

CHEMBIOCHEM

Supporting Information

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Supporting Information

for

Cloning and Heterologous Expression of the Aranciamycin Biosynthetic
Gene Cluster Revealed a New Flexible Glycosyltransferase

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S. E. Wohlert, A. Vente, and Andreas Bechthold*

MCF7 cells with aranciamycines

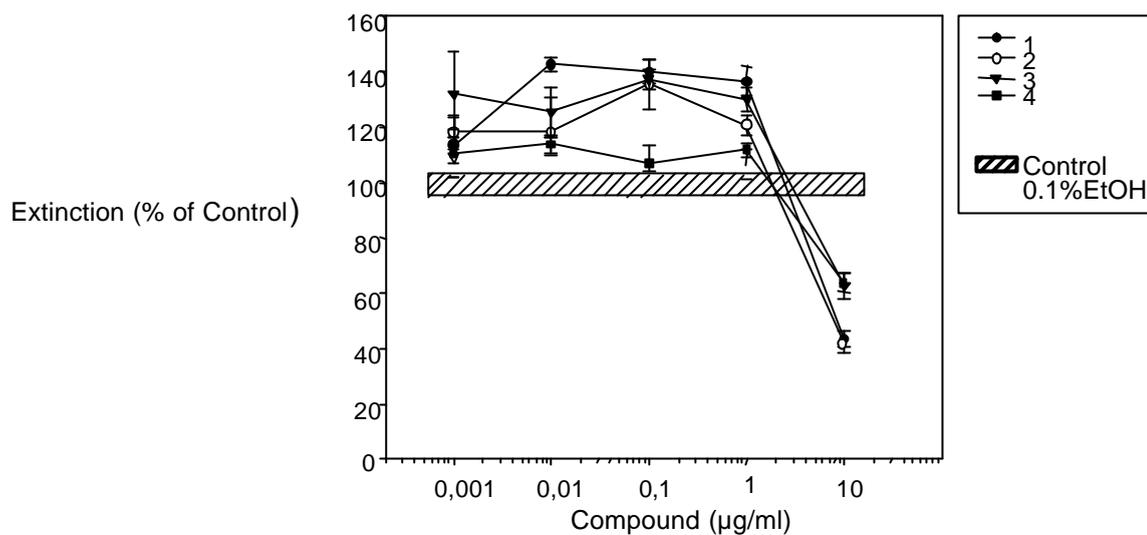


Figure S1. Activities of aranciamycins on MCF7 cell line. 1 – aranciamycin D; 2 – aranciamycin B; 3 – aranciamycin C; aranciamycinone.

MaTu cells with aranciamycines

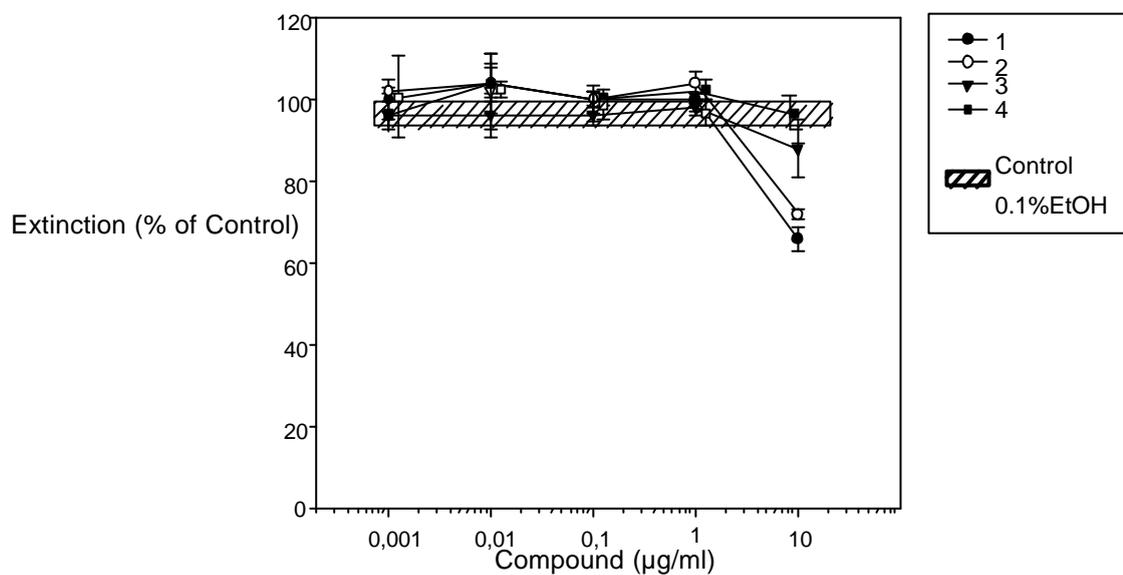
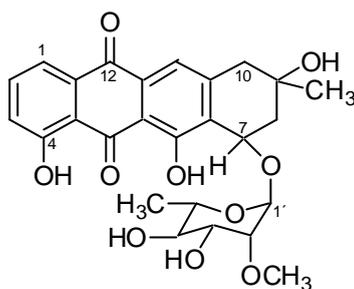


Figure S2. Activities of aranciamycins on Matu cell line. 1 – aranciamycin D; 2 – aranciamycin B; 3 – aranciamycin C; aranciamycinone.



aranciamycin A

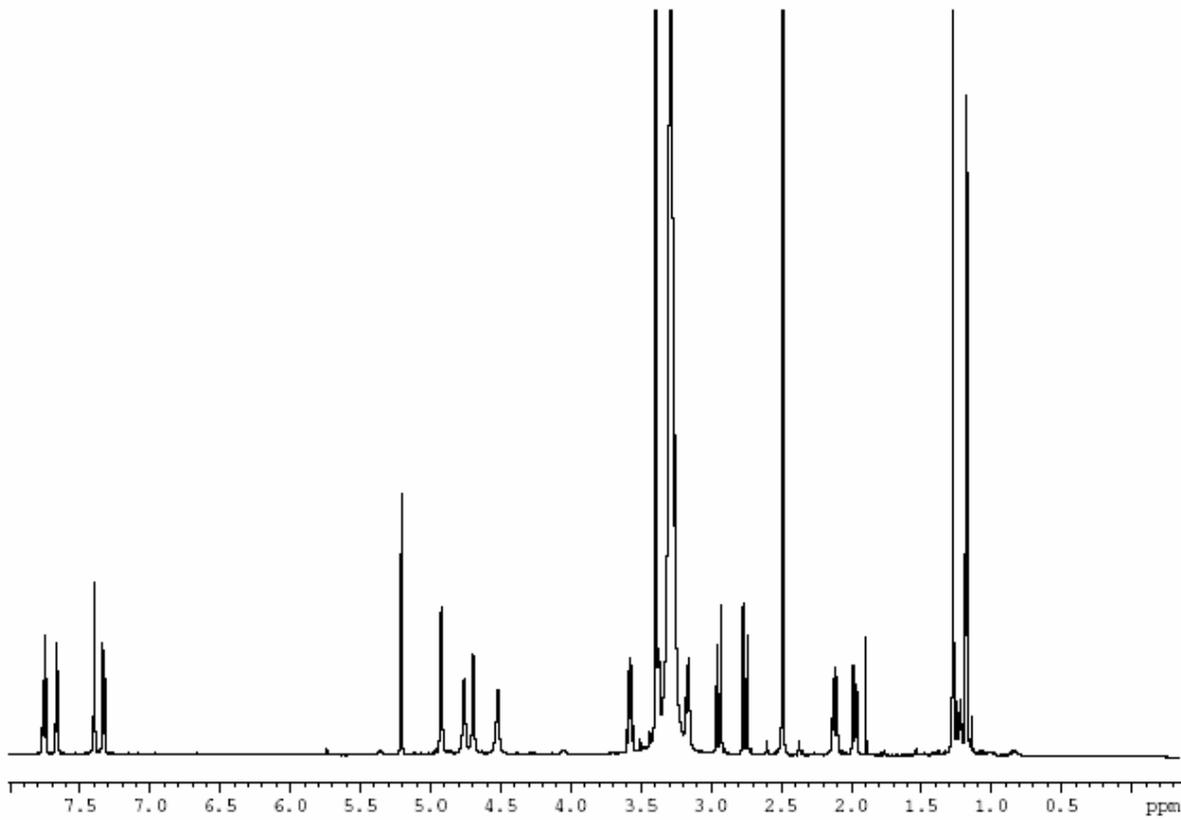
Molecular formula: C₂₆H₂₈O₁₀

Monoisotopic mass: 500.1682

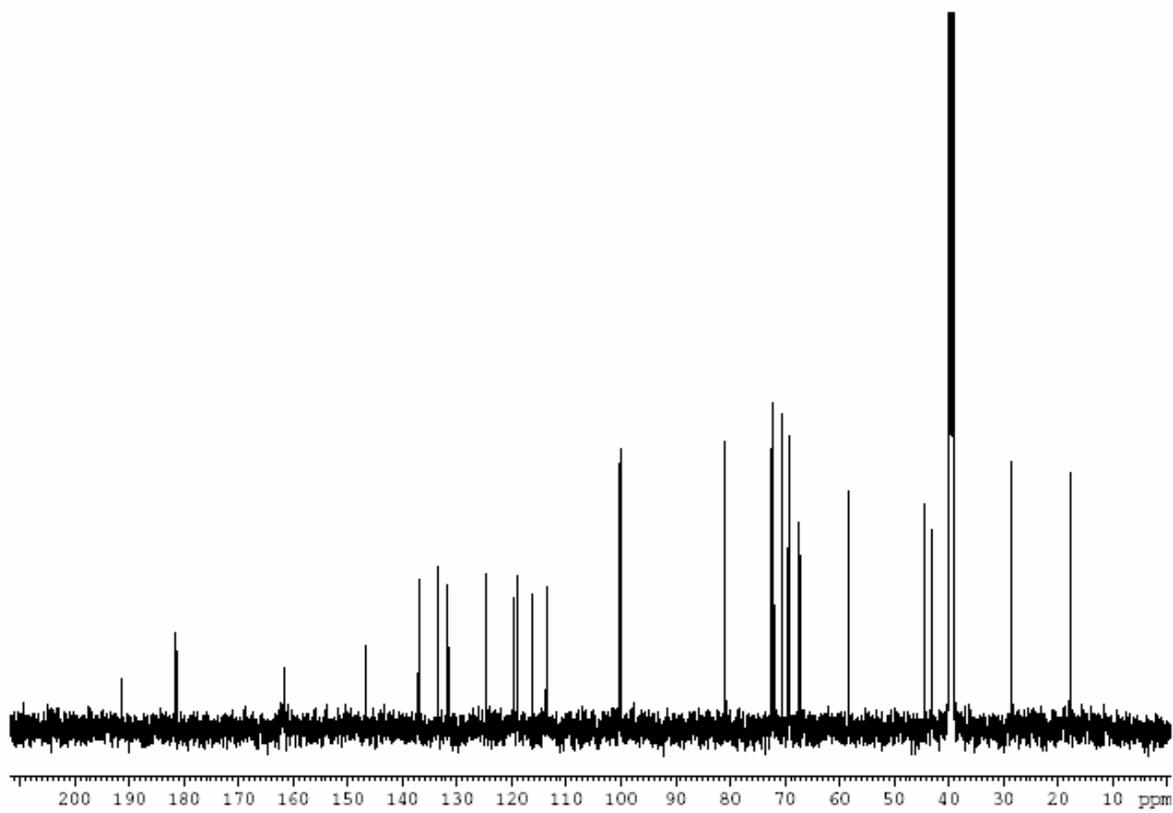
in [D₆]DMSO, T = 305K

Position	d ¹ H	d ¹³ C	COSY	HMBC
C-1	7.66 d, 1H, 7.7 Hz	118.9 d	7.75; (7.33)	181.4; 124.6; 116.1
C-2	7.75 dd, 1H, 8.2, 7.7 Hz	137.0 d	7.66; 7.33	161.6; 133.4; (124.6; 116.1)
C-3	7.33 d, 1H, 8.2 Hz	124.6 d	7.75; (7.66)	191.4; 161.6; 118.9; 116.1
C-4		161.6 s		
C-4-OH	12.5 br. s, 1H			
C-4a		116.1 s		
C=O-5		191.4 s		
C-5a		113.6 s		
C-6		162.0 s		
C-6-OH	12.5 br. s, 1H			
C-6a		131.4* s		
C-7	4.92 dd, 1H, 5.6, 5.1 Hz	72.5 d	2.11; 1.97	162.0; 146.7; 131.4; 100.1; 67.3; 43.1
C-8	2.11 dd, 1H, 13.8, 6.1 Hz 1.97 dd, 1H, 13.8, 5.1 Hz	43.1 t	4.92; 1.97 4.92; 2.11	131.4; 72.5; 67.3; 44.4; 28.4 131.4; 72.5; 67.3; 44.4; 28.4
C-9		67.3 s		
C-9-OH	4.69 br. s, 1H			67.3; 44.4; (28.4)
9-CH ₃	1.27 s, 3H	28.4 q		(146.7); 67.3; 44.4
C-10	2.94 d, 1H, 16.4 Hz 2.75 d, 1H, 16.4 Hz	44.4 t	2.75 2.94	146.7; 131.4; 67.3; 43.1; 28.4 146.7; 131.4; 67.3; 43.1; 28.4
C-10a		146.7 s		
C-11	7.39 s, 1H	119.6 d		181.4; 162.0; 131.8 / 131.4; 113.6; 72.5; 44.4
C-11a		131.8* s		
C=O-12		181.4 s		
C-12a		133.4 s		
C-1'	5.20 s, 1H	100.1 d	(3.25)	80.8; 72.5; 69.3
C-2'	3.25 (HMBC, peak hidden underneath H ₂ O peak)	80.8 d	5.20	72.0; 58.4
2'-O-CH ₃	3.39 s, 3H	58.4 q		
C-3'	3.37 m, 1H	70.4 d	4.51; 3.17	80.8
C-3'-OH	4.51 br. d, 1H, 5.1 Hz		3.37	
C-4'	3.17 m, 1H	72.0 d	4.76; 3.57; 3.37	80.8; 70.4; 69.3; 17.8
C-4'-OH	4.76 br. d, 1H, 5.1 Hz		3.17	70.4; 69.3
C-5'	3.57 dq, 1H, 9.2, 6.1 Hz	69.3 d	3.17 ; 1.17	72.0 ; (100.1)
5'-CH ₃	1.17 d, 3H, 6.1 Hz	17.8 q	3.57	72.0 ; 69.3 ;

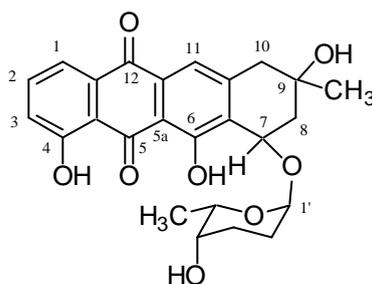
*Positions interchangeable



aranciamycin A, ^1H NMR



aranciamycin A, ^{13}C NMR

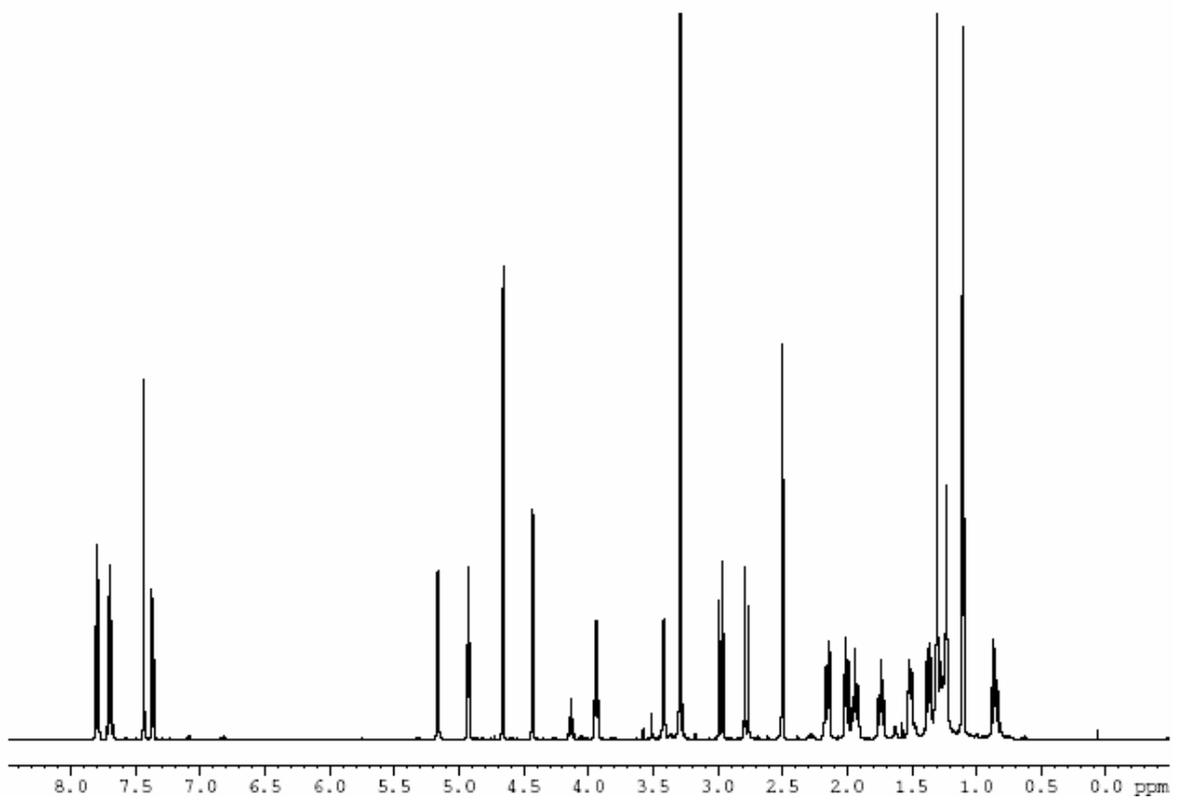


aranciamycin B

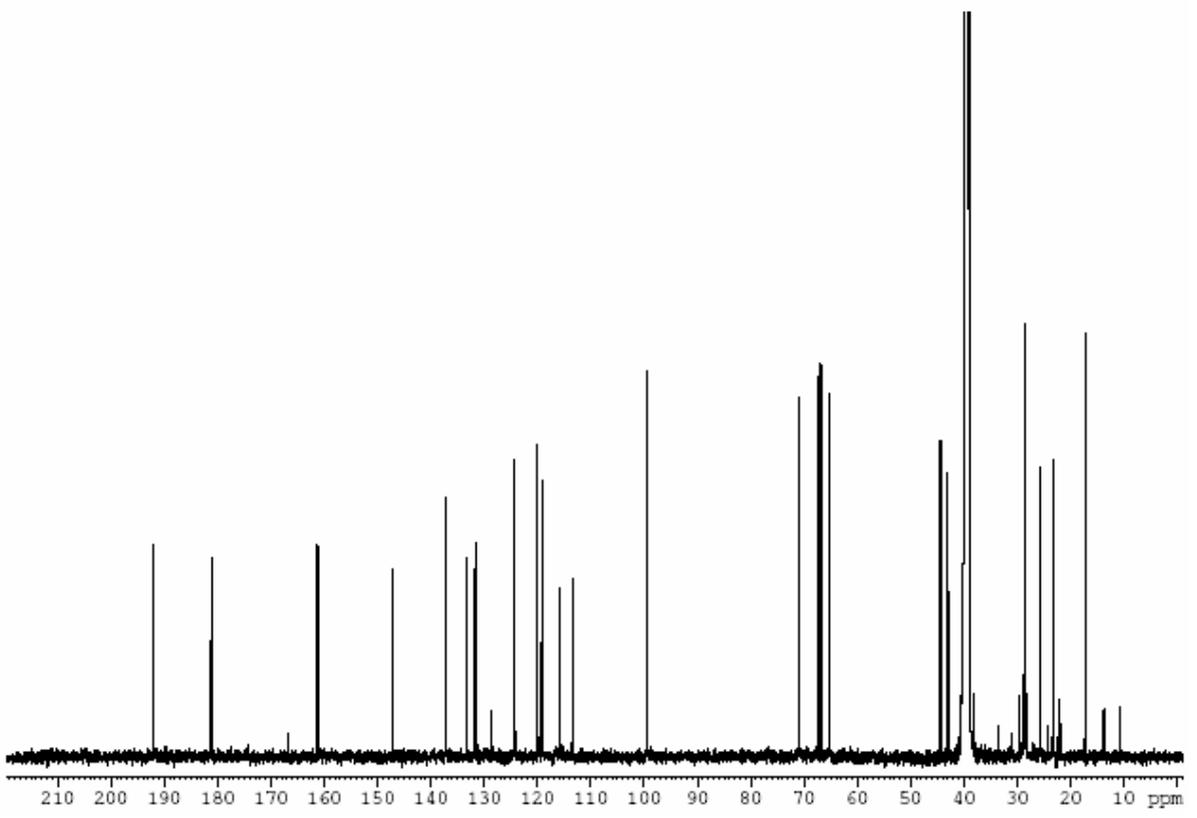
Molecular formula: $C_{25}H_{26}O_8$
 Monoisotopic mass: 454.1628

in $[D_6]DMSO$, $T = 305K$

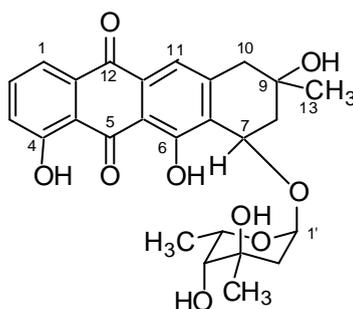
Position	d^1H	$d^{13}C$	COSY	HMBC
C-1	7.70 dd, 1H, 7.9, 1.1 Hz	119.20 d	7.80	191.83; 181.11; 124.20; 115.92
C-2	7.80 dd, 1H, 7.9, 7.9 Hz	137.23 d	7.70; 7.37	161.14; 133.27
C-3	7.37 dd, 1H, 7.9, 1.1 Hz	124.28 d	7.80	161.01; 133.72; 119.45; 116.28
C-4		161.22 s		
C-4-OH	11.89 s, 1H			
C-4a		115.86 s		
C=O-5		191.95 s		
C-5a		113.33 s		
C-6		161.35 s		
C-6-OH	12.59 s, 1H			
C-6a		131.85 s		
C-7	4.93 t, 1H, 5.8 Hz	71.05 d	2.14; 1.99	161.78; 147.60; 131.92; 99.68; 67.67; 42.96
C-8	2.14 dd, 1H, 14.2, 6.3 Hz 1.99 dd, 1H, 14.2, 6.3 Hz	42.95 t	4.95; 1.99 4.95; 2.14	131.70; 70.98; 67.73; 44.35; 28.76
C-9		67.40 s		
C-9-OH	4.66 s, 1H			
CH ₃ -9	1.30 s, 3H	28.72 q		67.32; 43.96; 148.0
C-10	2.97 d, 1H, 16.8 Hz 2.77 d, 1H, 16.8 Hz	44.42 t	2.77 2.97	146.95; 132.13; 120.09; 67.67; 42.75; 28.35
C-10a		147.16 s		
C-11	7.44 s, 1H	120.0 d		191.83; 181.25; 146.89; 131.83; 113.43; 44.31;
C-11a		n.d.		
C=O-12		181.16 s		
C-12a		133.30 s		
C-1'	5.16 d, 1H, 3.2 Hz	99.49 d	3.43; 1.93	70.99; 66.80; 25.77
C-2'e C-2'a	1.93 m, 1H 1.36 m, 1H	23.29 t	5.16; 1.74; 1.51 5.16; 1.93	99.85; 65.30; 25.68 99.45; 65.52; 25.,77
C-3'e C-3'a	1.74 m, 1H 1.51 m, 1H	25.65 t	3.43; 1.93; 1.51 3.43; 1.74	99.47; 23.19 99.68; 66.16
C-4'	3.42 m, 1H	65.31 d	4.43; 1.74; 1.51	23.62
C-4'-OH	4.43 d, 1H, 4.7 Hz		3.42	
C-5'	3.93 q, 1H, 6.3 Hz	66.83 d	1.12	99.90; 65.52; 17.60
CH ₃ -5'	1.10 d, 3H, 6.3 Hz	17.26 q	3.95	66.87; 65.54



aranciamycin B, ¹H NMR



aranciamycin B, ¹³C NMR

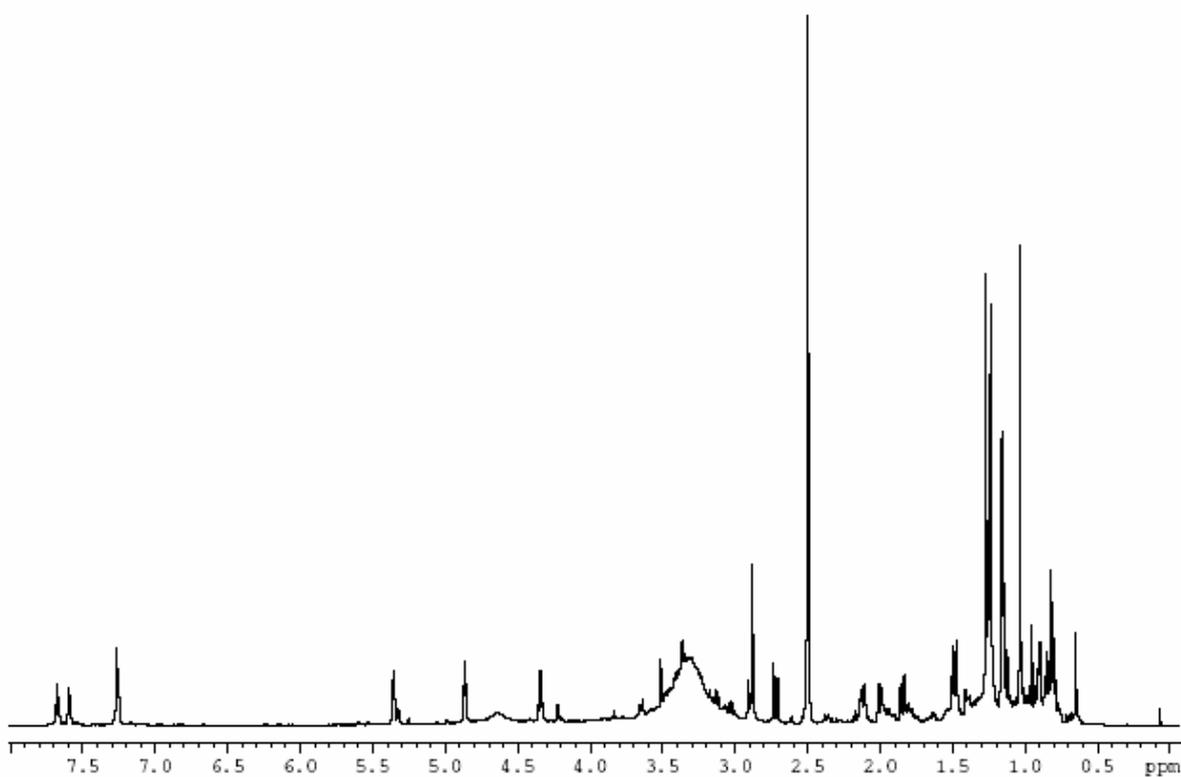


aranciamycin C

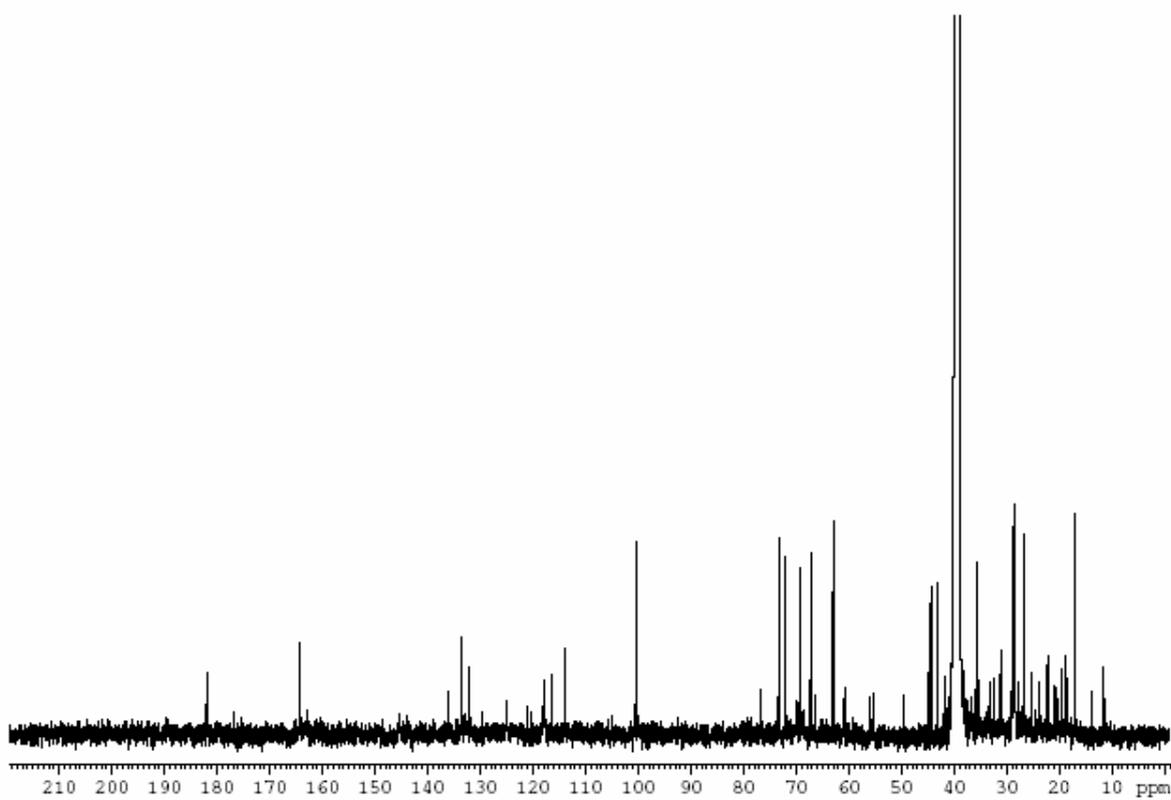
Molecular formula: C₂₆H₂₈O₉
 Monoisotopic mass: 484.1733

In [D₆]DMSO

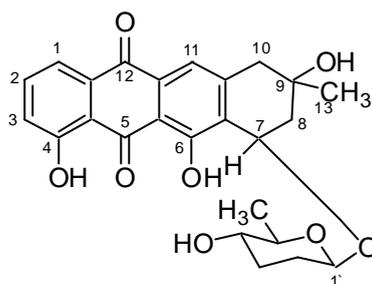
Position	d ¹ H (ppm)	d ¹³ C (ppm)	COSY	HMBC
C-1	7.59 d, 1H, 7.6 Hz	117.9 d	7.67	116.5; 125.3; 181.9
C-2	7.67 dd, 1H, 8.0, 7.6 Hz	135.8 d	7.25; 7.59	132.1; 162.9
C-3	7.25 d (br), 1H, 8.0 Hz	125.3 d	7.67	116.5; 117.9
C-4	-	162.9 s		
C-4a	-	116.5 s		
C-5	-	n. d.		
C-5a	-	113.8 s		
C-6	-	163.1 s		
C-6a	-	133.5 s		
C-7	4.86 dd, 1H, 6.0, 4.6 Hz	72.4 d	1.99; 2.11	43.2; 67.3; 100.2; 133.5; 145.7
C-8	2.11 dd, 1H, 13.8 Hz, 6.0 Hz 1.99 dd, 1H, 13.8 Hz, 4.6 Hz	43.2 t	1.99; 4.86 2.11; 4.86	133.5 28.7; 44.4; 67.3; 72.4;
C-9	-	67.3 s		
C-9-CH ₃	1.27 s, 3H	28.7 q	-	43.2; 44.4; 67.3
C-10	2.72 d, 1H, 16.1 Hz 2.88 d (br), 1H, 16.1 Hz	44.4 t	2.88 2.72	28.7; 43.2; 67.3; 118.0; 133.5; 145.7
C-10a	-	145.7 s		
C-11	7.25 s, 1H	118.0 d	-	44.4; 133.5; 181.9
C-11a	-	124.9 s		
C-12	-	181.9 s		
C-12a	-	132.1 s		
C-1'	5.36 d (br), 1H, 3.8 Hz	100.2 d	1.84	63.1; 69.2; 73.4
C-2'	1.84 m, 1H 1.49 m, 1H, ,	35.9 t	1.49; 5.36 1.84; (5.36)	27.0; 69.2; 100.2
C-3'	-	69.2 s		
C-3'-CH ₃	1.03 s, 3H	27.0 q	-	35.9; 69.2; 73.4; 100.2
C-4'	2.89 m, 1H	73.4 d	4.34	35.9; 63.1; 100.2
C-5'	4.34 q, 1H, 6.2 Hz	63.1 d	1.16; (2.89)	17.3; 73.4; 100.2
C-5'-CH ₃	1.16 d, 1H, 6.2 Hz	17.3 q	4.34	73.4; 63.1
OH	4.60, 8.35, 2H			



aranciamycin C, ^1H NMR



aranciamycin C, ^{13}C NMR



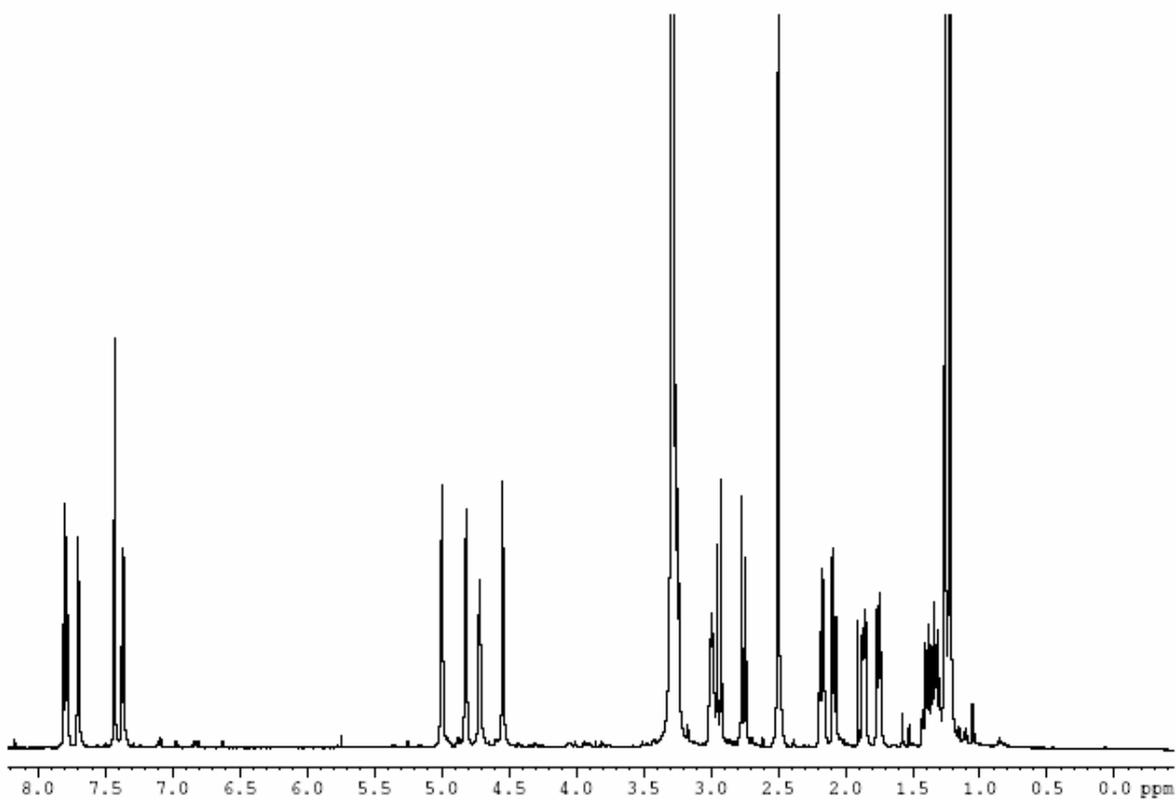
aranciamycin D

Molecular formula: C₂₅H₂₆O₈
 Monoisotopic mass: 454.1628

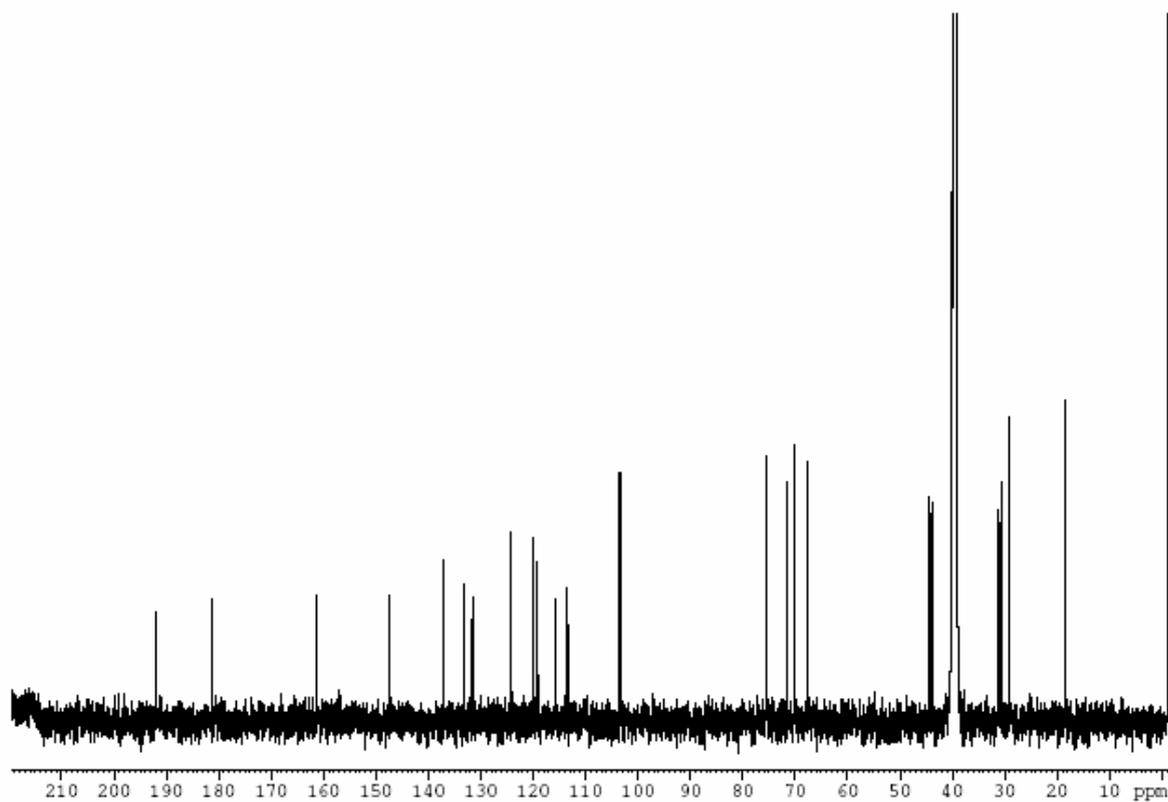
in [D₆]DMSO, T = 305K

Position	d ¹ H	d ¹³ C	COSY	HMBC
C-1	7.70 d, 1H 7.6, 1.0 Hz	119.14 d	7.80	181.52; 124.36; 115.99
C-2	7.80 dd, 1H, 8.6, 7.6 Hz	137.21 d	7.70; 7.37	161.75; 133.60
C-3	7.37 d, 1H, 8.6, 1.0 Hz	124.36 d	7.80	119.40; 115.53
C-4		161.41 s		
C-4-OH	12.25 s, 1H			
C-4a		115.90 s		
C=O-5		191.88 s		
C-5a		113.44 s		
C-6		161.32 s		
C-6-OH	12.25 s, 1H			
C-6a		131.49* s		
C-7	4.99 dd, 1H, 6.0, 5.0 Hz	71.59 d	2.17; 2.08	161.22; 147.41; 131.56; 103.55; 67.72; 43.83
C-8	2.17 dd, 1H, 13.6, 6.0 Hz 2.08 dd, 1H, 13.6, 5.0 Hz	43.77 t	2.08 2.17	131.41; 71.62; 67.78; 44.37; 29.38
C-9		67.61 s		
C-9-OH	4.55 s, 1H			67.72; 44.03; 29.21
CH ₃ -9	1.26 s, 3H	29.24 q		67.57; 44.81; 43.18
C-10	2.94 d, 1H, 16.6 Hz 2.76 d, 1H, 16.6 Hz	44.32 t	2.76 2.94	147.51; 131.84; 120.29; 67.69; 43.77; 29.13
C-10a		147.46 s		
C-11	7.43 s, 1H	119.93 d	2.94; 2.76	191.83; 181.52; 131.67; 113.41; 44.22
C-11a		131.61* s		
C=O-12		181.25 s		
C-12a		133.32 s		
C-1'	4.82 dd, 1H, 9.1, 2.0 Hz	103.44 d	1.75; 1.34	71.63; 30.85
C-2'e	1.75 m, 1H	30.60 t	1.34	103.62; 70.16; 30.85
C-2'a	1.34 m, 1H		1.75	103.62; 67.60; 31.03
C-3'e	1.86 m, 1H	31.11 t	1.40	103.74; 75.59; 70.0; 30.68
C-3'a	1.40 m, 1H		1.86; 2.99	70.0; 30.47
C-4'a	2.99 m, 1H	70.14 d	1.40; 1.86; 3.25; 4.72	147.66; 67.41; 18.63
C-4'-OH	4.72 d, 1H, 5.0 Hz			75.91; 70.0; 31.09
C-5'a	3.25 m, 1H, 9.2, 6.0 Hz	75.49 d	2.99; 1.22	103.52; 70.22; 31.11; 18.65
CH ₃ -5'e	1.22 d, 3H, 6.0 Hz	18.43 q	3.25	75.85; 69.59

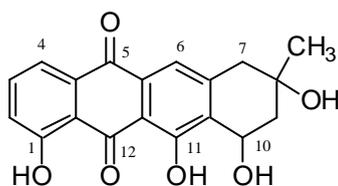
*Positions interchangeable



aranciamycin D, ^1H NMR



aranciamycin C, ^{13}C NMR



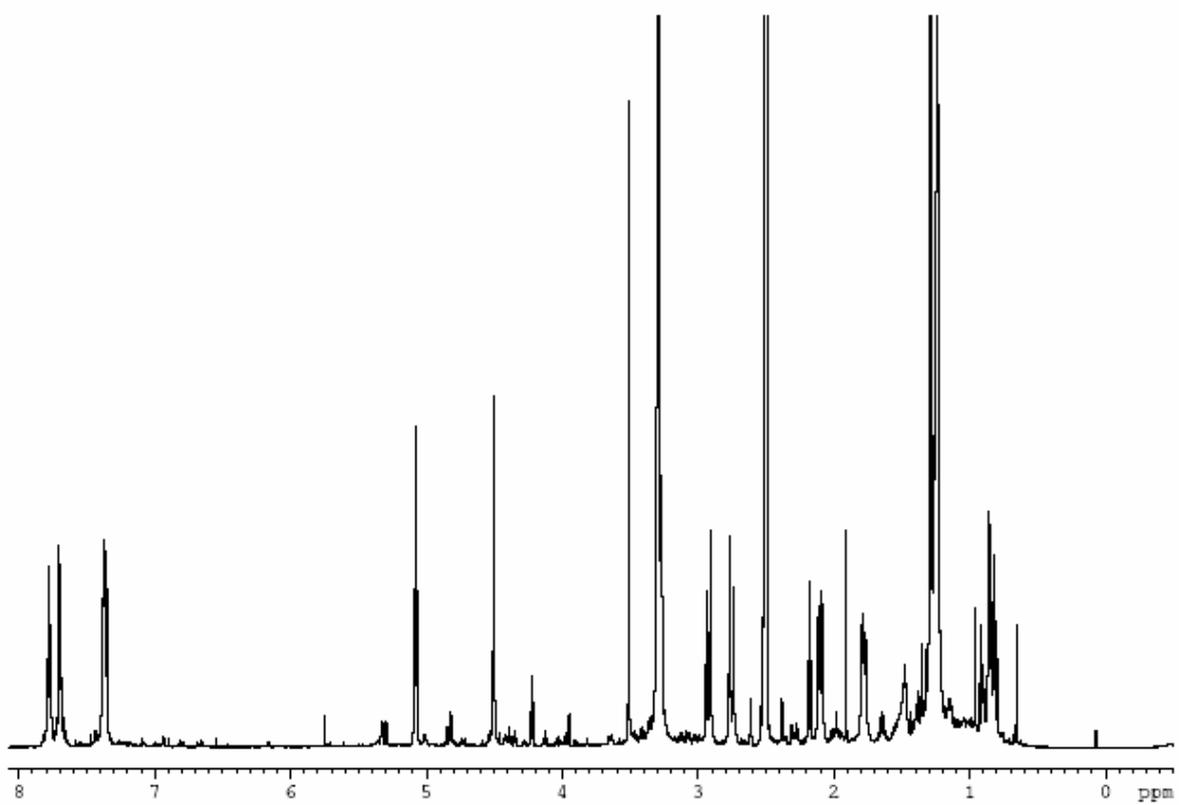
demethoxyaranciamycinone

Molecular formula: C₁₉H₁₆O₆
 Monoisotopic mass: 340.0974

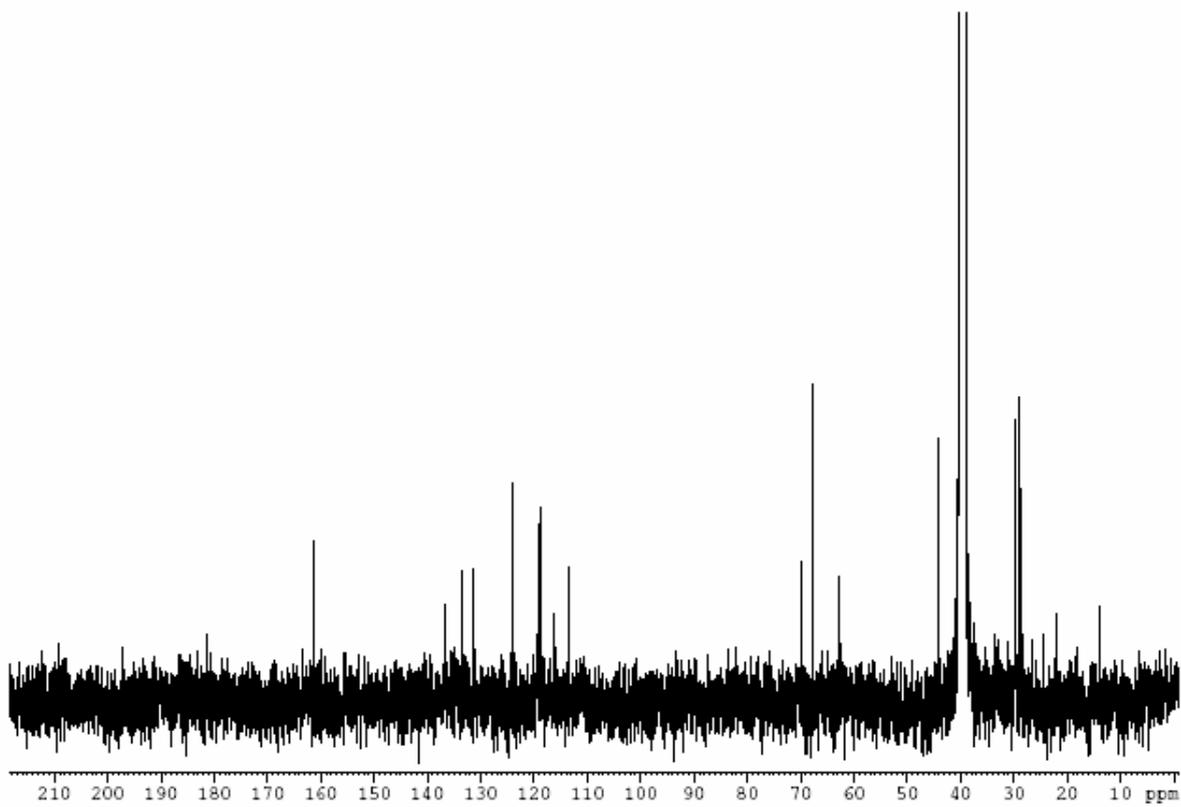
in [D₆]DMSO, T = 305K

Position	d ¹ H	d ¹³ C	COSY	HMBC
C-1		161.32 s		
C-1-OH	11.7-12.7 s, 1H			
C-2	7.36 d, 1H, 7.2 Hz	124.18 d	7.78	119.08; 116.39
C-3	7.78 dd, 1H, 8.27.2 Hz	136.80 d	7.70; 7.36	161.40; 133.58
C-4	7.70 d, 1H, 8.2 Hz	118.89 d	7.78	181.63; 124.32; 116.39
C-4a		133.38 s		
C=O-5		181.51 s		
C-5a		131.40 s		
C-6	7.38 s, 1H	119.21 d		181.97; 134.59; 113.83; 44.57
C-6a		146.27 s		
C-7	2.92 d, 1H, 17.4 Hz 2.75 d, 1H, 17.4 Hz	44.16* t	2.75 2.92	146.40; 135.01; 119.54; 68.19; 44.34; 29.94
C-8		67.71 s		
C-8-OH	4.50 s, 1H			67.75; 44.23
CH ₃ -8	1.28 s, 3H	29.61 q		67.61; 44.10
C-9	2.09 m, 1H 1.77 dd, 1H, 13.3, 6.7 Hz	44.25* t	1.77 2.09	134.80; 67.97; 62.60; 44.12; 29.51 134.80; 67.97; 62.60; 44.12
C-10	5.08 d, 1H, 6.7, 6.1 Hz	62.71 d	2.09; 1.77	146.19; 134.98; 68.03; 44.14
C-10-OH	n.d.			
C-10a		134.80 s		
C-11		n.d.		
C-11-OH	11.7-12.7 s, 1H			
C-11a		113.58 s		
C=O-12		n.d.		
C-12a		116.34 s		

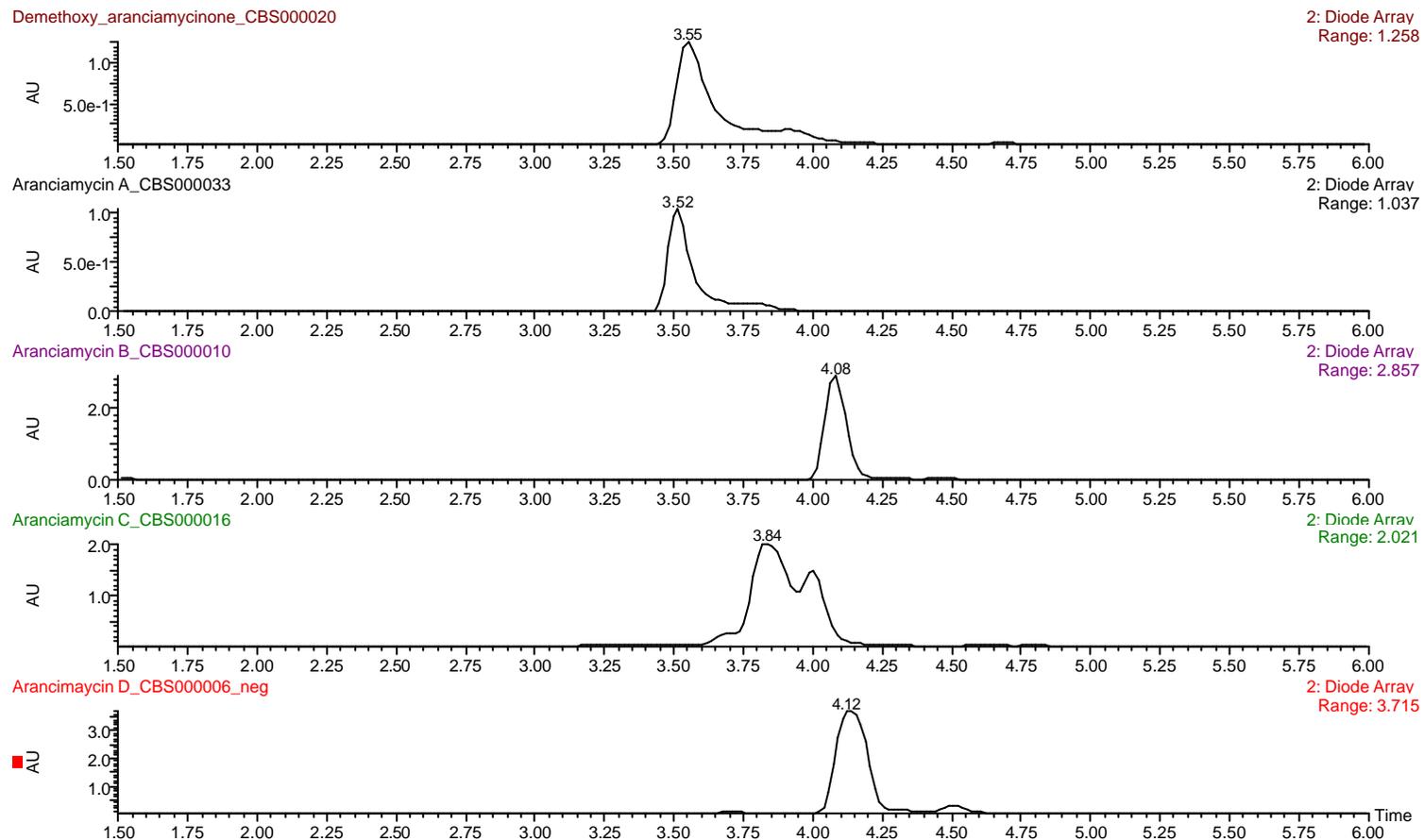
* positions interchangeable.



demethoxyaranciamycinone, ^1H NMR



demethoxyaranciamycinone, ^{13}C NMR

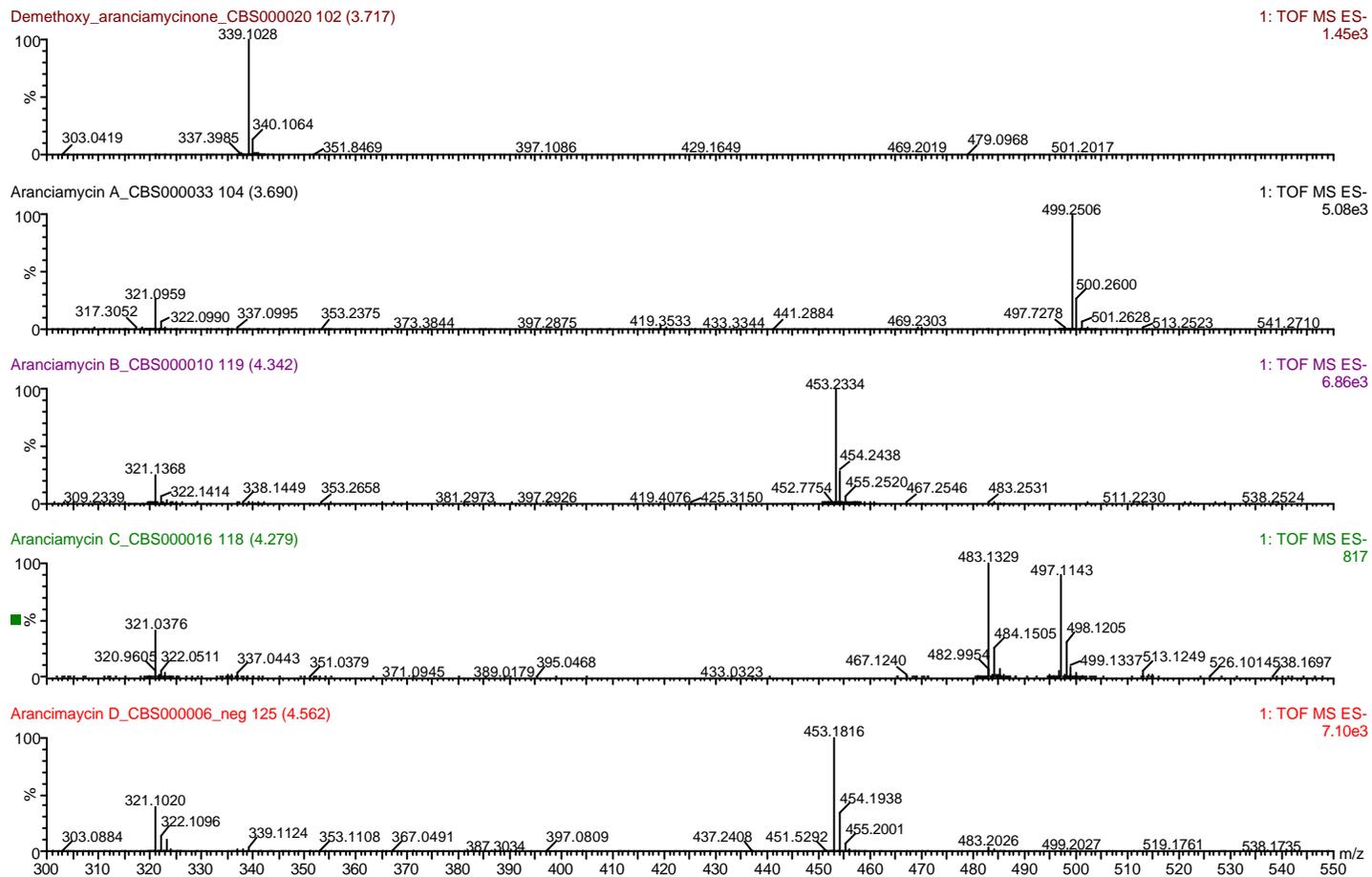


BPI UV-chromatograms [200 – 795 nm, 1.5 – 6.0 min., BPI], Demethoxyaranciamycinone, Aranciamycins A – D (BPI: base peak intensity)

Demethoxyaranciamycinone $t_R = 3.55$ min

Aranciamycin A $t_R = 3.52$ min Aranciamycin B $t_R = 4.08$ min

Aranciamycin C $t_R = 3.84$ min Aranciamycin D $t_R = 4.12$ min



ESI- MS-spectra for the corresponding, respective UV-chromatogram peaks [m/z 300-550] Demethoxyaranciamycinone, Aranciamycins A – D

Demethoxyaranciamycinone $M = 340.0974$ ($C_{19}H_{16}O_6$)

Aranciamycin A $M = 500.1682$ ($C_{26}H_{28}O_{10}$) Aranciamycin B $M = 454.1628$ ($C_{25}H_{26}O_8$)

Aranciamycin C $M = 484.1733$ ($C_{26}H_{28}O_9$) Aranciamycin D $M = 454.1628$ ($C_{25}H_{26}O_8$)