Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

Biosynthetic rivalry of *o*-aminophenol-carboxylic acids initiates production of *hemi*-actinomycins in *Streptomyces antibioticus*

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FIGURES

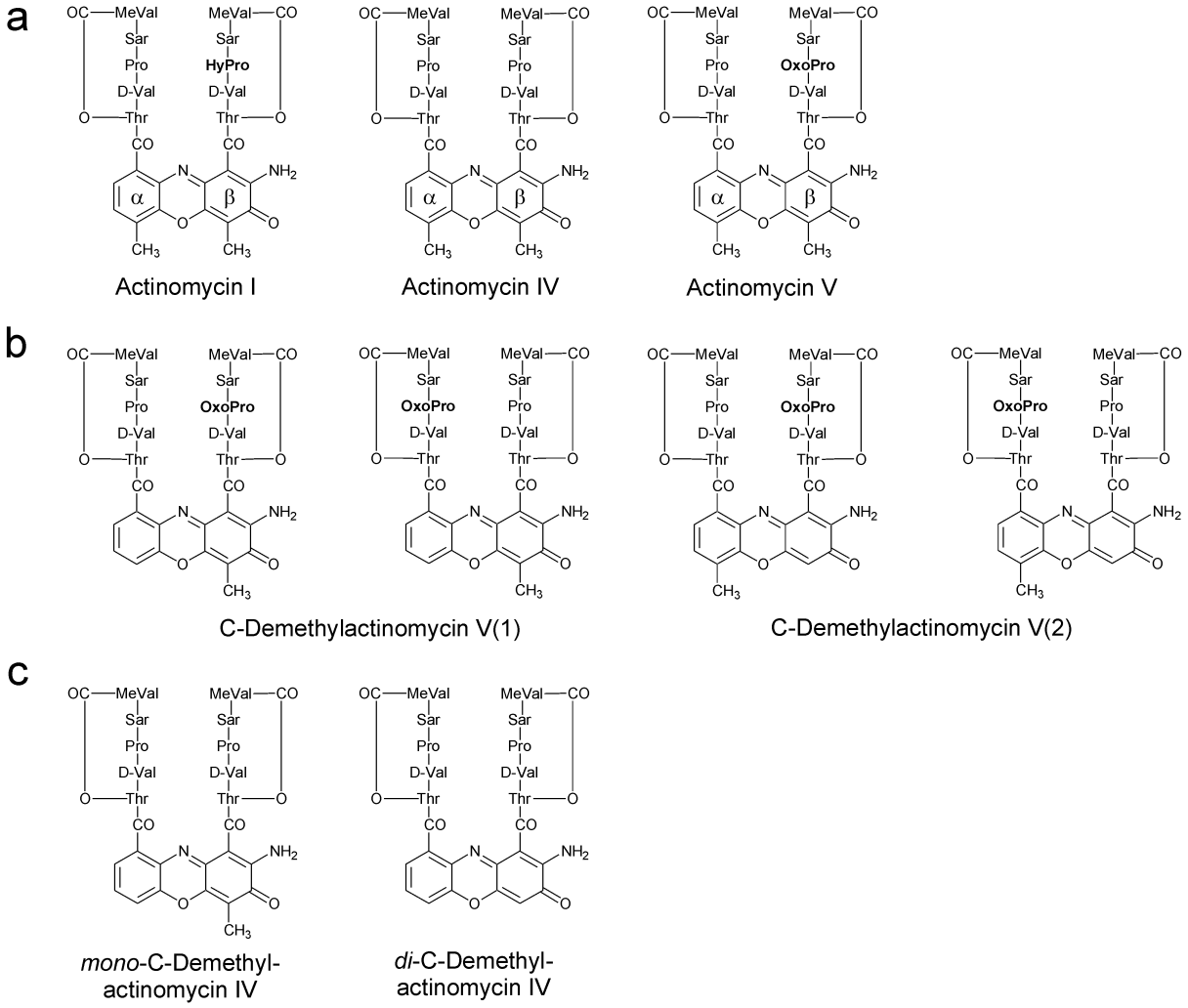

Figure S1: Structures of the different actinomycins of the Actinomycin X complex of *S. antibioticus*. a: actinomycin I, IV and V., **b**: Structures of the possible four isomeric *mono*-C-demethylactinomycins V. **c**: Structures of *mono*-C-demethylactinomycin IV ($\mathbf{D_{IV}}$) and *di*-C-demethylactinomycin IV ($\mathbf{DD_{IV}}$). Sar: N-methylglycine, MeVal: N-methyl-L-valine, HyPro: 4-hydroxyproline, OxoPro: 4-oxoproline.

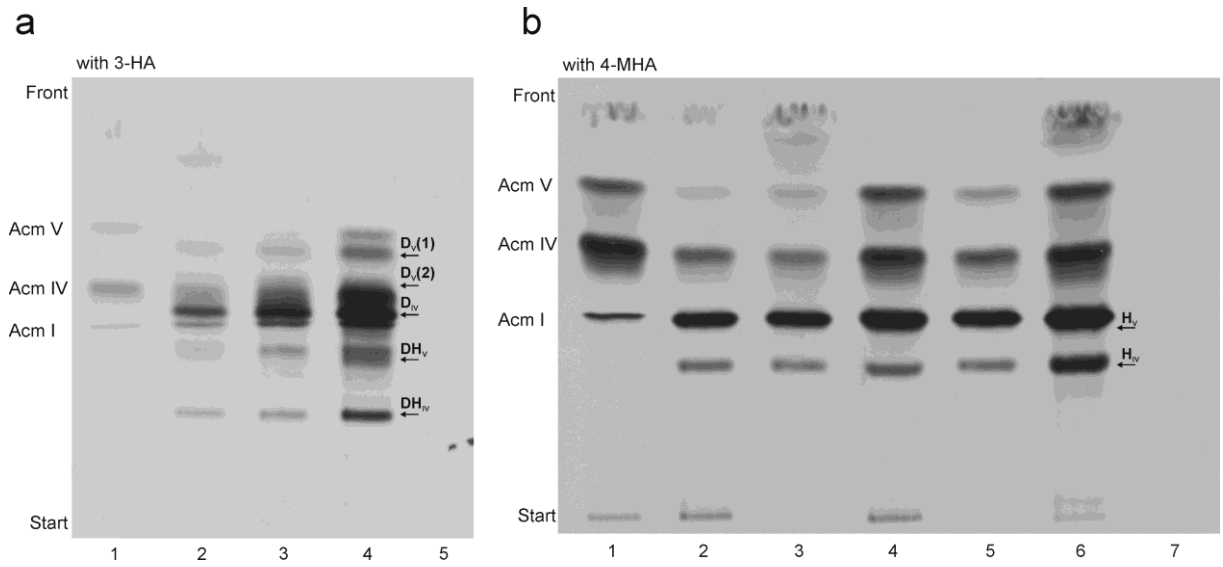

Figure S2: Incorporation of ^{14}C-labeled amino acid precursors into the pentapeptide lactone rings of C-demethylactinomycins, C-demethyl-*hemi*-actinomycins (a) and *hemi*-actinomycins (b) elaborated by *S. antibioticus*. a: TLC separations of extracts from radioincorporation experiments of *S. antibioticus* mycelium with various radioactive amino acids in the presence of 250 µM 3-HA. Lane 2: Incubation with ^{14}C-threonine, Lane 3: with ^{14}C-proline, Lane 4: with (^{14}C-methyl)-L-methionine, Lane 5: with ^{14}C-glutamic acid, Lane 1: with ^{14}C-threonine with omission of 3-HA. Glutamic acid is no constituent of the peptide chains of any actinomycin and therefore is not incorporated. **b:** TLC separations of extracts from radioincorporation experiments of *S. antibioticus* mycelium with various amino acids in the presence of 250 µM 4-MHA. Lane 2: Incubation with ^{14}C-valine, Lane 3: with ^{14}C-threonine, Lane 4: with ^{14}C-proline, Lane 5: with ^{14}C-glycine, Lane 6: with ^{14}C-methyl-L-methionine, Lane 7: with ^{14}C-glutamic acid, Lane 1: with ^{14}C-valine with omission 4-MHA. Symbols at the different bands denote new compounds as follows: **$D_V(1)$**: *mono*-C-demethylactinomycin V(1), **$D_V(2)$**: *mono*-C-demethylactinomycin V(2), **D_{IV}**: *mono*-C-demethylactinomycin IV, **DH_{IV}**: *mono*-C-demethyl-*hemi*-actinomycin V, **DH_V**: *mono*-C-demethyl-*hemi*-actinomycin IV, **H_V**: *hemi*-actinomycin V, **H_{IV}**: *hemi*-actinomycin IV. 3-HA: 3-hydroxyanthranilic acid, 4-MHA: 4-methyl-3-hydroxyanthranilic acid, Acm: actinomycin. Separation was on silica thin-layer plates using solvent system I. Time of exposure of the chromatogram to x-ray film was 3 d.

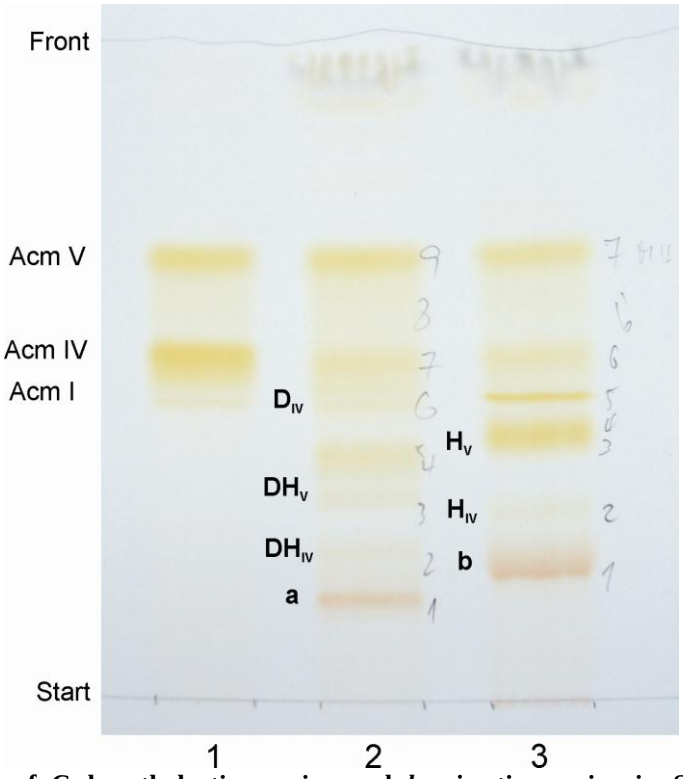

Figure S3: Formation of C-demethylactinomycins and *hemi*-actinomycins in *S. antibioticus*. Lane 1: Separation of actinomycin mixture formed in mycelial suspensions. Lane 2: Separation of actinomycins formed in the presence of 250 µM 3-hydroxyanthranilic acid (3-HA). Lane 3: Separation of actinomycins formed in the presence of 250 µM 4-methyl-3-hydroxyanthranilic acid (4-MHA). Separation was on silica plates using solvent system I. **a**: cinnabarinic acid, **b**: actinocin, $\mathbf{D_{IV}}$: *mono*-C-demethylactinomycin IV, $\mathbf{DH_{IV}}$: *mono*-C-demethyl-*hemi*-actinomycin V, $\mathbf{DH_V}$: *mono*-C-demethyl-*hemi*-actinomycin IV, $\mathbf{H_V}$: *hemi*-actinomycin V, $\mathbf{H_{IV}}$: *hemi*-actinomycin IV, Acm: actinomycin.

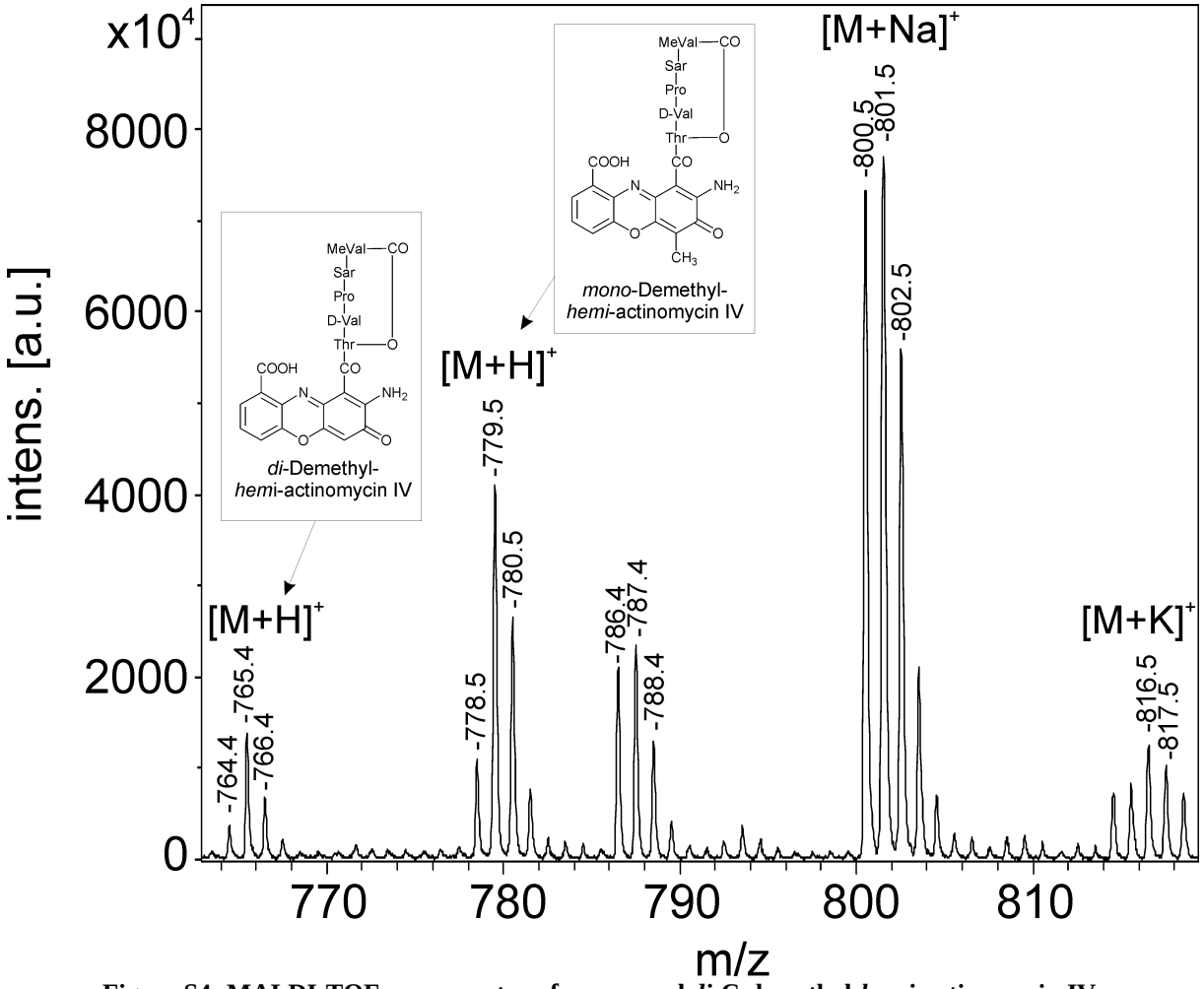

Figure S4: MALDI-TOF mass spectra of *mono*- and *di*-C-demethyl-*hemi*-actinomycin IV.

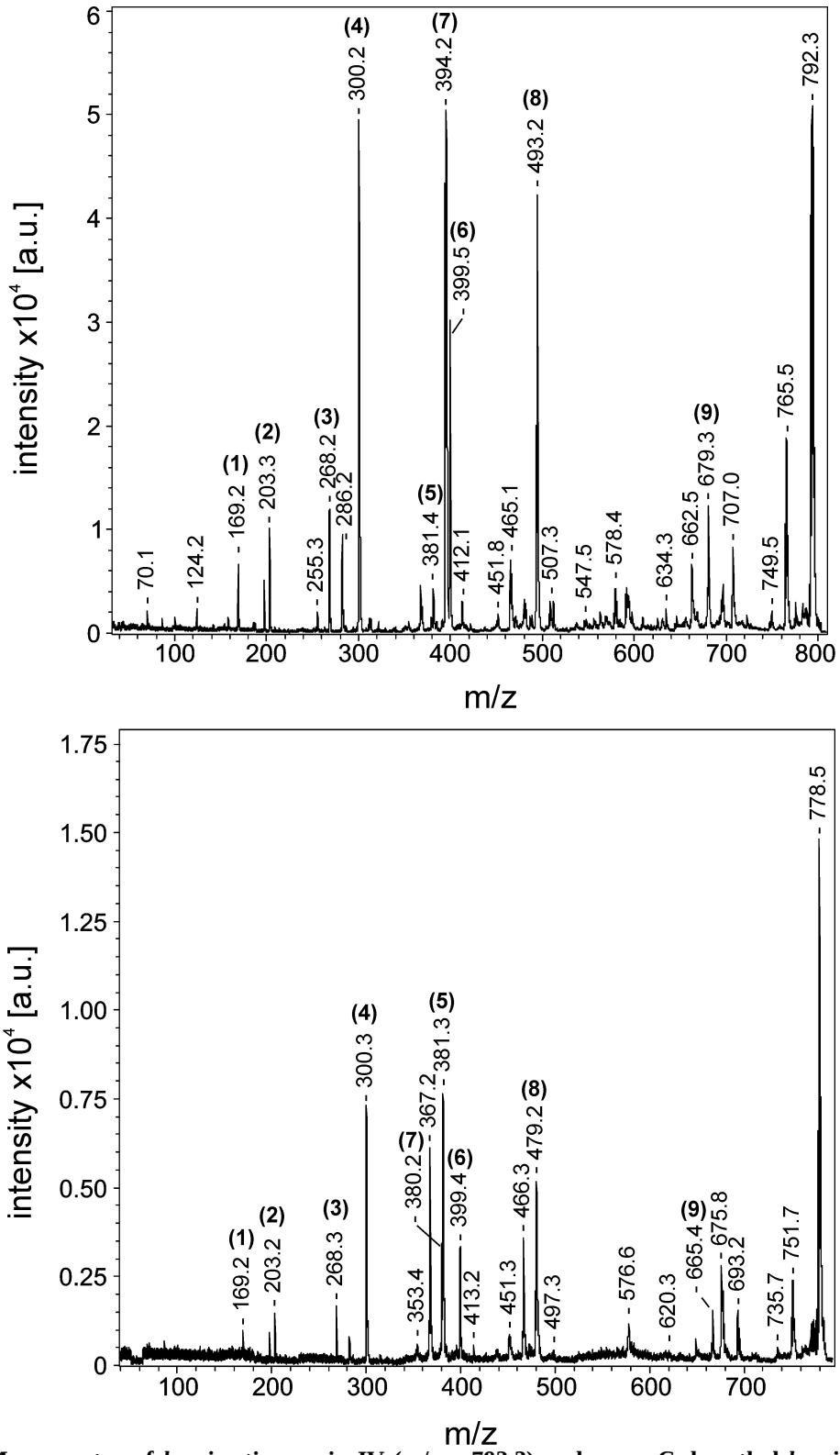

Figure S5: Mass spectra of *hemi*-actinomycin IV (m/z = 792.3) and *mono*-C-demethyl-*hemi*-actinomycin IV (m/z = 778.5) from *S. antibioticus* obtained by PSD-MALDI-TOF mass spectrometric analysis.

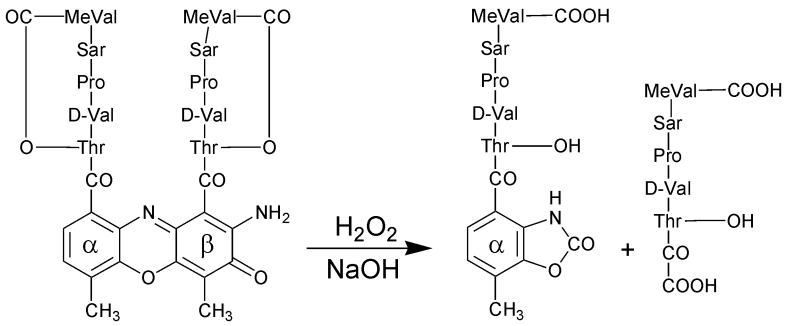

Figure S6: Reaction scheme of alkaline oxidative cleavage of actinomycin IV by NaOH/H_2O_2. Sar: N-methylglycine, MeVal: N-methyl-L-valine. (after Bullock E. and A.W. Johnson *J. Chem. Soc.*, 1957, **0**, 1602-1607.)

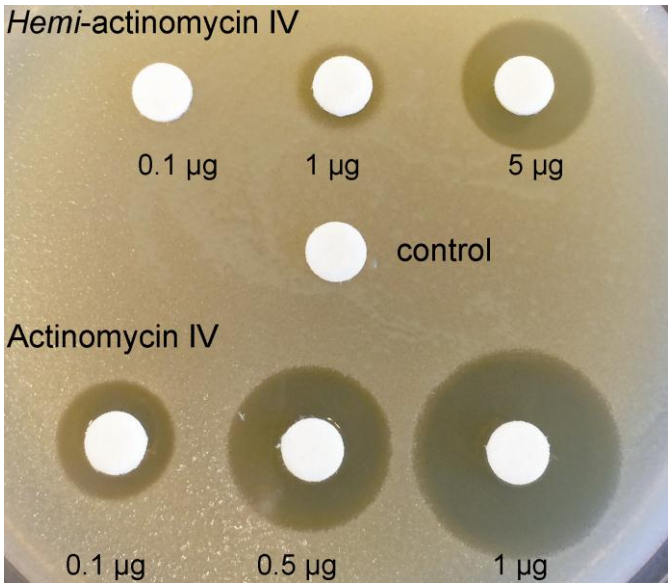

Figure S7: Antimicrobial activity of *Hemi*-actinomycin IV against *Bacillus subtillis*. Plate diffusion tests were performed as described in Material and Methods. Shown are *in vivo* inhibitory activity of *hemi*-actinomycin IV and actinomycin IV against *B. subtillis*. The indicated amounts of compounds dissolved in ethanol were spotted on paper discs (6 mm diameter, Schleicher and Schuell) and after drying were laid on a soft top nutrient agar (Difco) inoculated with 0.1 % (v/v) of an overnight culture of *Bacillus subtilis* ATCC 6633. After incubation at 30 °C over night a diameter of inhibition zones were determined.

TABLES

Fragment ions of *hemi*-actinomycin D species obtained by PSD-MALDI-TOF mass spectrometry

	hemiact D		demethyl-hemiact D	didemethyl-hemiact D
	$[M + H]^+ = 792.6$		$[M + H]^+ = 778.5$	$[M + H]^+ = 764.4$
fragments	m/z			
	calc.	found	found	found
Fragment ions of the peptide lactone moiety:				
VP	197.2	197.3	197.3	197.2
PMeG	169.1	169.2 (1)	169.2	169.2
MeGMeV	185.1	185.4	185.4	185.6
H-MeGMeV-OH	203.1	203.3 (2)	203.2	203.3
VPMeG	268.2	268.2 (3)	268.3	268.3
PMeGMeV	282.2	282.3	282.3	282.3
H-PMeGMeV-OH	300.2	300.2 (4)	300.3	300.4
VPMeGMeV	381.3	381.4 (5)	381.3	381.4
H-VPMeGMeV-OH	399.3	399.5 (6)	399.4	399.4
TVPMeGMeV	482.3	482.3	482.2	n.f.
Fragment ions generated by elimination of parts of the peptide lactone moiety from hemiactinomycin D species:				
Loss of:				
V	693.3	693.5	n.f.	665.2.
P	695.3	695.4	681.6	667.5
MeV	679.3	679.3 (9)	665.4	651.5
VP	596.2	596.3	582.2	n.f.
H-VP-OH	578.2	578.2	564.1	n.f.
H-VPMeG-OH	507.2	507.2	493.2	497.3
VPMeGMeV	412.1	412.2	398.1	384.0
H-VPMeGMeV-OH	394.1	394.2 (7)	380.2	n.f.
PMeGMeV	511.2	511.3	497.2	483.6
H-PMeGMeV-OH	493.2	493.2 (8)	479.2	n.f.
MeGMeV	608.3	608.3	594.5	580.2
H-MeGMeV-OH	590.3	590.3	576.4	562.3
TVPMeGMeV	311.1	311.1	287.0	n.f.

Table S1: The numbers set in parenthesis behind the masses of *hemi*-actinomycin D - fragments correspond to the numbers indicating prominent mass peaks in the corresponding PSD-MALDI-TOF mass spectra shown in Figure S5.

	Actinomycin	R_f-values	Mol. Wt.
*	Actinomycin I	0.42-0.47	1271.42
*	Actinomycin IV	0.52-0.58	1255.42
*	Actinomycin V	0.65-0.69	1269.40
	mono-C-Demethylactinomycin I	0.43-0.48	1257.39
*	*mono*-C-Demethylactinomycin IV	0.49-0.55	1241.39
*	*di*-C-Demethylactinomycin IV	0.49-0.55	1227.36
*	*mono*-C-Demethylactinomycin V (1)	0.62-0.64	1255.37
*	*mono*-C-Demethylactinomycin V (2)	0.58-0.6	1255.37
*	*di*-C-Demethylactinomycin V	0.58-0.64	1241.35
*	*Hemi*-actinomycin IV	0.30-0.35	791.85
*	*Hemi*-actinomycin V	0.40-0.44	805.83
*	*mono*-C-Demethyl-*hemi*-actinomycin IV	0.23-0.26	777.82
*	*di*-C-Demethyl-*hemi*-actinomycin IV	0.23-0.26	763.79
*	*mono*-C-Demethyl-*hemi*-actinomycin V	0.37-0.4	791.80

Table S2: List of actinomycins of the X complex from *Streptomyces antibioticus* and demethyl- and *hemi*-derivatives found in this study. C-Demethylactinomycins found also in natural conditions are marked with an asterisk. Separation was on silica plates using solvent system I.