d, 18.0
			4.81	d, 18.0				4.69	d, 18.0
	*N*Me	35.2	2.88	s		*N*Me	35.2	2.88	s
MeVal	1	169.9	–	–	MeAla	1	170.5	–	–
	2	71.8	3.07	m		2	60.8	3.60	q, 7.0
	3	28.9	2.60	m		3	13.5	1.42	d, 6.5
	4	21.5	0.99	d, 6.5		*N*Me	36.9	2.94	s
	5	19.1	0.80	d, 6.5					
	*N*Me	39.3	3.03	s					

Chromophore

δ_H 2.21 (s, 3H, 12-H_3), 2.54 (s, 3H, 11-H_3), 7.43 (d, *J* = 7.5 Hz, 1H, 8-H), 7.44 (d, *J* = 7.5 Hz, 1H, 7-H).

δ_C 7.9 (CH_3, C-12), 15.0 (CH_3, C-11), 102.6 (C, C-1), 115.4 (C, C-4), 127.0 (CH, C-8), 128.7 (C, C-6), 130.2 (C, C-9), 131.6 (CH, C-7), 132.2 (C, C-9a), 141.4 (C, C-5a), 145.0 (C, C-4a), 147.1 (C, C-10a), 151.2 (C, C-2), 167.9 (C, C-13), 174.9 (C, C-14), 178.5 (C, C-3).

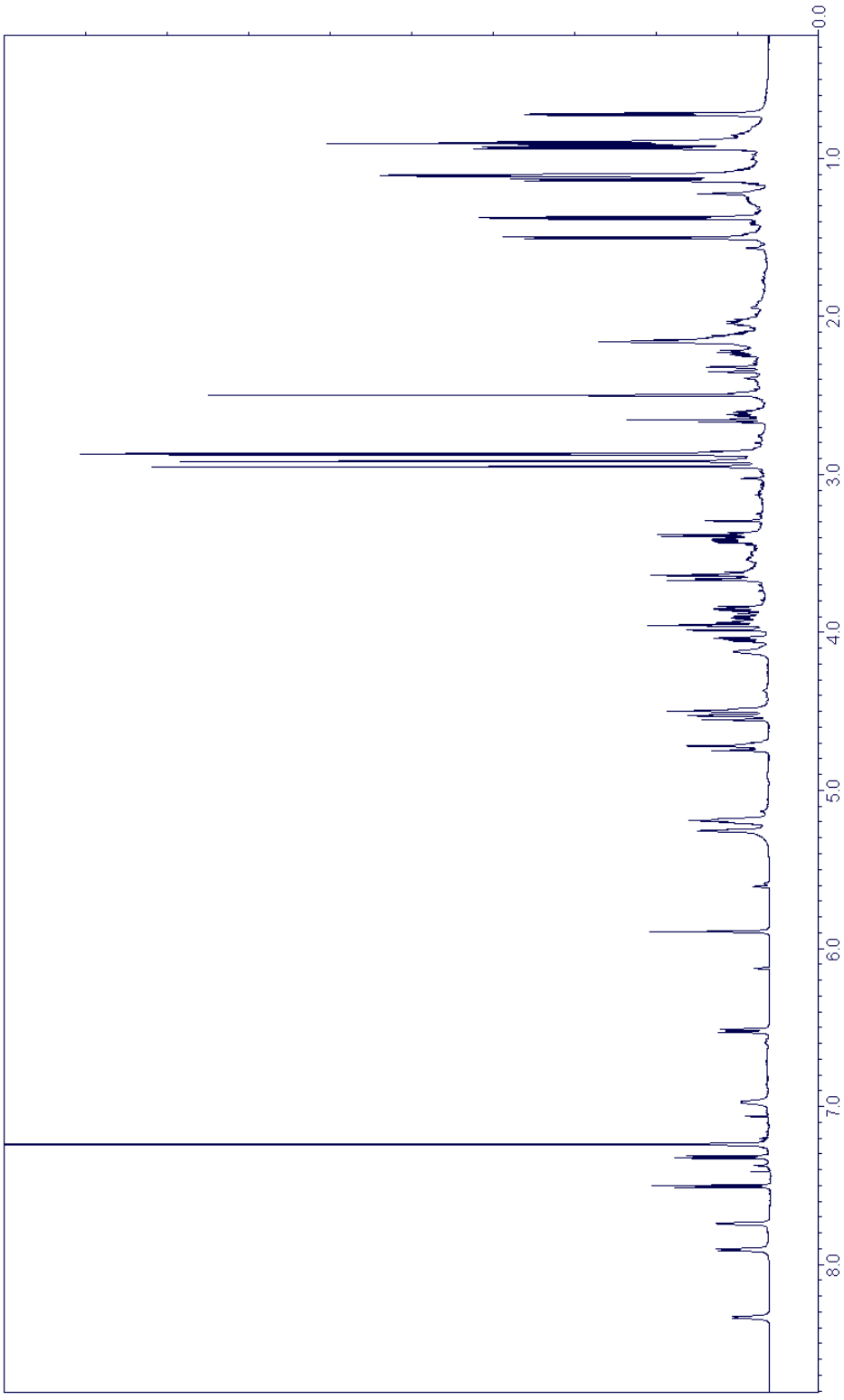


Figure S4: ^{1}H NMR spectrum of actinomycin Y_1 (**1**) ($CDCl_3$, 600 MHz).

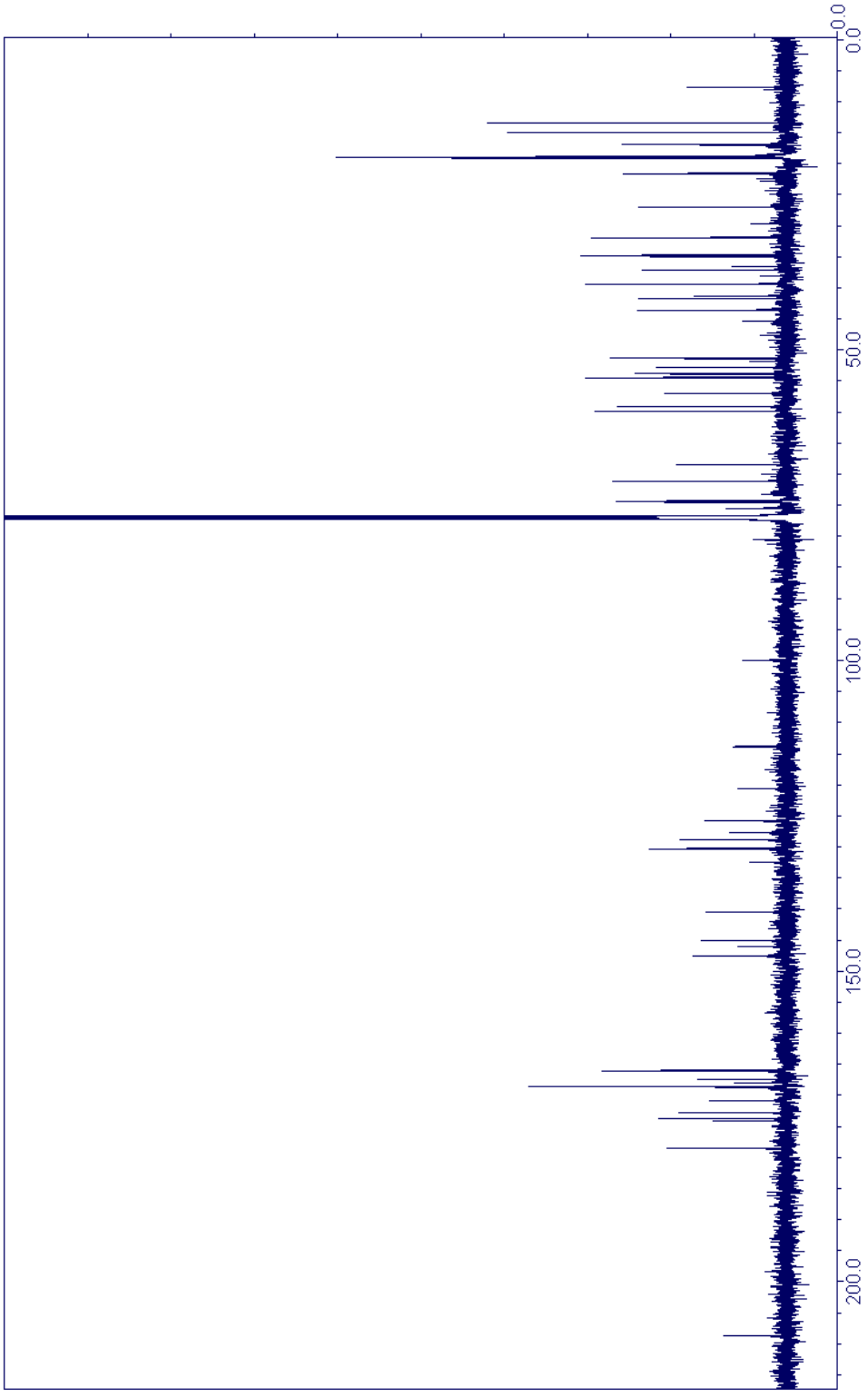


Figure S5: ^{13}C NMR spectrum of actinomycin Y_1 (**1**) ($CDCl_3$, 150 MHz).

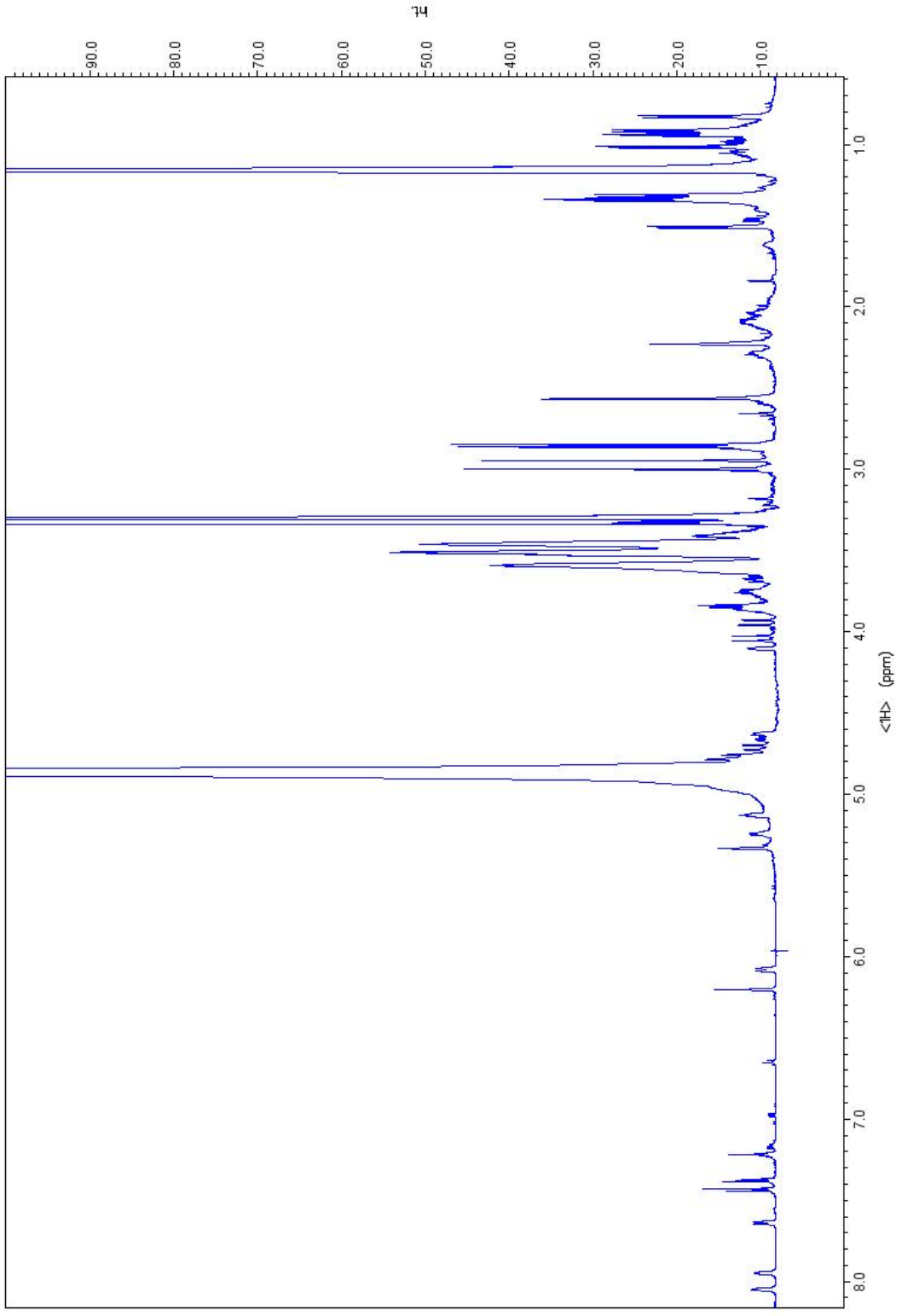


Figure S6: ^{1}H NMR spectrum of actinomycin Y_2 (**2**) (CD_3OD, 600 MHz).

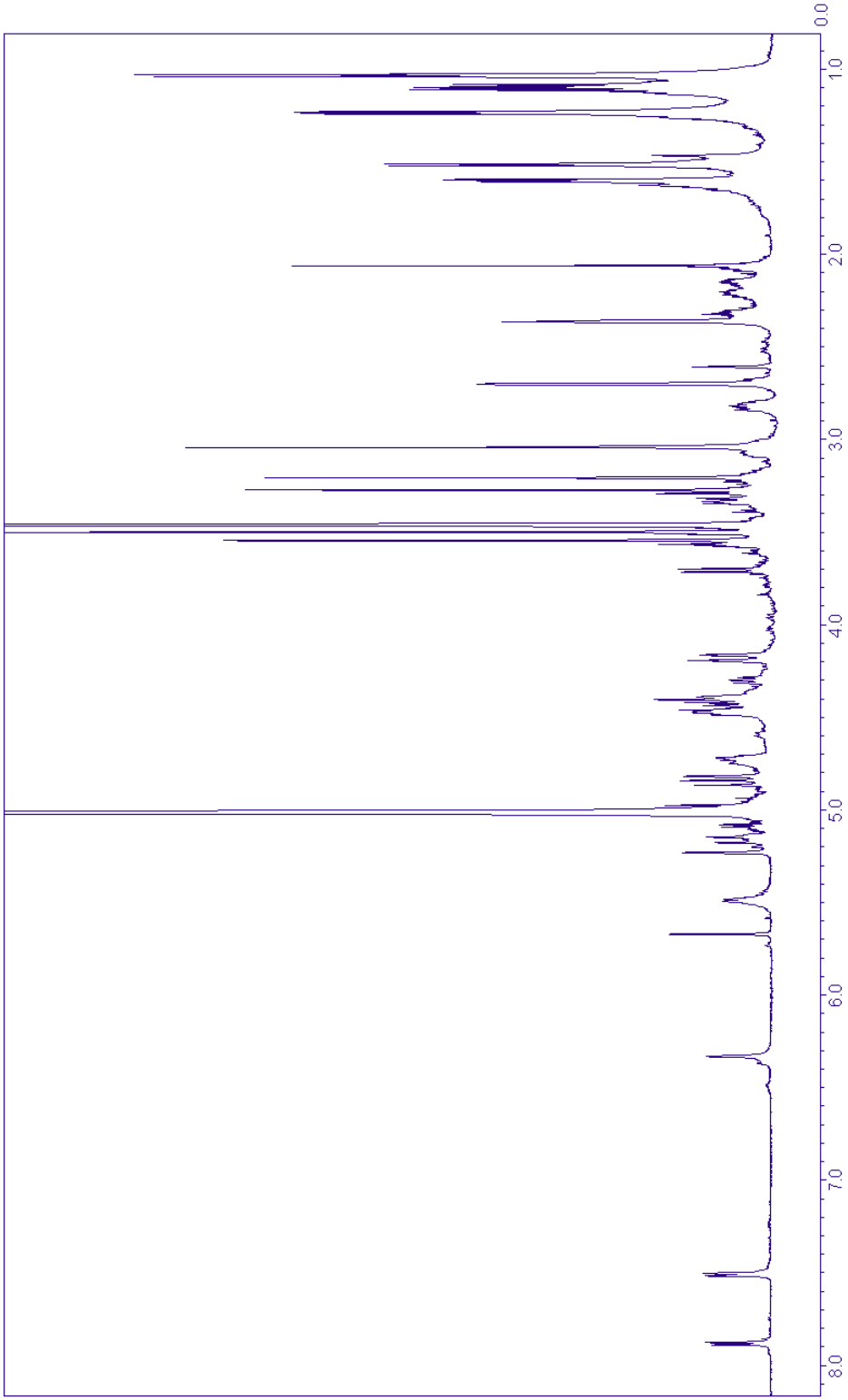


Figure S7: ^{1}H NMR spectrum of actinomycin Y_3 (**3**) (CD_3OD, 600 MHz).

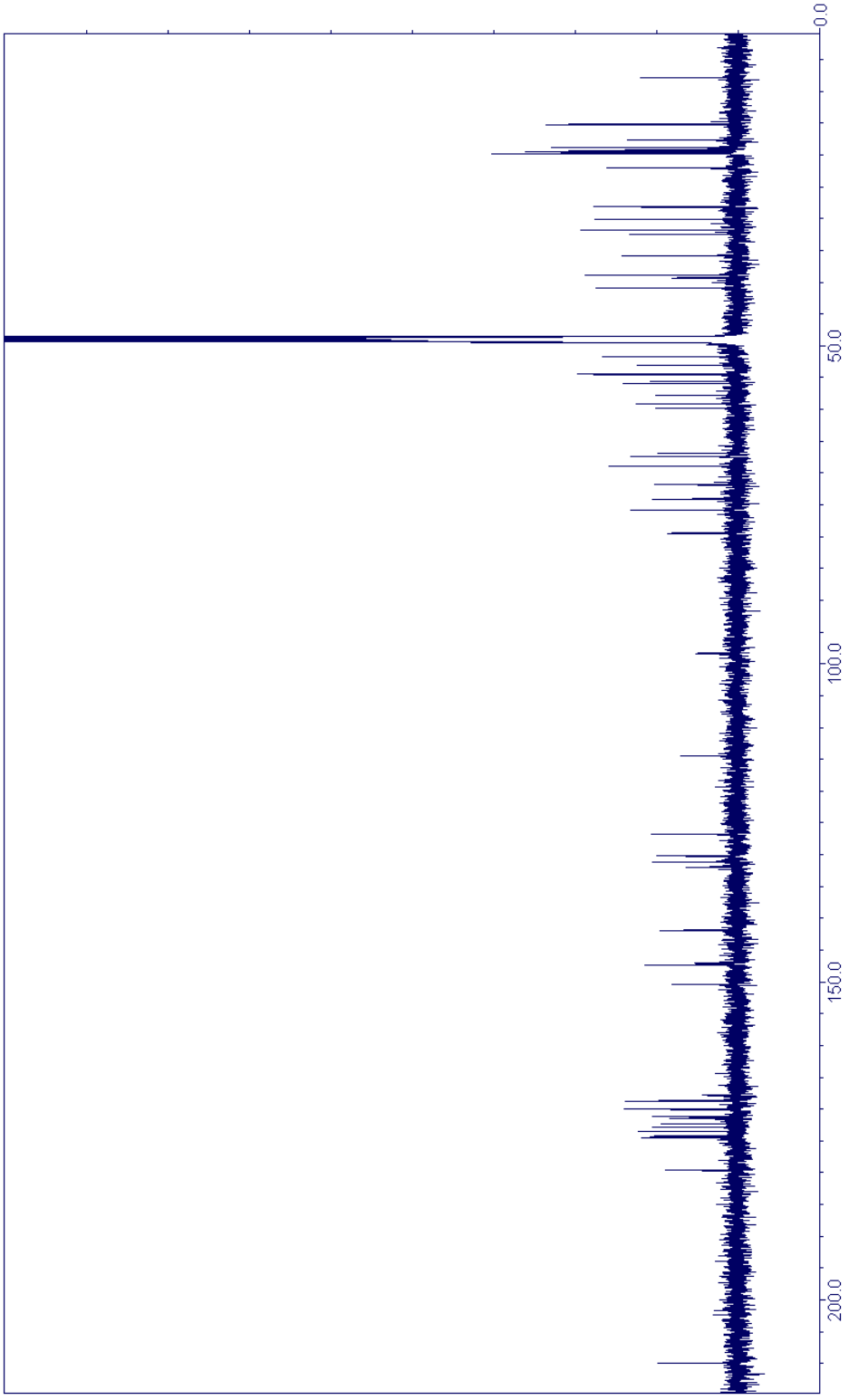


Figure S8: ^{13}C NMR spectrum of actinomycin Y_3 (**3**) (CD_3OD, 150 MHz).

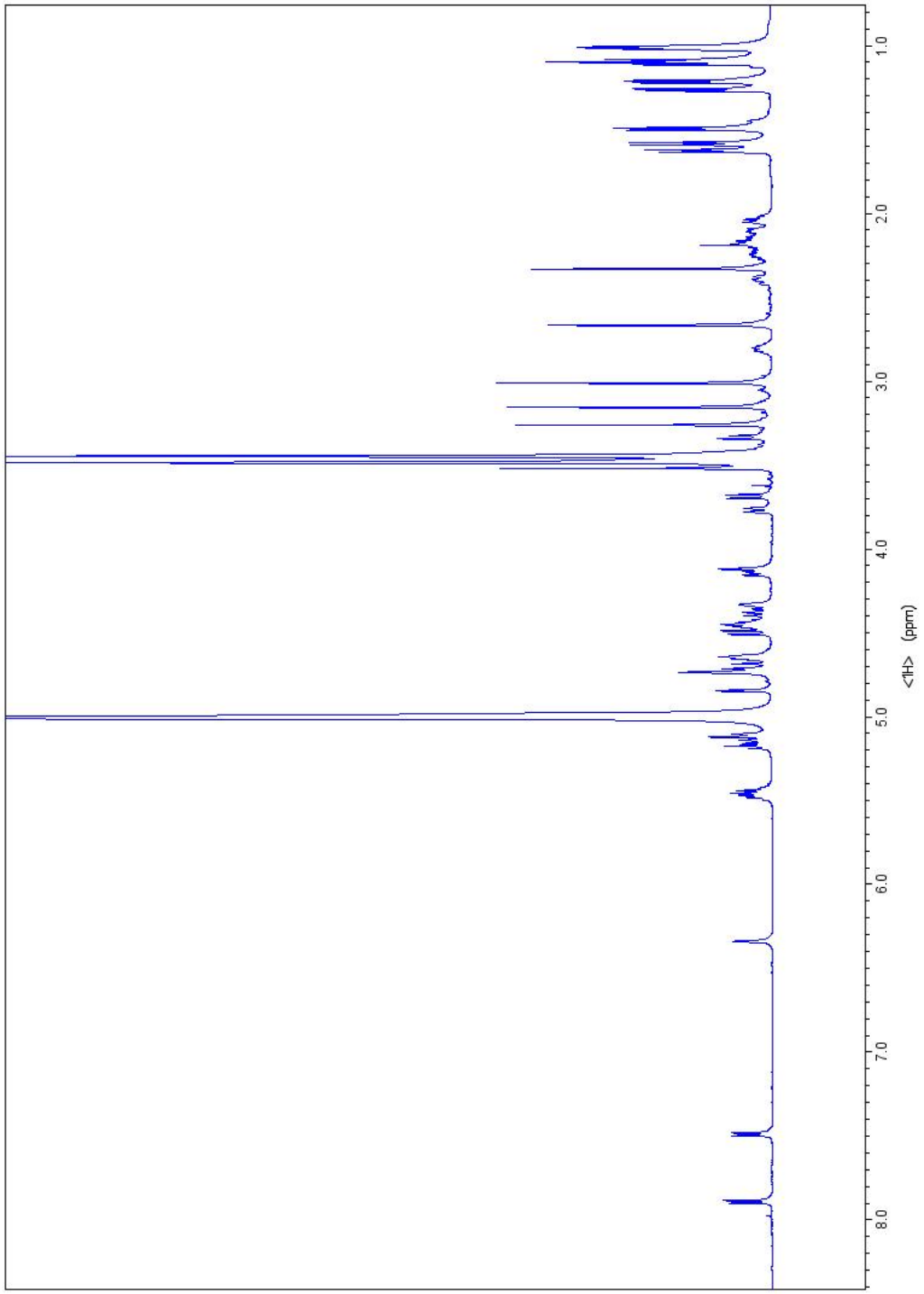


Figure S9: ^{1}H NMR spectrum of actinomycin Y_4 (**4**) (CD_3OD, 600 MHz).

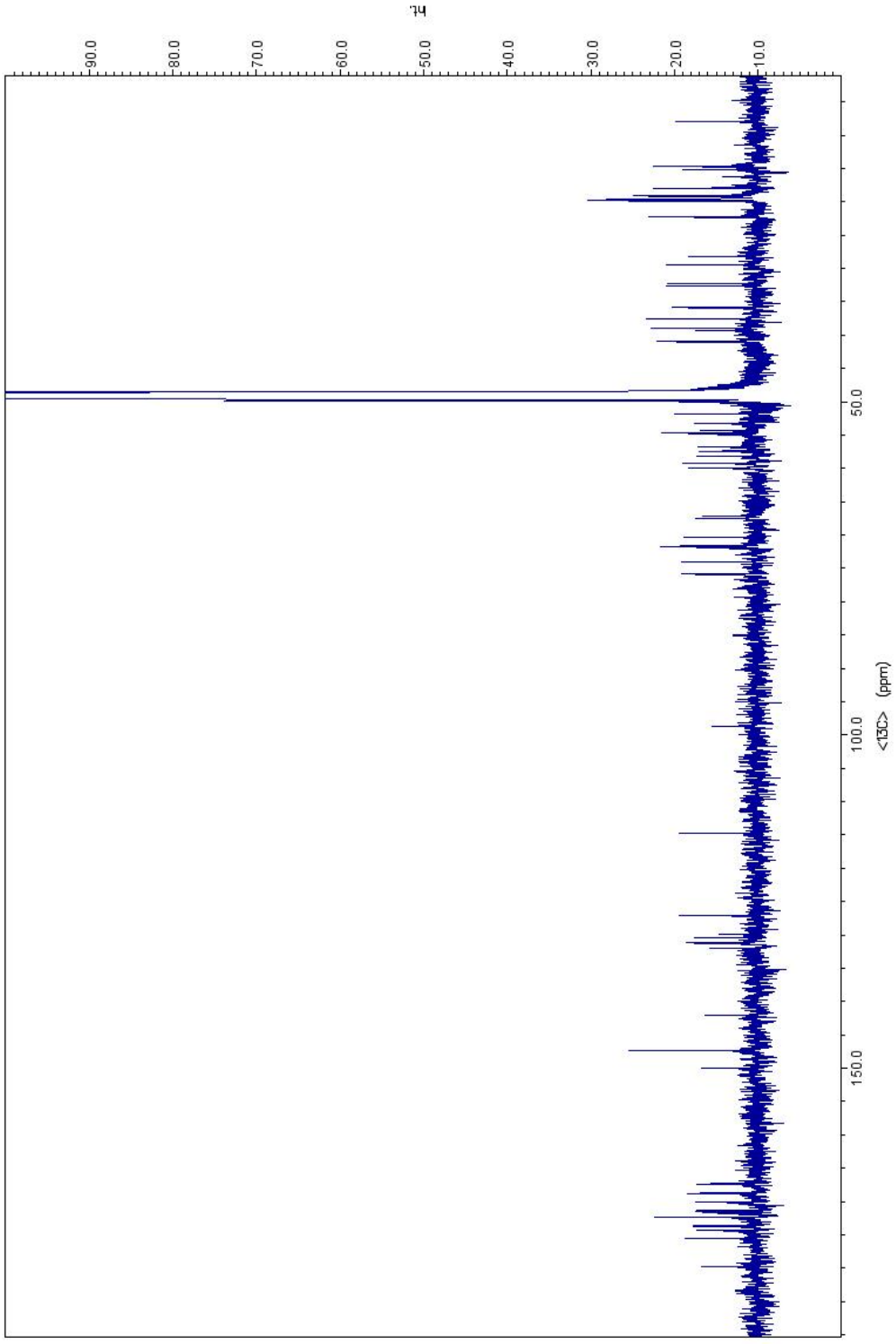


Figure S10: ^{13}C NMR spectrum of actinomycin Y_4 (**4**) (CD_3OD, 150 MHz).

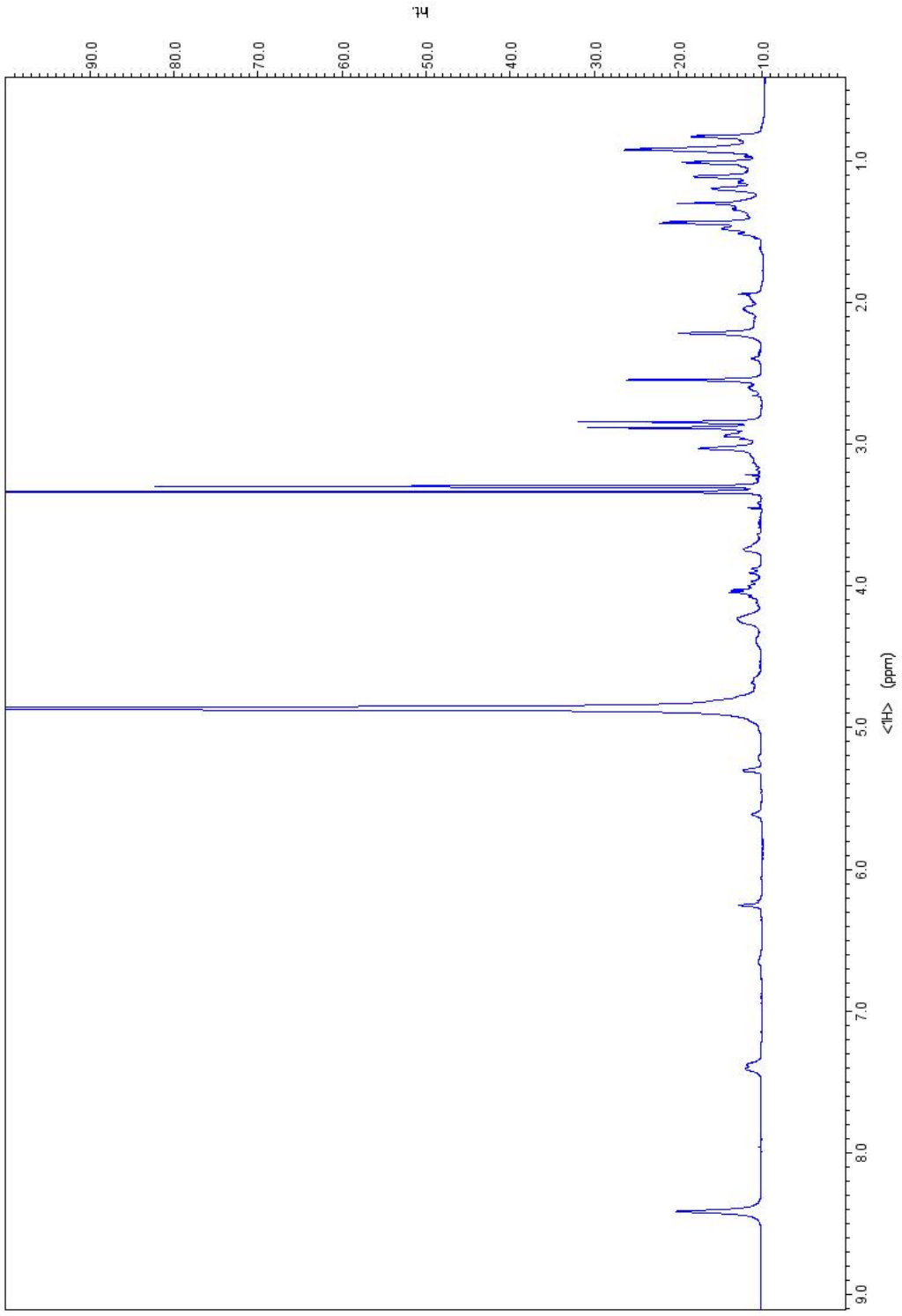


Figure S11: ^{1}H NMR spectrum of actinomycin Y_5 (**5**) (CD_3OD, 600 MHz).

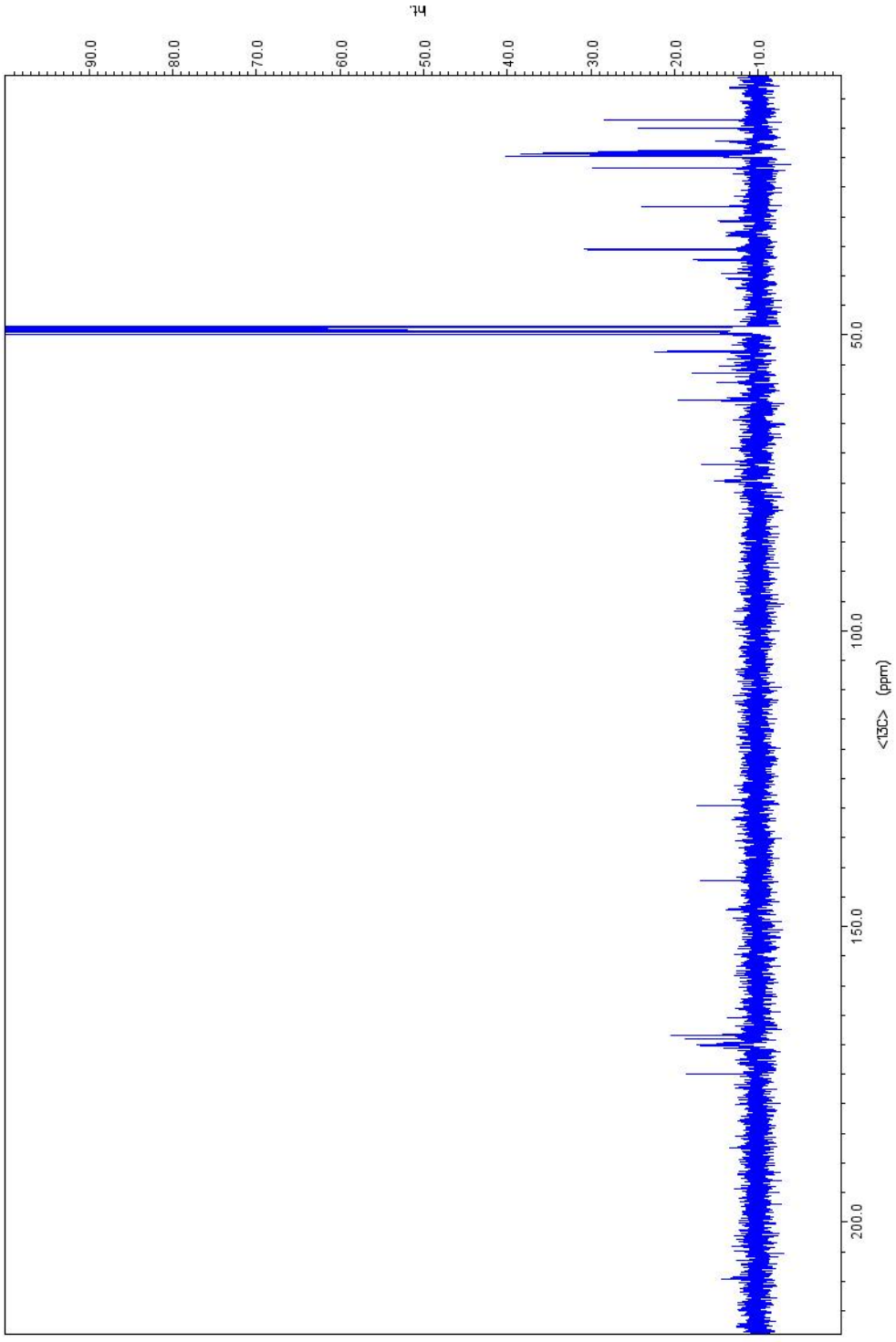


Figure S12: ^{13}C NMR spectrum of actinomycin Y_5 (**5**) (CD_3OD, 150 MHz).

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