Supporting Information

A NOVEL MITHRAMYCIN ANALOGUE WITH HIGH ANTITUMOR ACTIVITY AND LESS TOXICITY GENERATED BY COMBINATORIAL BIOSYNTHESIS

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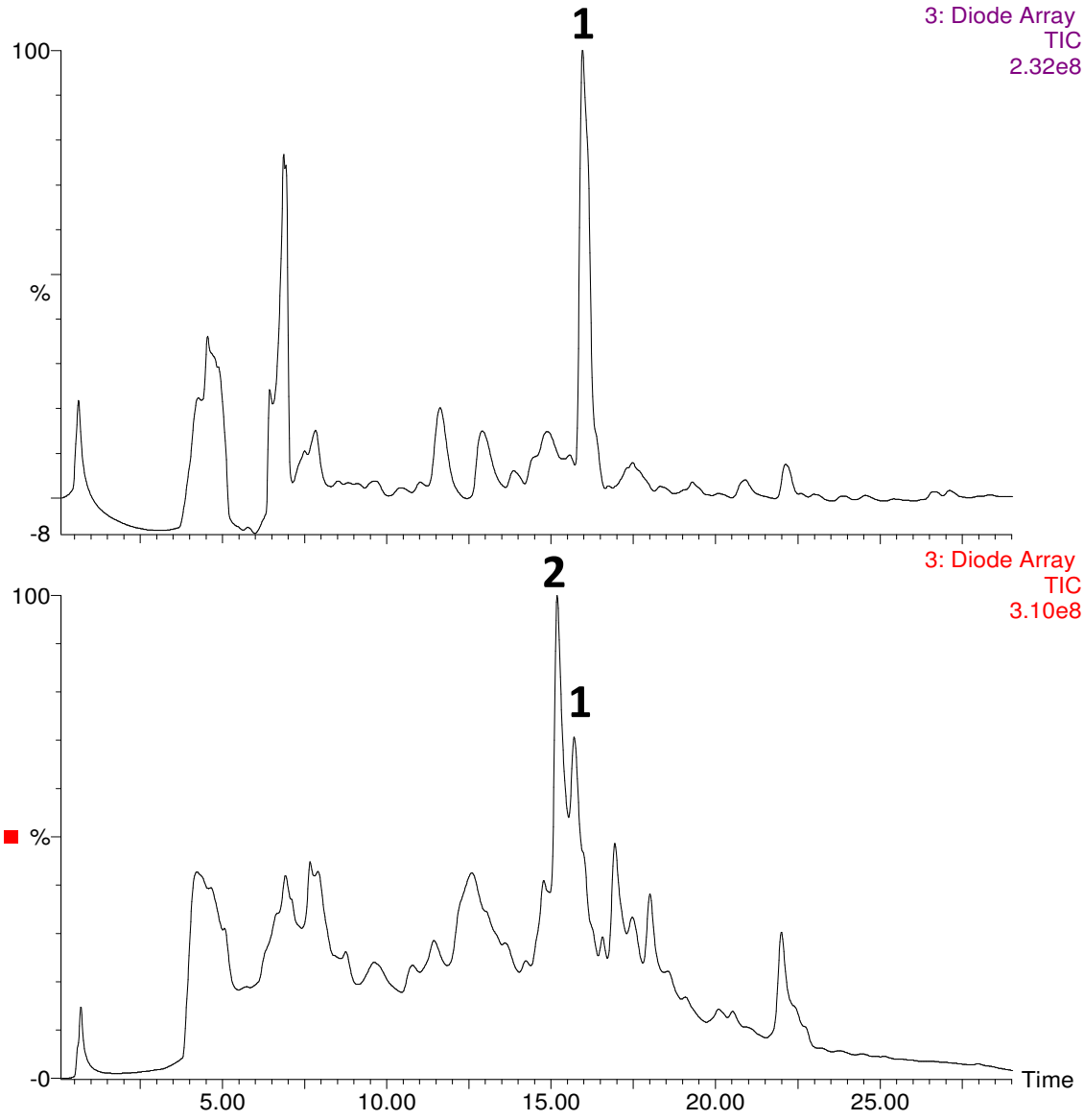


Figure S0, A:
upper trace HPLC chromatogram traces of an extract from wildtype *Streptomyces argillaceus* mithramycin (**1**) R_t=15.9 min^{-1}; A, lower trace: Extract from *Streptomyces argillaceus* (pKOL): mithramycin (**1**) R_t=15.9 min^{-1}, demycarosyl-3D-β-D-digitoxosyl mithramycin (**2**) R_t=15.3 min^{-1}.

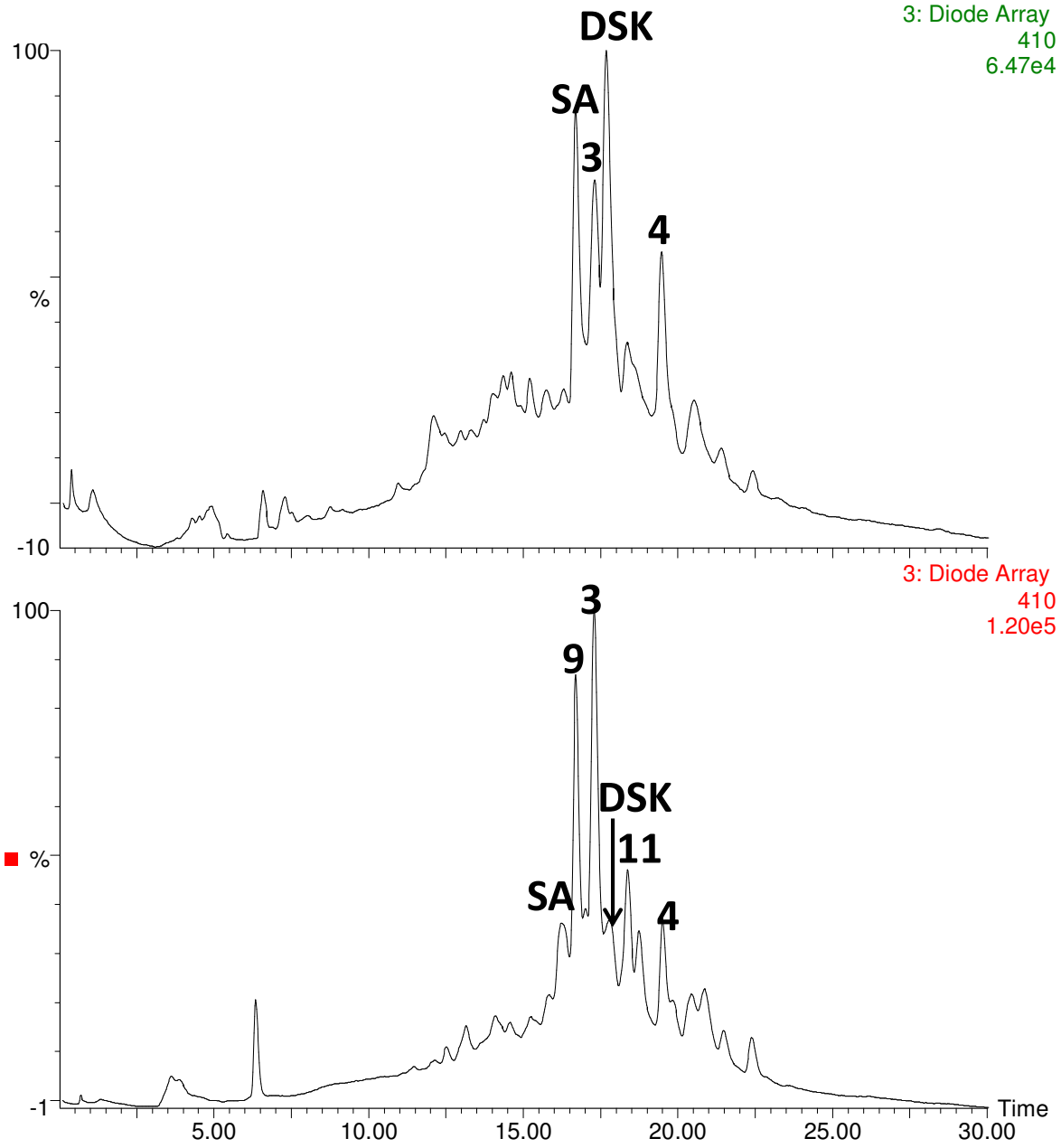


Figure S0, B:
upper trace: HPLC chromatogram of an extract from *Streptomyces argillaceus* M3W1: mithramycin SK (**3**) R_{rel}=17.1 min^{-1}, mithramycin SA (**SA**) R_{rel}=16.4 min^{-1}, demycarosyl-mithramycin SK (**DSK**) R_{rel}=17.6 min^{-1}, and mithramycin SDK (**4**) R_{rel}=19.2 min-1. B, lower trace: Extract from *Streptomyces argillaceus* M3W1(pKOL): mithramycin SK (**3**) R_{rel}=17.1 min^{-1}, mithramycin SA (**SA**) R_{rel}=16.4 min^{-1}, demycarosyl-mithramycin SK (DSK) R_{rel}=17.6 min^{-1}, and mithramycin SDK (**3**) R_{rel}=19.2 min^{-1}, demycarosyl-3D-β-D-digitoxosyl mithramycin SK (**5**) R_{rel}=16.6 min^{-1}, and demycarosyl-3D-β-D-digitoxosyl mithramycin SDK (**6**) R_{rel}=18.6 $min^{-}1$.

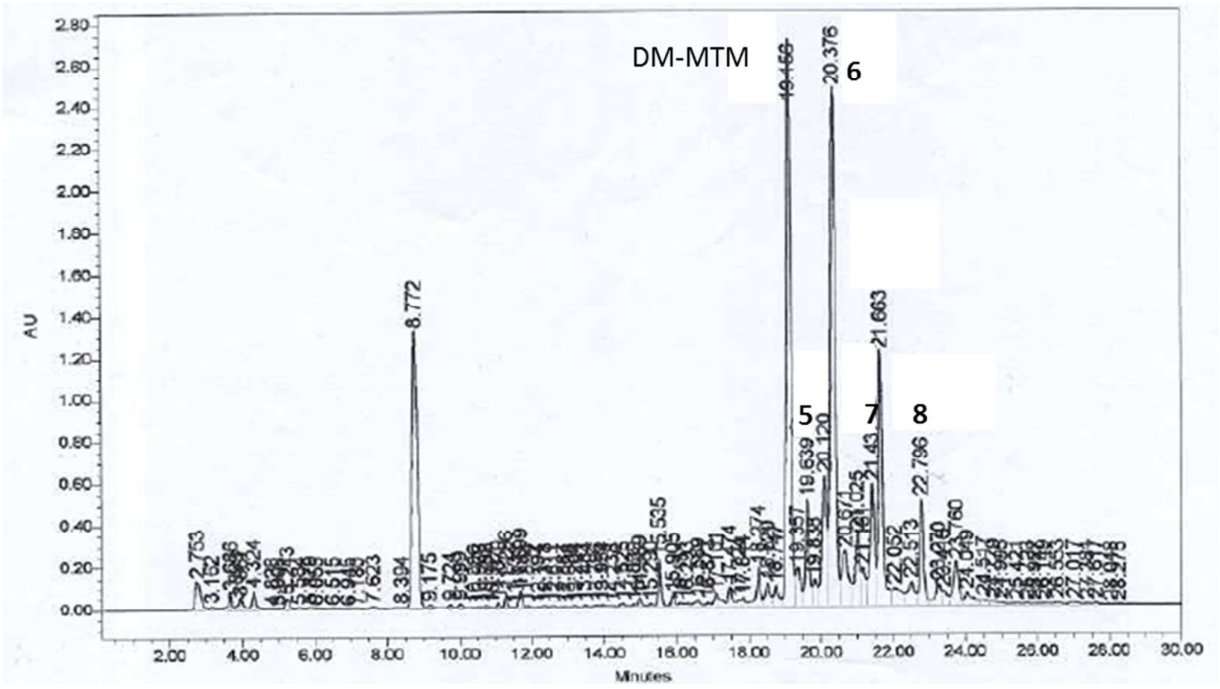


Figure S0, C:
HPLC chromatogram of an extract from *Streptomyces argillaceus* M7C1-pFL845: demycarosyl-mithramycin (DM-MTM) R_{rel}=19.2 min^{-1}, dideolivosyl-6-β-D-amicetosyl-demycarosyl-2-*O*-β-D-oliosyl-3C-β-D-olivosyl-mithramycin (**5**) R_{rel}=19.6 min^{-1} , dideolivosyl-6-β-D-amicetosyl-demycarosyl-mithramycin (**6**) R_{rel}=20,4 min^{-1}, deoliosyl-demycarosyl-3C-β-D-amicetosyl-mithramycin (**7**) R_{rel}=21.4 min^{-1} and dideolivosyl-6-β-D-amicetosyl-deoliosyl-demycarosyl-3C-β-D-amicetosyl-mithramycin (**8**) R_{rel}=22.8 min^{-1}.

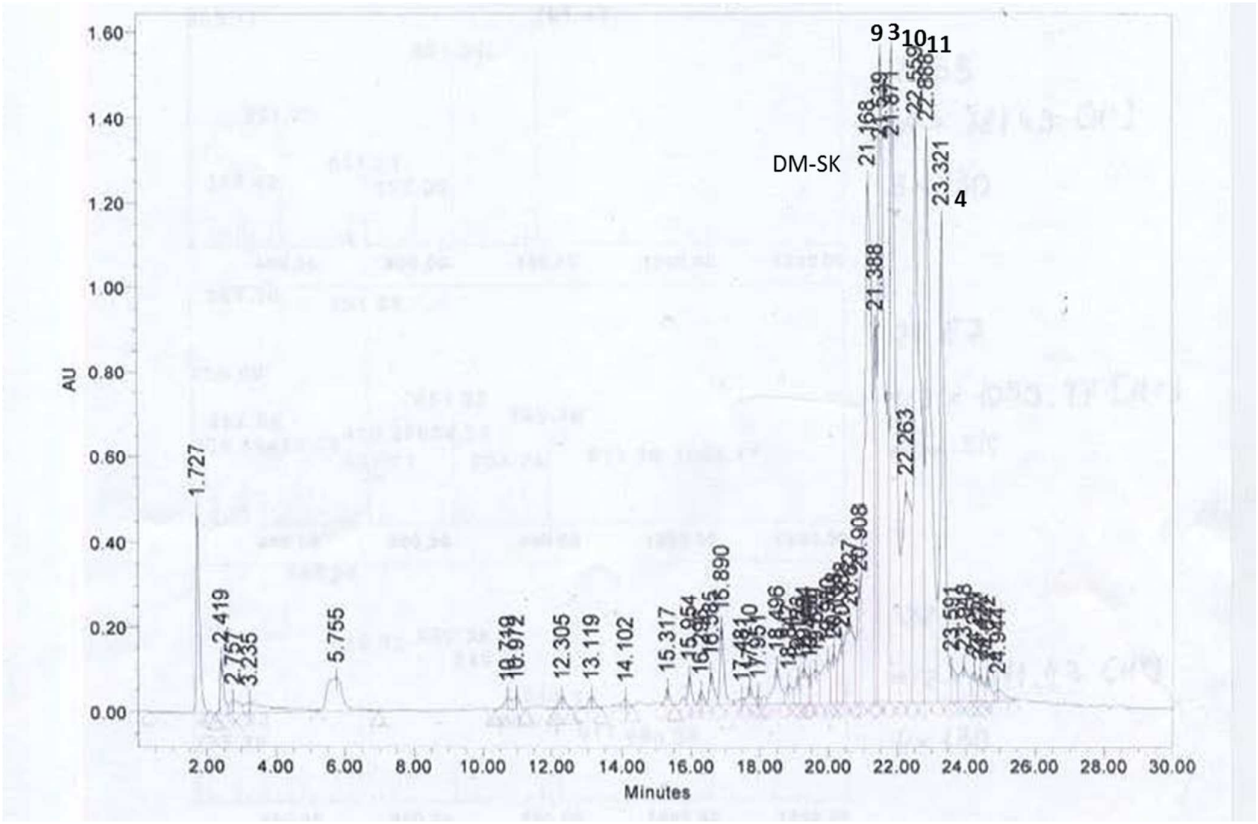


Figure S0, D:
HPLC chromatogram of an extract from *Streptomyces argillaceus* M3W1-pMP3*BII: demycarosyl-mithramycin SK (DM-SK) R_{ref}=21.2 min^{-1}, demycarosyl-3D-β-D-digitoxosyl-mithramycin SK (**9**) R_{ref}=21.5 min^{-1}, mithramycin SK (**3**) R_{ref}=21.9 min^{-1}, demycarosyl-mithramycin SDK (**10**) R_{ref}=22.5 min^{-1}, demycarosyl-3D-β-D-digitoxosyl-mithramycin SDK (**11**) R_{ref}=22.9 min^{-1} and mithramycin SDK (**4**) R_{ref}=23.3 min^{-1}.

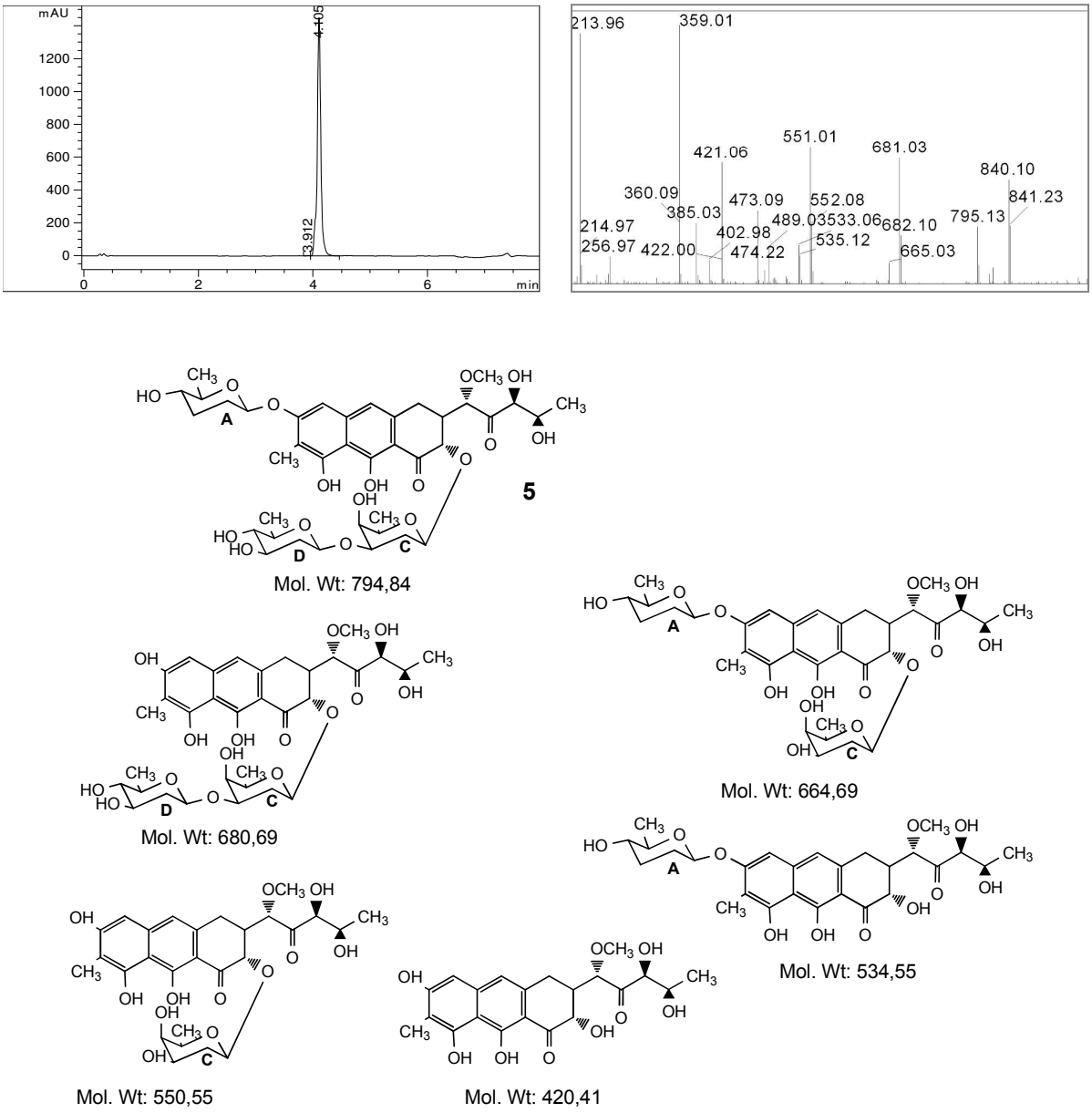
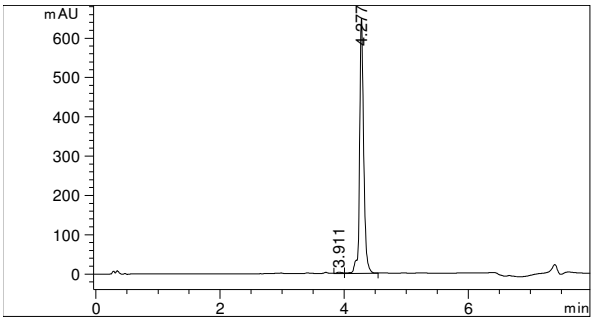
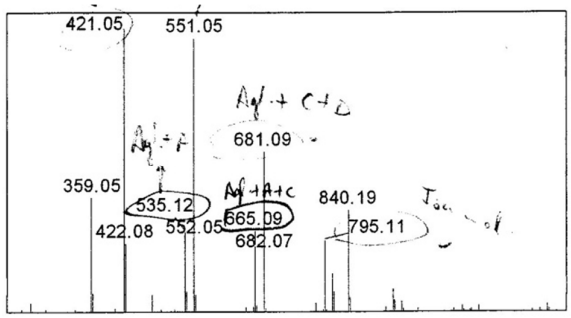
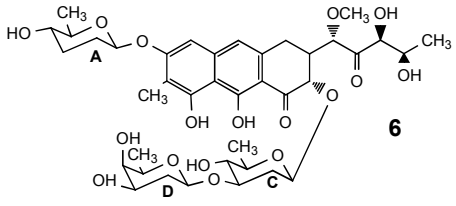


Figure S1: HPLC-MS and fragmentation pattern of the compound **5**.

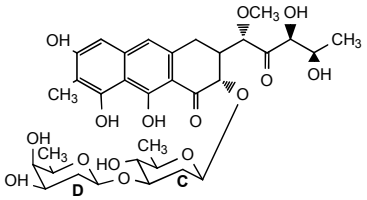




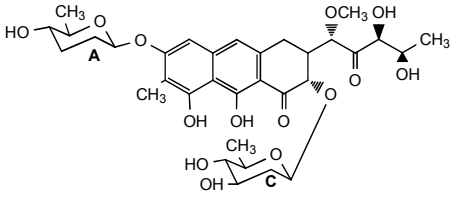


6

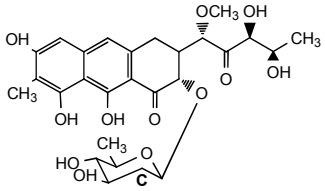
Mol. Wt: 794,84



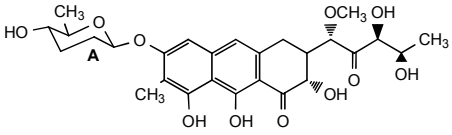
Mol. Wt: 680,69



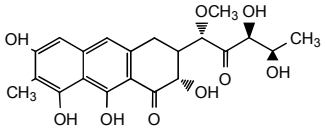
Mol. Wt: 664,69



Mol. Wt: 550,55



Mol. Wt: 534,55



Mol. Wt: 420,41

Figure S2: HPLC-MS and fragmentation pattern of the compound **6**.

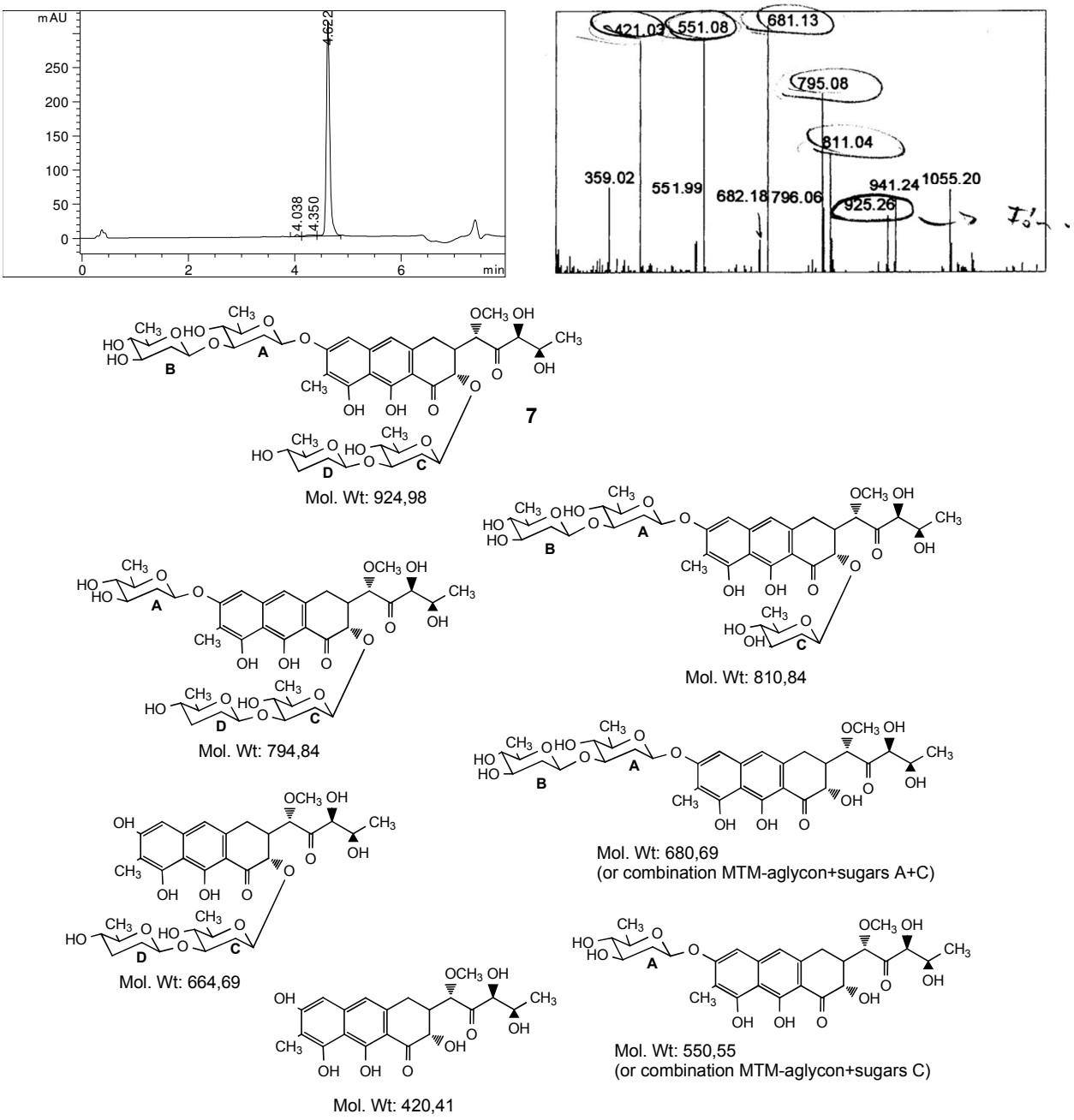
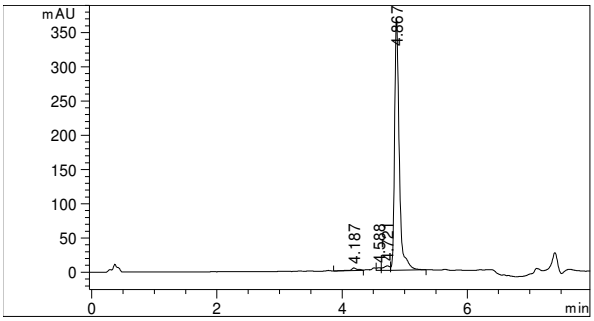
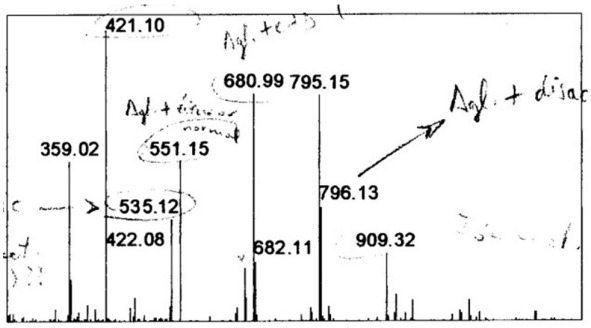
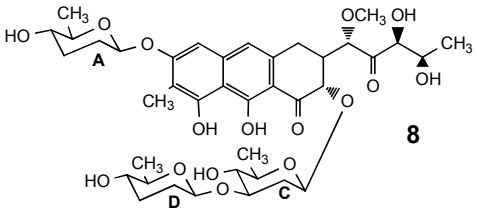


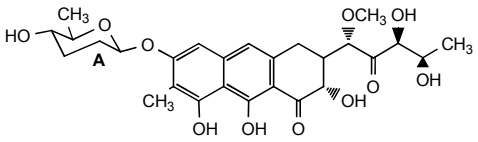
Figure S3: HPLC-MS and fragmentation pattern of the compound **7**.



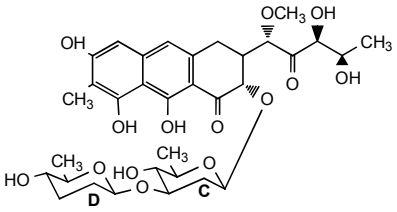




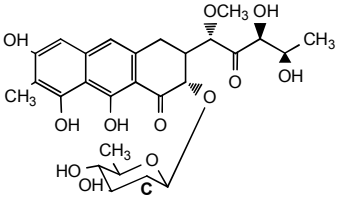
Mol. Wt: 778,84



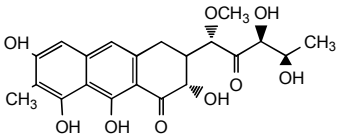
Mol. Wt: 534,55



Mol. Wt: 664,69
(or combination MTM-aglycon+sugars A+C)



Mol. Wt: 550,55



Mol. Wt: 420,41

Figure S4: HPLC-MS and fragmentation pattern of the compound **8**.

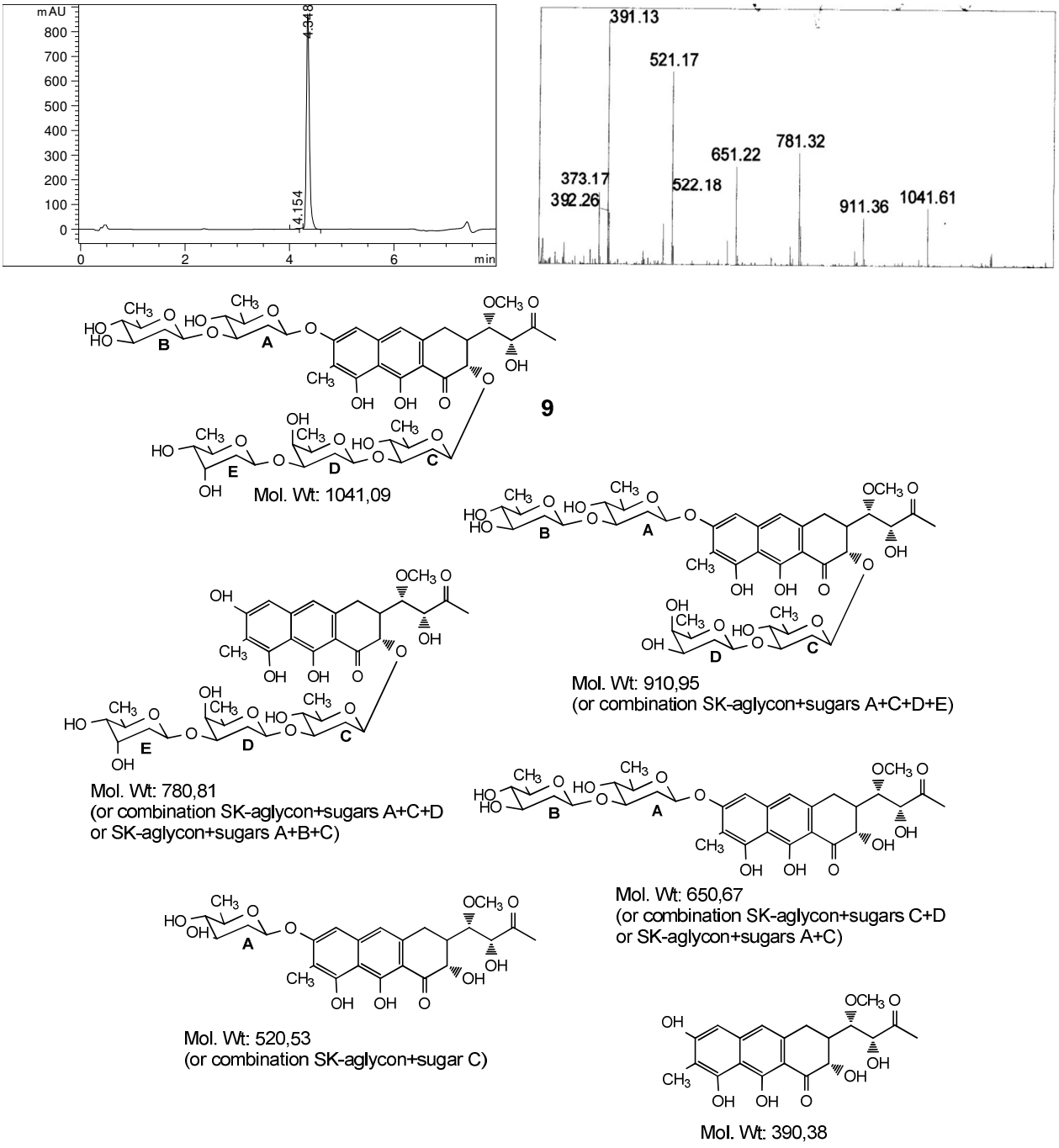


Figure S5: HPLC-MS and fragmentation pattern of the compound **9**.

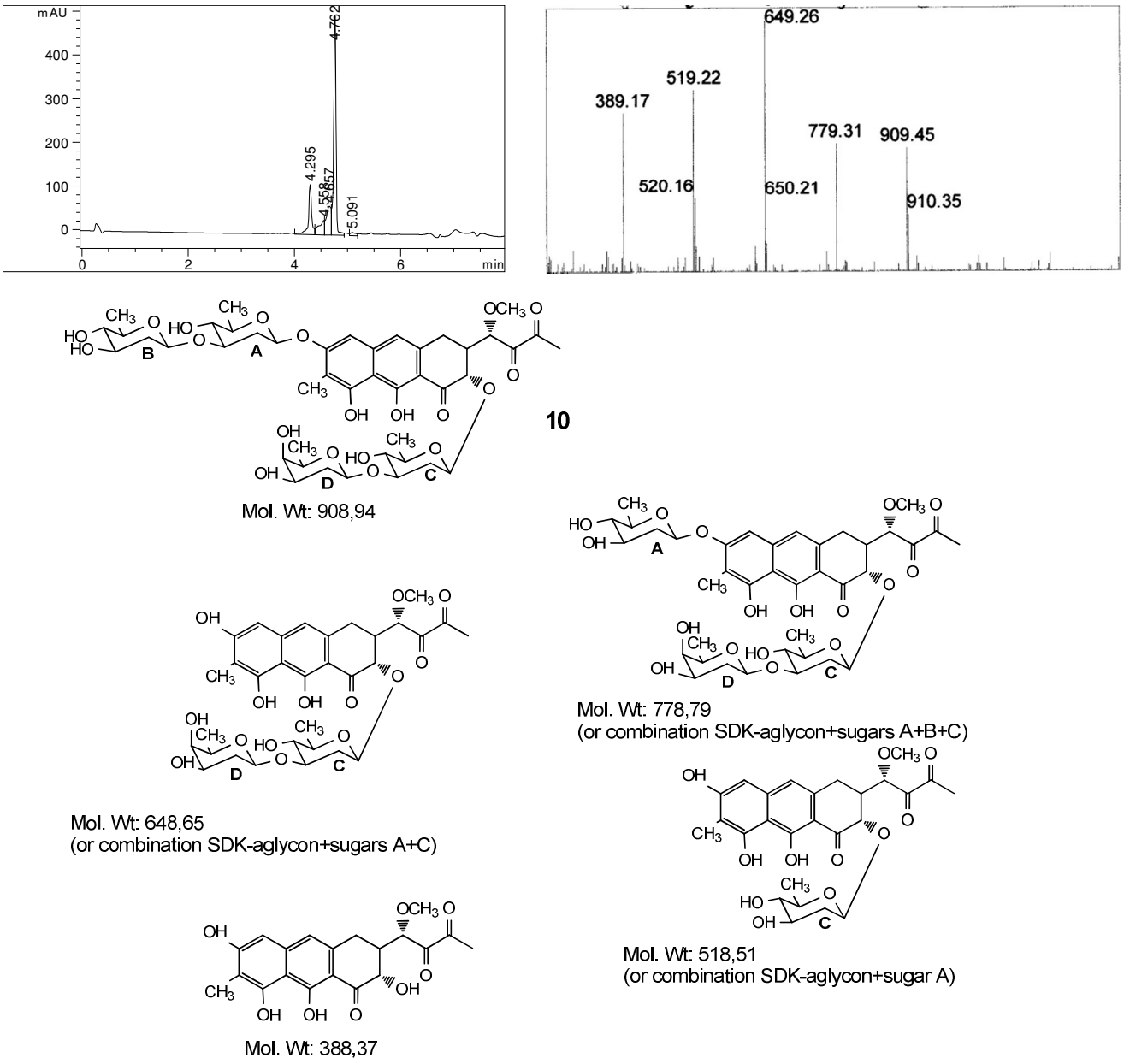


Figure S6: HPLC-MS and fragmentation pattern of the compound **10**.

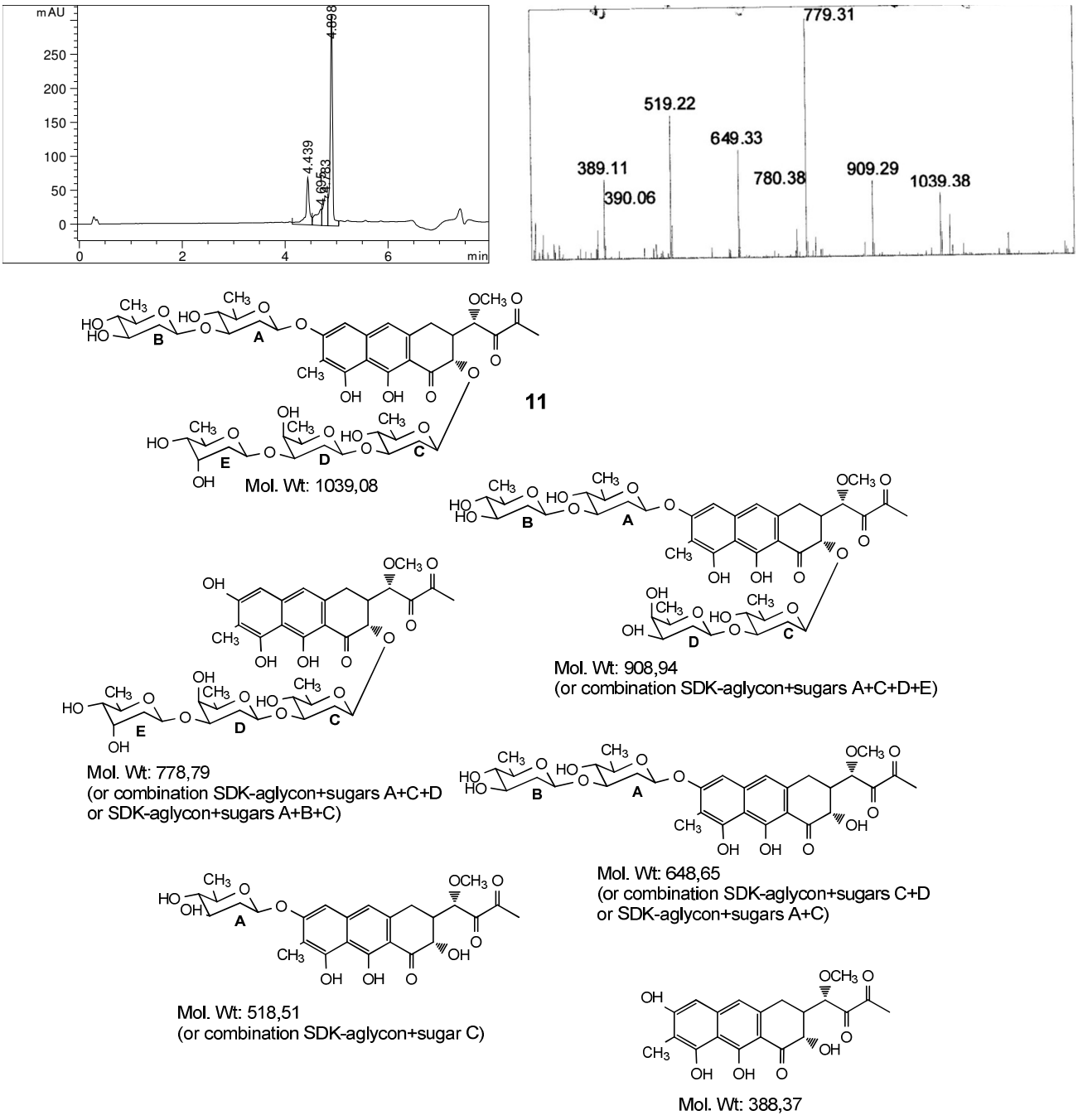


Figure S7: HPLC-MS and fragmentation pattern of the compound **11**.

Table S1: NMR data for dideolivosyl-6-β-D-amicetosyl-demycarosyl-2-*O*-β-D-oliosyl-3C-β-D-olivosyl-MTM (**5**) in acetone-*d*6 (^{1}H, 600 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)	^{13}C-NMR (δ in ppm)	Multiplicity
1		200.1	C
2	4.60 brs	77.3	CH
3	2.80 (overlapped)	41.3	CH
4_{ax}	2.69 dd (16.8, 3.6)	27.3	CH_2
4_{eq}	2.90 (overlapped with 4C)		
4a		135.8	C
5	6.95 (s)	101.3	CH
6		159.6	C
7		110.7	C
7-CH_3	2.16 (s)	7.5	CH_3
8		155.8	C
8a		107.7	C
9		164.0	C
9a		109.0	C
10	6.90 (s)	116.9	CH
10a		138.2	C
1'	4.65 s	83.9	CH
1'-OCH_3	3.35 (s)	58.3	CH_3
2'		211.6	C
3'	4.30 (overlapped with 4')	79.3	CH
4'	4.30 (overlapped with 3')	67.4	CH
5'	1.25 d (6.2)	19.3	CH_3
1A	5.38 brd (8.6)	98.5	CH
$2A_{ax}$	1.90 (overlapped with $2D_{eq}$)	30.3	CH_2
$2A_{eq}$	2.10 (overlapped)		
$3A_{ax}$	1.65 m (signal not resolved)	30.8	CH_2
$3A_{eq}$	2.10 (overlapped)		
4A	3.3 m (signal not resolved)	70.5	CH
5A	3.58 dq (8.6, 6.4)	76.2	CH
6A	1.32 d (6.4)	17.8	CH_3
1C	4.65 dd (9.0, 2.5)	100.2	CH
$2C_{ax}$	1.73 ddd (12.0, 12.0, 9.0)	34.8	CH_2
$2C_{eq}$	1.90 (overlapped with $2A_{ax}$)		
3C	3.75 m (signal not resolved)	68.6	CH_2
4C	3.51 t (3.9)	69.8	CH
5C	3.65 brq partially overlapped (6.5)	70.9	CH
6C	1.28 d (6.1)	16-1	CH_3
1D	4.85 brd (9.2)	98.3	CH
$2D_{ax}$	1.65 brq (12.0)	37-3	CH_2
$2D_{eq}$	2.54 brdd (12.0, 5.4)		
3D	3.65 (overlapped with 5D)	81.3	CH
4D	2.90 t partially overlapped with 4_{eq}	75.2	CH
5D	3.21 dq (9.0, 5.9)	72.1	CH
6D	1.15 d (5.9)	17.3	CH_3

Table S2: NMR data for dideolivosyl-6-β-D-amicetosyl-demycarosyl-MTM (**6**) in acetone-*d*6 (^{1}H, 600 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)	^{13}C-NMR (δ in ppm)	Multiplicity
1		203.4	C
2	4.82 d (11.6)	76.6	CH
3	2.80 (overlapped)	42.4	CH
4_{ax}	2.69 dd (16.3, 3.4)	26.9	CH_2
4_{eq}	3.00 (overlapped)		
4a		136.0	C
5	6.92 (s)	101.2	CH
6		159.7	C
7		110.6	C
7-CH_3	2.16 (s)	7.51	CH_3
8		155.8	C
8a		107.5	C
9		164.0	C
9a		108.1	C
10	6.88 (s)	116.6	CH
10a		138.7	C
1'	4.87 d (1.1)	81.1	CH
1'-OCH_3	3.47 (s)	58.3	CH_3
2'		210.9	C
3'	4.30 (overlapped with 4')	78.8	CH
4'	4.30 (overlapped with 3')	68.0	CH
5'	1.29 d (5.9)	19.2	CH_3
1A	5.39 dd (9.4, 1.6)	98.5	
$2A_{ax}$	1.90 m (complex signal)	30.2	
$2A_{eq}$	2.10 (overlapped with $2A_{eq}$)		
$3A_{ax}$	1.65 (overlapped with $2C_{ax}$)	30.6	
$3A_{eq}$	2.10 (overlapped with $2A_{eq}$)		
4A	3.30 m (partially overlapped with 5C)	70.4	
5A	3.58 dq (8.6, 6.2)	76.3	
6A	1.29 d (6.2)	17.8	
1C	5.14 d (9.7, 1.7)	100.5	
$2C_{ax}$	1.65 (overlapped with $3A_{ax}$)	37.6	
$2C_{eq}$	2.53 ddd (12.4, 5.2, 1.7)		
3C	3.70 (overlapped with 5D)	81.3	
4C	3.00 (overlapped)	75.3	
5C	3.32 dq partially overlapped (9.1, 6.0)	72.2	
6C	1.33 d (6.0)	16.1	
1D	4.70 d partially overlapped with OH (8.5)	100.0	
$2D_{ax}$	1.77 brq (11.9)	34.8	
$2D_{eq}$	1.94 brdd (12.4, 4.4)		
3D	3.82 m (signal not resolved)	68.5	
4D	3.54 brt (3.4)	69-8	
5D	3.70 (overlapped with 3C)	71.0	
6D	1.32 d (6.8)	17.6	

Table S3: NMR data for deoliosyl-demycarosyl-3C-β-D-amicetosyl-MTM (**7**) in acetone-*d*6 (^{1}H, 600 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)
1	
2	4.80 signal not resolved
3	2.80 (overlapped)
4_{ax}	2.70 brd (15.1)
4_{eq}	3.00 (overlapped)
4a	
5	6.94 (s)
6	
7	
7-CH_3	2.16 (s)
8	
8a	
9	
9a	
10	6.90 (s)
10a	
1'	4.86 brs
1'-OCH_3	3.46 (s)
2'	
3'	4.30 (overlapped with 4')
4'	4.30 (overlapped with 3')
5'	1.27 d (6.0)
1A	5.42 brd (10.0)
$2A_{ax}$	1.95 (overlapped with $2D_{eq}$)
$2A_{eq}$	2.48 brdd (11.6, 3.8)
3A	3.77 ddd partially overlapped with OH (12.0, 8.8, 5.6)
4A	3.07 t (8.8)
5A	3.55 dq partially overlapped (8.8, 6.0)
6A	1.33 d (6.0)
1B	4.76 dd (9.8, 1.9)
$2B_{ax}$	1.60 (overlapped)
$2B_{eq}$	2.20 ddd (12.2 4.9, 1.9)
3B	3.59 ddd partially overlapped (12.2, 9.3, 4.9)
4B	3.00 (overlapped with 4C)
5B	3.40 (overlapped with 5D)
6B	1.31 d (6.1)
1C	5.15 brd (8.8)
$2C_{ax}$	1.60 (overlapped)
$2C_{eq}$	2.53 brdd (12.0, 4.5)
3C	3.72 m (signal not resolved)
4C	3.00 (overlapped with 4B)
5C	3.33 dq (9.0, 6.0)
6C	1.32 d (6.0)
1D	4.66 d partially overlapped with OH (8.2)
$2D_{ax}$	1.60 (overlapped)

$2D_{eq}$	1.95 (overlapped with $2A_{ax}$)
$3D_{ax}$	1.60 (overlapped)
$3D_{eq}$	2.05 (overlapped)
4D	3.20 m (signal not resolved)
5D	3.40 (overlapped with 5B)
6D	1.28 d (6.4)

Table S4: NMR data for dideolivosyl-6-β-D-amicetosyl-deoliosyl-demycarosyl-3C-β-D-amicetosyl-MTM (**8**) in acetone-*d*6 (^{1}H, 600 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)
1	
2	4.80 brs
3	2.80 (overlapped)
4_{ax}	2.69 dd (16.8, 3.6)
4_{eq}	3.00 (overlapped with 4C)
4a	
5	6.92 (s)
6	
7	
7-CH_3	2.16 (s)
8	
8a	
9	
9a	
10	6.88 (s)
10a	
1'	4.86 brs
1'-OCH_3	3.47 (s)
2'	
3'	4.30 (overlapped with 4')
4'	4.30 (overlapped with 3')
5'	1.28 d (6.2)
1A	5.38 brd (8.8)
$2A_{ax}$	1.90 m (signal not resolved)
$2A_{eq}$	2.10 (overlapped)
$3A_{ax}$	1.55 (overlapped)
$3A_{eq}$	2.10 (overlapped)
4A	3.28 m (signal not resolved)
5A	3.58 dq (8.4, 6.4)
6A	1.31 d (6.4)
1C	5.15 brd (9.2)
$2C_{ax}$	1.65 brq partially overlapped (12.0)
$2C_{eq}$	2.54 brdd (12.0, 5.4)
3C	3.72 ddd (11.8, 8.5, 5.4)
4C	3.00 (overlapped with 4_{eq})
5C	3.33 dq partially overlapped with 4A (9.0, 5.9)
6C	1.32 d (5.9)
1D	4.66 brs

$2D_{ax}$	1.55	(overlapped)
$2D_{eq}$	1.55	(overlapped)
$3D_{ax}$	1.55	(overlapped)
$3D_{eq}$	1.95	m partially overlapped
4D	3.20	m (signal not resolved)
5D	3.40	dq (9.0, 6.1)
6D	1.29	d (6.1)

Table S5: NMR data for demycarosyl-3D-β-D-digitoxosyl-MTM-SK (**9**) in acetone-*d*6 (^{1}H, 600 MHz; ^{13}C, 150 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)	^{13}C-NMR (δ in ppm)	Multiplicity
1		202.1	C
2	4.76 d (11.3)	78.0	CH
3	2.50 (overlapped)	43.4	CH
4_{ax}	3.17 dd (15.7, 3.6)	29.7	CH_2
4_{eq}	3.00 (overlapped)		
4a		135.2	C
5	6.92 (s)	101.2	CH
6		159.7	C
7		110.1	C
7-CH_3	2.16 (s)	7.6	CH_3
8		152.2	C
8a		107.2	C
9		164.0	C
9a		107.9	C
10	6.92 (s)	116.5	CH
10a		138.8	C
1'	4.25 dd (3.4, 1.5)	78.9	CH
1'-OCH_3	3.57 (s)	59.6	CH_3
2'	4.32 brd (3.4)	79.1	CH
3'		209.5	C
4'	2.34 (s)	25.8	CH_3
1A	5.42 dd (9.6, 1.9)	96.7	CH
$2A_{ax}$	1.88 ddd (12.0, 12.0, 9.6)	37.1	CH_2
$2A_{eq}$	2.50 (overlapped)		
3A	3.78 ddd partially overlapped (12.0, 8.9, 5.0)	80.9	CH
4A	3.08 t (8.9)	75.0	CH
5A	3.55 (overlapped)	72.3	CH
6A	1.34 d (6.1)	17.6	CH_3
1B	4.77 dd (9.5, 1.7)	99.5	CH
$2B_{ax}$	1.56 ddd (12.0, 12.0, 9.5)	39.6	CH_2
$2B_{eq}$	2.20 ddd (12.0, 4.9, 1.7)		
3B	3.55 (overlapped)	71.0	CH
4B	3.00 (overlapped)	77.2	CH
5B	3.40 dq (9.2, 6.2)	72.3	CH
6B	1.32 d (6.2)	17.2	CH_3
1C	5.14 dd (9.6, 1.6)	100.4	CH
$2C_{ax}$	1.62 ddd (12.1, 12.0, 9.6)	37.5	CH_2

$2C_{eq}$	2.50 (overlapped)		
3C	3.70 (overlapped)	81.4	CH
4C	3.00 (overlapped)	75.3	CH
5C	3.33 dq (8.9, 6.4)	72.3	CH
6C	1.33 d (6.4)	17.2	CH_3
1D	4.70 dd (9.5, 1.5)	99.8	CH
$2D_{ax}$	1.81 ddd (12.0, 12.0, 9.5)	32.1	CH_2
$2D_{eq}$	1.96 (signal not resolved)		
3D	3.90 dt (11.9, 4.0)	76.5	CH
4D	3.70 (overlapped)	68.5	CH
5D	3.70 (overlapped)	70.6	CH
6D	1.31 d (6.2)	16.1	CH_3
1E	5.03 dd (9.6, 1.9)	96.9	CH
$2E_{ax}$	1.70 ddd (13.0, 9.6, 2.9)	38.3	CH_2
$2E_{eq}$	1.92 ddd (13.0, 2.0, 1.9)		
3E	4.05 brs	67.8	CH
4E	3.20 dd (9.5, 2.9)	72.8	CH
5E	3.70 (overlapped)	69.6	CH
6E	1.23 d (6.2)	16.1	CH_3

Table S6: NMR data for demycarosyl-MTM-SDK (**10**) in acetone-*d*6 (^{1}H, 600 MHz; ^{13}C, 150 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)	^{13}C-NMR (δ in ppm)	Multiplicity
1		204.1	C
2	4.76 (overlapped with 1B)	76.9	CH
3	2.74 t (12.1)	42.3	C
4_{ax}	2.95 (overlapped)	27.3	CH_2
4_{eq}	2.64 d (15.5)		
4a		135.5	C
5	6.90 (s)	100.9	CH
6		159.5	C
7		110.3	C
7-CH_3	2.18 (s)	7.5	CH_3
8		152.4	C
8a		107.4	C
9		164.5	C
9a		107.9	C
10	6.90 (s)	116.3	CH
10a		138.5	C
1'	5.07 d (2.0)	79.6	CH
1'-OCH_3	3.45 (s)	58.4	CH_3
2'		198.3	C
3'		198.3	C
4'	2.34 (s)	23.6	CH_3
1A	5.39 d (9.7)	96.8	CH
$2A_{ax}$	1.87 brq (11.9)	37.1	CH_2
$2A_{eq}$	2.48 brdd (11.3, 4.9)		
3A	3.80 (overlapped with 3D)	81.3	CH
4A	3.08 t (9.0)	74.9	CH

5A	3.55 (overlapped with 4D)	72.2	CH
6A	1.34 d (6.1)	17.5	CH_3
1B	4.76 (overlapped with 2)	99.5	CH
$2B_{ax}$	1.55 ddd (12.0, 12.0, 9.7)	39.5	CH_2
$2B_{eq}$	2.21 ddd (12.0, 5.0, 1.7)		
3B	3.55 (overlapped with 4D)	70.9	CH
4B	3.00 t coincident with 4C (8.9)	77.1	CH
5B	3.39 dq (8.9, 5.9)	72.2	CH
6B	1.31 d (5.9)	17.2	CH_3
1C	5.10 dd (9.7, 1.6)	100.6	CH
$2C_{ax}$	1.63 brq (12.2)	37.5	CH_2
$2C_{eq}$	2.53 ddd (12.0, 5.2, 1,6)		
3C	3.70 (overlapped with 5D)	81.6	CH
4C	3.00 t coincident with 4B (8.9)	75.3	CH
5C	3.31 dq (8.9, 5.6)	72.2	CH
6C	1.33 d (5.6)	17.5	CH_3
1D	4.69 d (10.0)	100.0	CH
$2D_{ax}$	1.77 brq (11.9)	34.8	CH_2
$2D_{eq}$	1.92 (signal not resolved)		
3D	3.80 (overlapped with 3A)	68.4	CH
4D	3.55 (overlapped with 5A)	69.7	CH
5D	3.70 (overlapped with 3C)	70.9	CH
6D	1.31 d (6.0)	16.1	CH_3

Table S7: NMR data for demycarosyl-3D-β-D-digitoxosyl-MTM-SDK (**11**) in acetone-*d*6 (^{1}H, 600 MHz; ^{13}C, 150 MHz)

Position	^{1}H-NMR (δ in ppm, multiplicity, *J* (H,H) in Hz)	^{13}C-NMR (δ in ppm)	Multiplicity
1		203.8	C
2	4.78 d (13.1)	76.8	CH
3	2.74 t (13.1)	42.4	C
4_{ax}	3.05 (overlapped)	27.3	CH_2
4_{eq}	2.65 brd (16.3)		
4a		135.8	C
5	6.92 (s)	101.2	CH
6		159.6	C
7		110.2	C
7-CH_3	2.15 (s)	7.5	CH_3
8		154.0	C
8a		107.2	C
9		164.0	C
9a		108.2	C
10	6.92 (s)	116.4	CH
10a		138.6	C
1'	5.06 d (1.4)	79.7	CH
1'-OCH_3	3.45 (s)	58.4	CH_3
2'		198.2	C
3'		198.3	C
4'	2.37 (s)	23.7	CH_3

1A	5.38	d (9.2)	96.8	CH
$2A_{ax}$	1.89	brq (11.0)	37.1	CH_2
$2A_{eq}$	2.55	dd (12.0, 5.0)		
3A	3.75	(overlapped with 4D and 5E)	80.9	CH
4A	3.08	t (9.0)	75.0	CH
5A	3.54	dq (9.0, 6.1)	72.3	CH
6A	1.34	d (6.1)	17.8	CH_3
1B	4.75	dd overlapped with 2 (9.7, 1.4)	99.5	CH
$2B_{ax}$	1.55	ddd (12.0, 12.0, 9.7)	39.6	CH_2
$2B_{eq}$	2.21	ddd (12.0, 4.9, 1.4)		
3B	3.58	ddd (12.0, 8.9, 4.9)	71.0	CH
4B	3.00	t coincident with 4C (8.9)	77.2	CH
5B	3.40	dq (8.9, 6.2)	72.3	CH
6B	1.31	d (6.2)	17.2	CH_3
1C	5.10	d (9.4)	100.6	CH
$2C_{ax}$	1.62	brq (12.0)	37.5	CH_2
$2C_{eq}$	2.55	dd (12.0, 4.8)		
3C	3.70	(overlapped with 5D)	81.5	CH
4C	3.00	t coincident with 4B (8.9)	75.3	CH
5C	3.32	dq (8.9, 6.2)	72.3	CH
6C	1.32	d (6.2)	17.5	CH_3
1D	4.68	brs	99.9	CH
$2D_{ax}$	1.81	brq (12.0)	32.1	CH_2
$2D_{eq}$	1.95	(signal not resolved)		
3D	3.90	(signal not resolved)	76.5	CH
4D	3.76	(overlapped with 3A and 5E)	68.5	CH
5D	3.70	brq (overlapped with 3C)	70.6	CH
6D	1.31	d (6.2)	16.1	CH_3
1E	5.04	dd (9.6, 1.6)	96.9	CH
$2E_{ax}$	1.70	ddd (12.8, 9.6, 2.8)	38.3	CH_2
$2E_{eq}$	2.01	dt (12.8, 2.6)		
3E	4.05	brd (2.8)	67.8	CH
4E	3.20	dd (9.2, 2.3)	72.8	CH
5E	3.75	(overlapped with 3A and 4D)	69.6	CH
6E	1.24	d (6.1)	14.7	CH_3

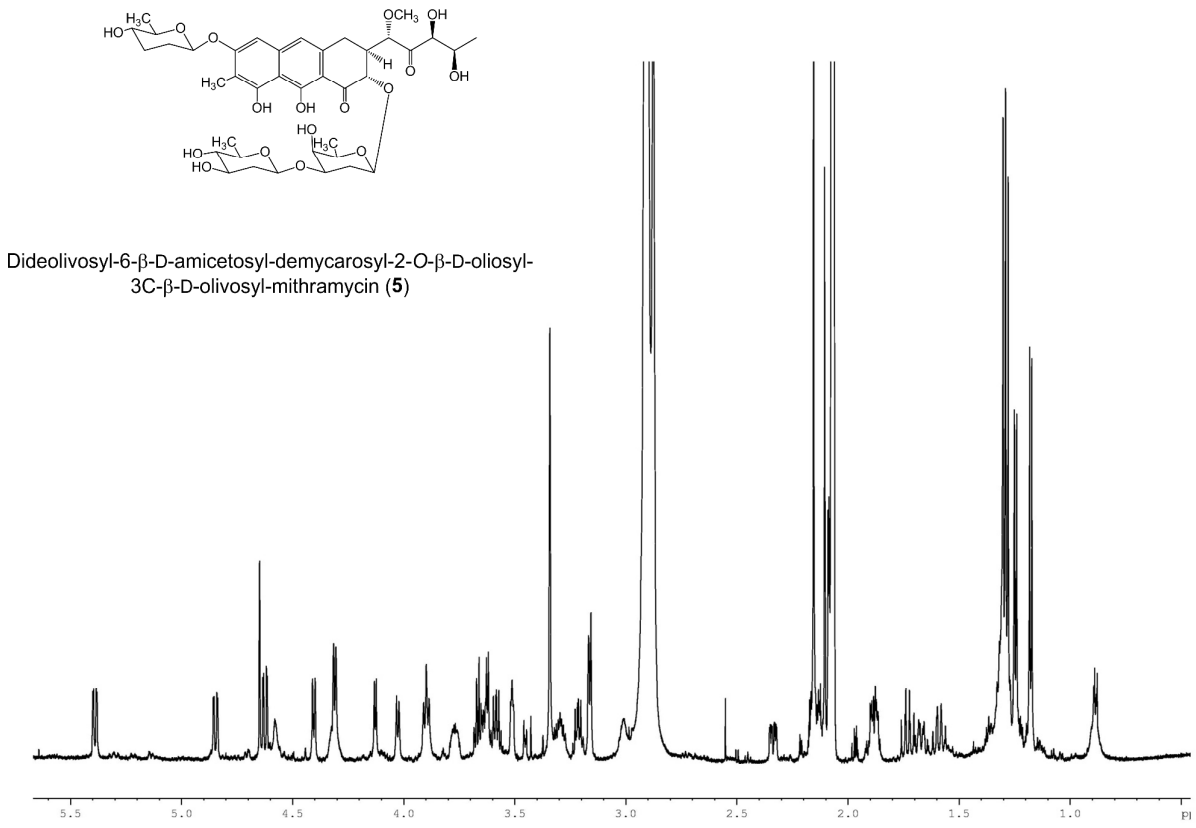


Figure S8: ^{1}H-NMR expanded chart for **5** in acetone-*d*6 (600 MHz)

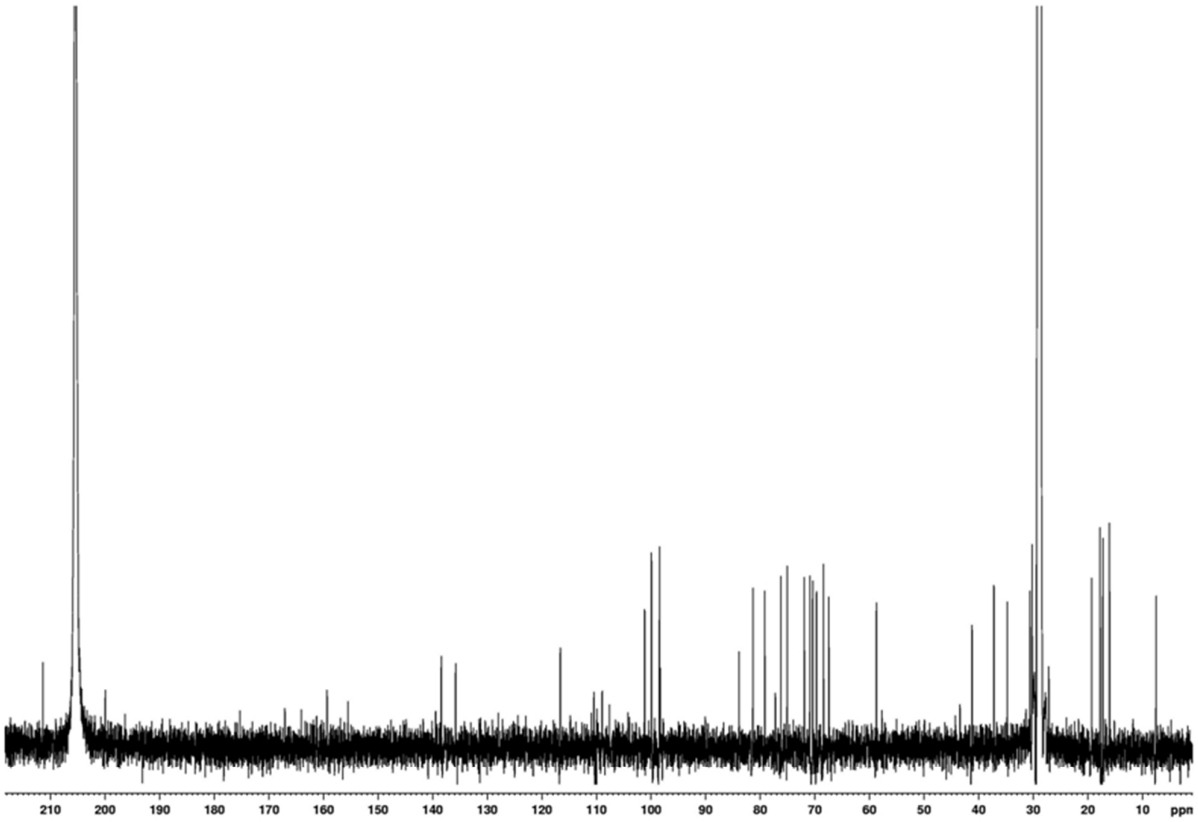


Figure S9: ^{13}C-NMR chart for **5** in acetone-*d*6 (150 MHz)

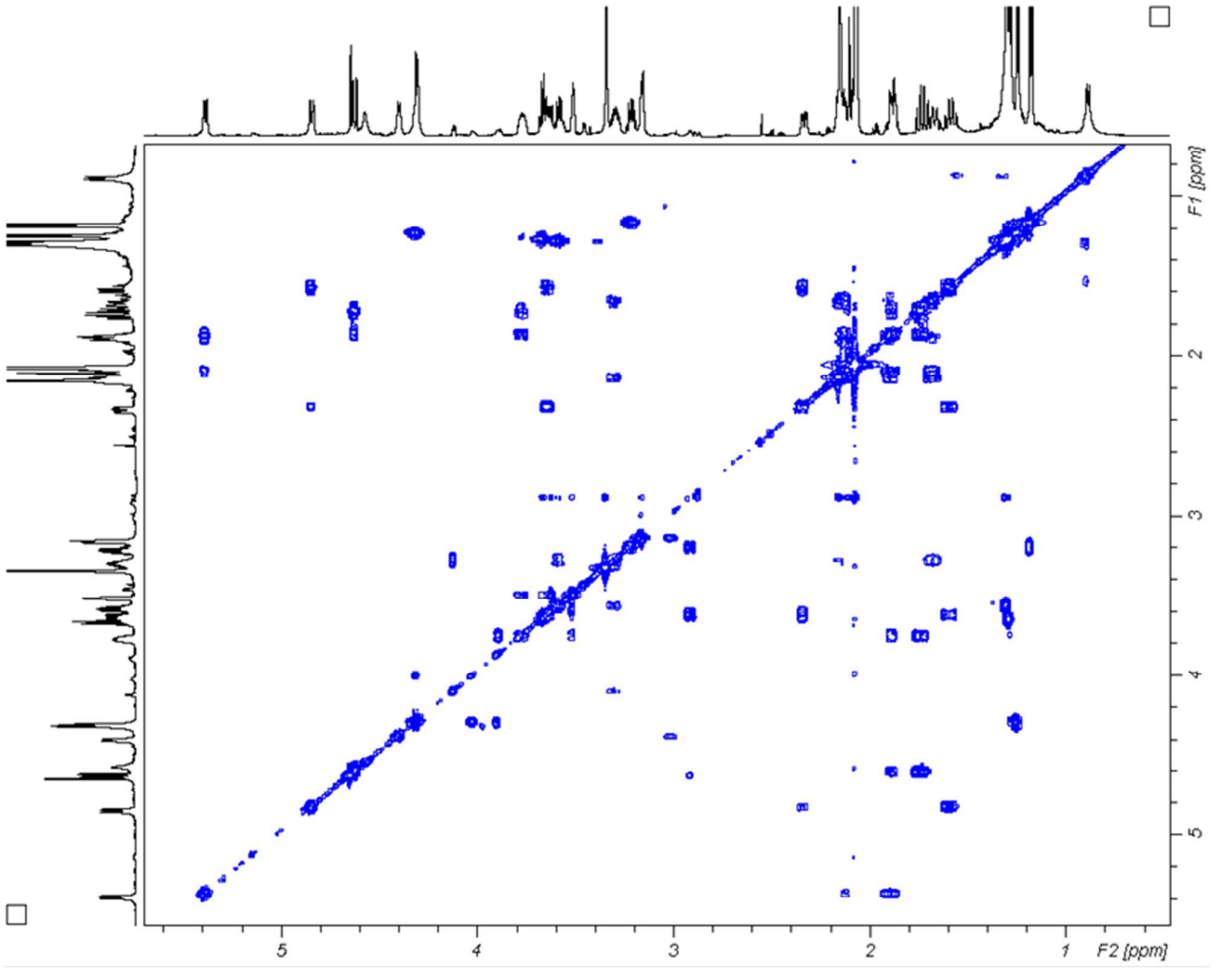


Figure S10: COSY chart for **5** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

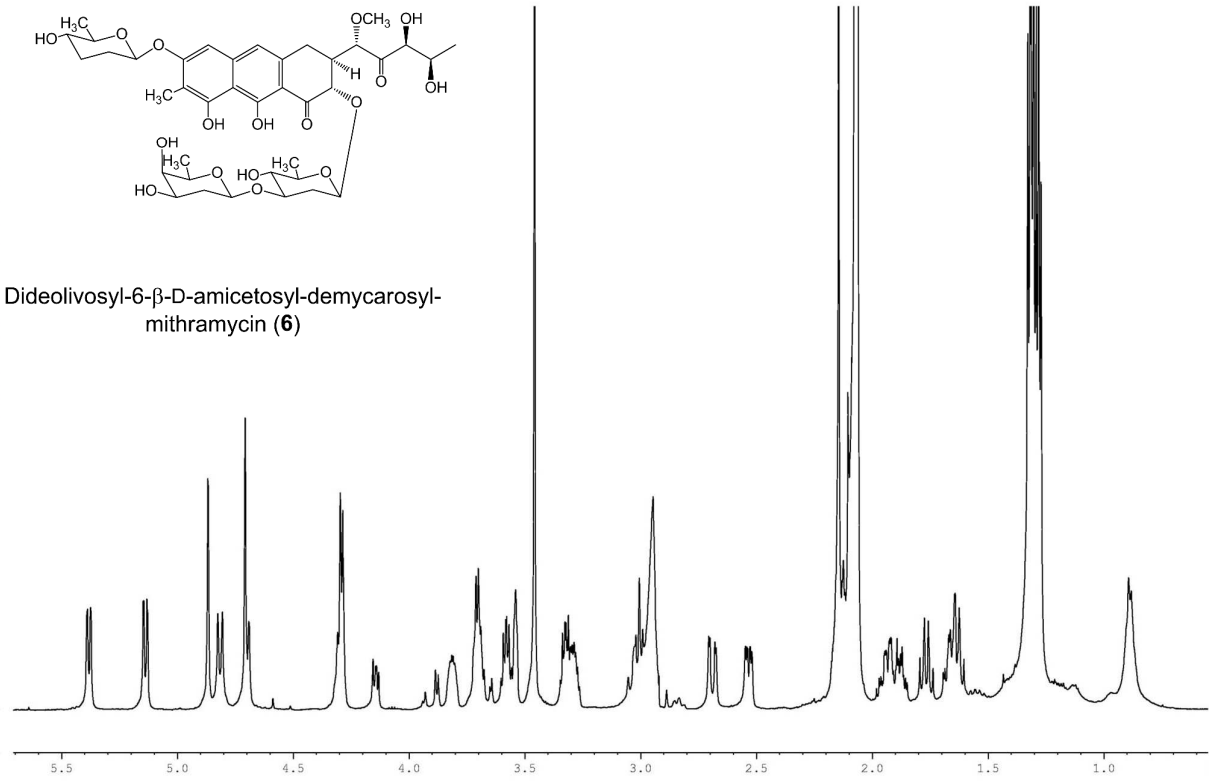


Figure S11: ^{1}H-NMR expanded chart for **6** in acetone-*d*6 (600 MHz)

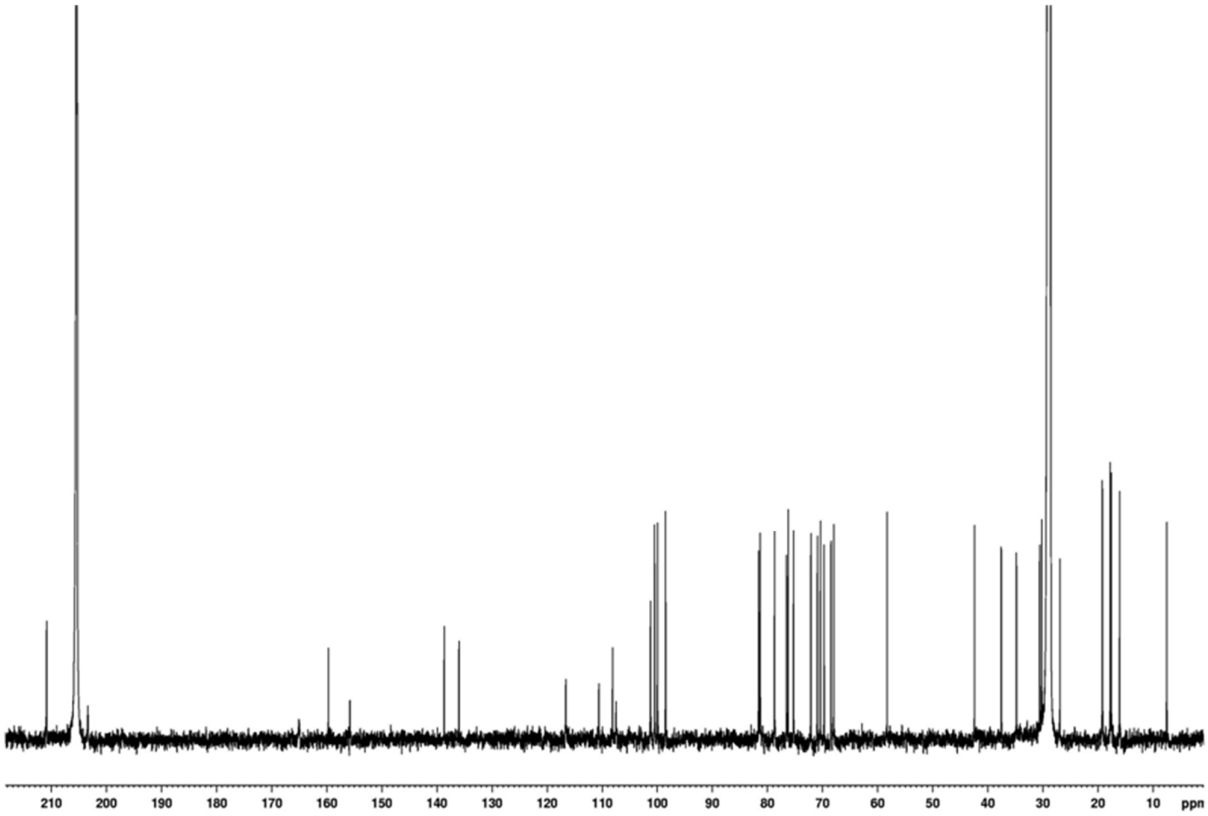


Figure S12: ^{13}C-NMR chart for **6** in acetone-*d*6 (150 MHz)

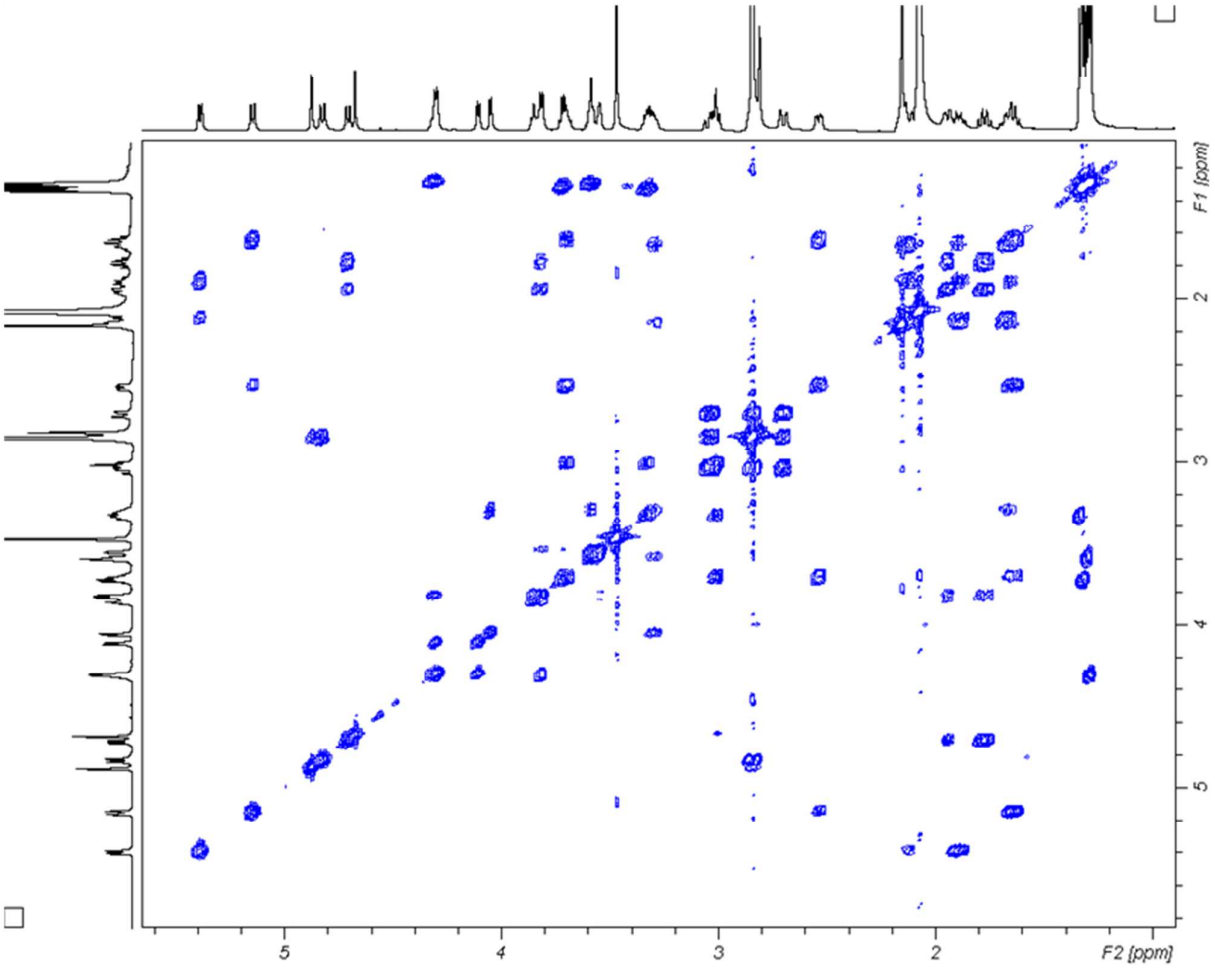


Figure 13: COSY chart for **6** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

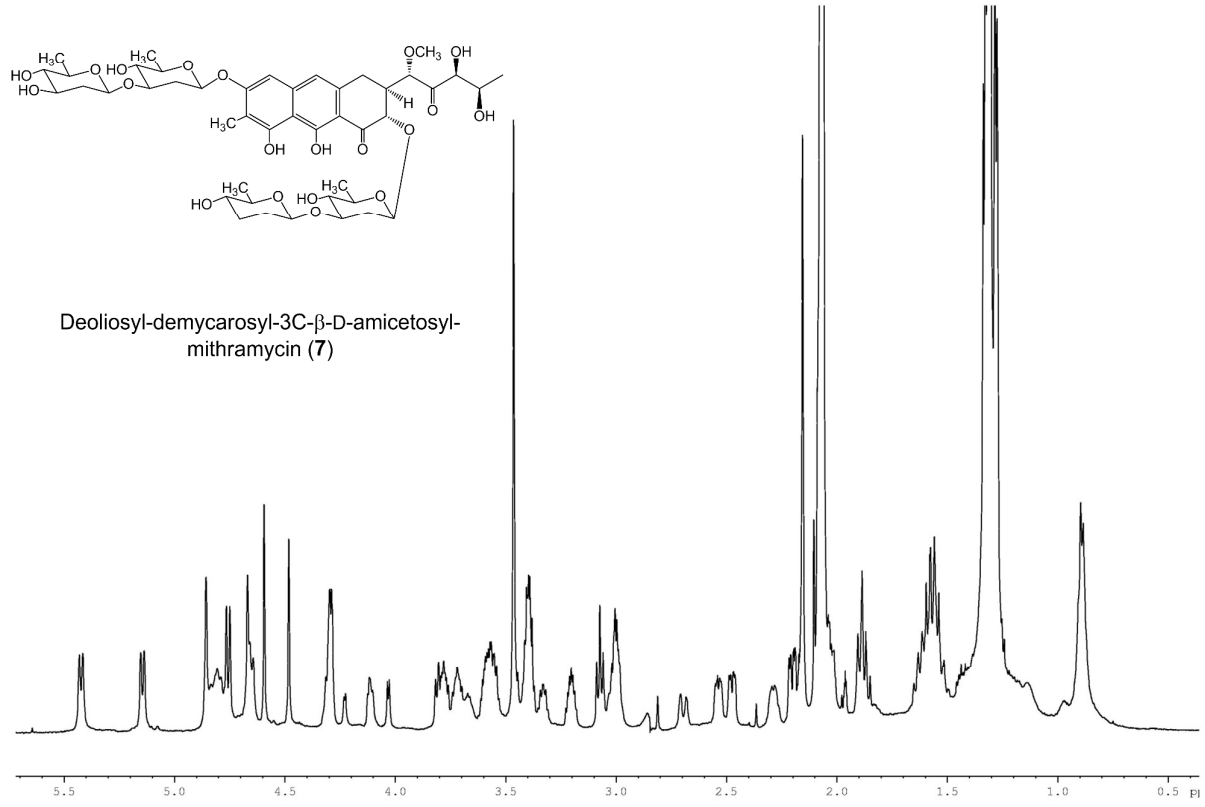


Figure S14: ^{1}H-NMR expanded chart for **7** in acetone-*d*6 (600 MHz)

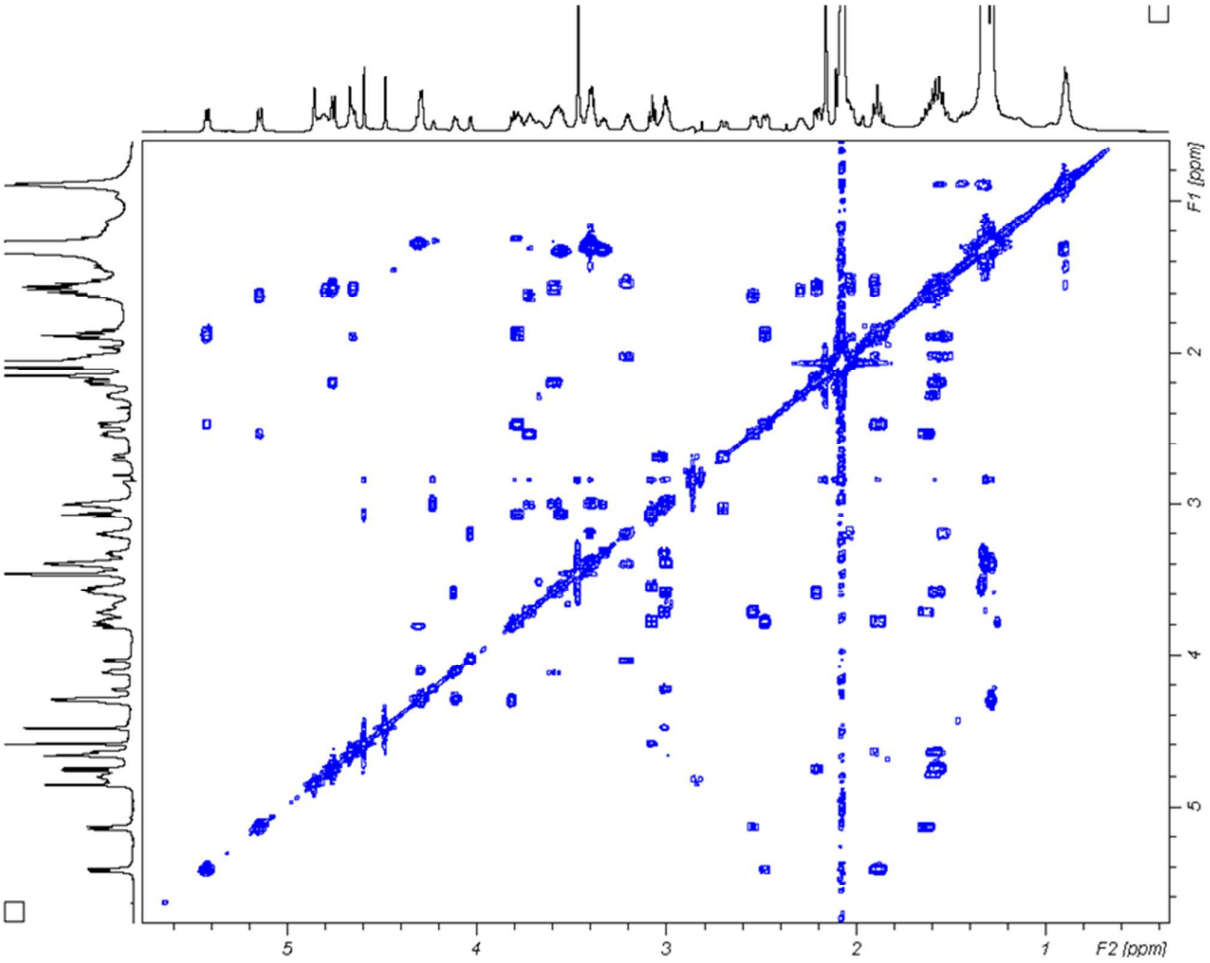


Figure S15: COSY chart for **7** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

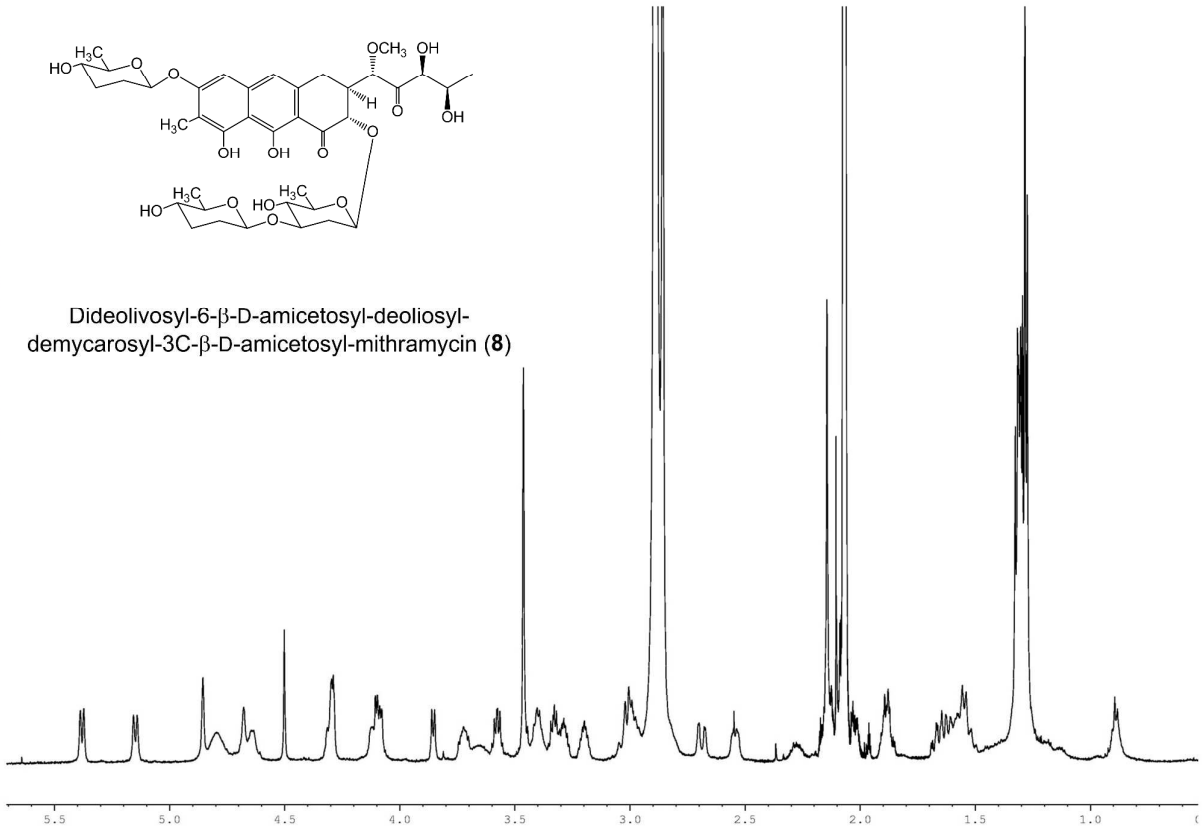


Figure S16: ^{1}H-NMR expanded chart for **8** in acetone-*d*6 (600 MHz)

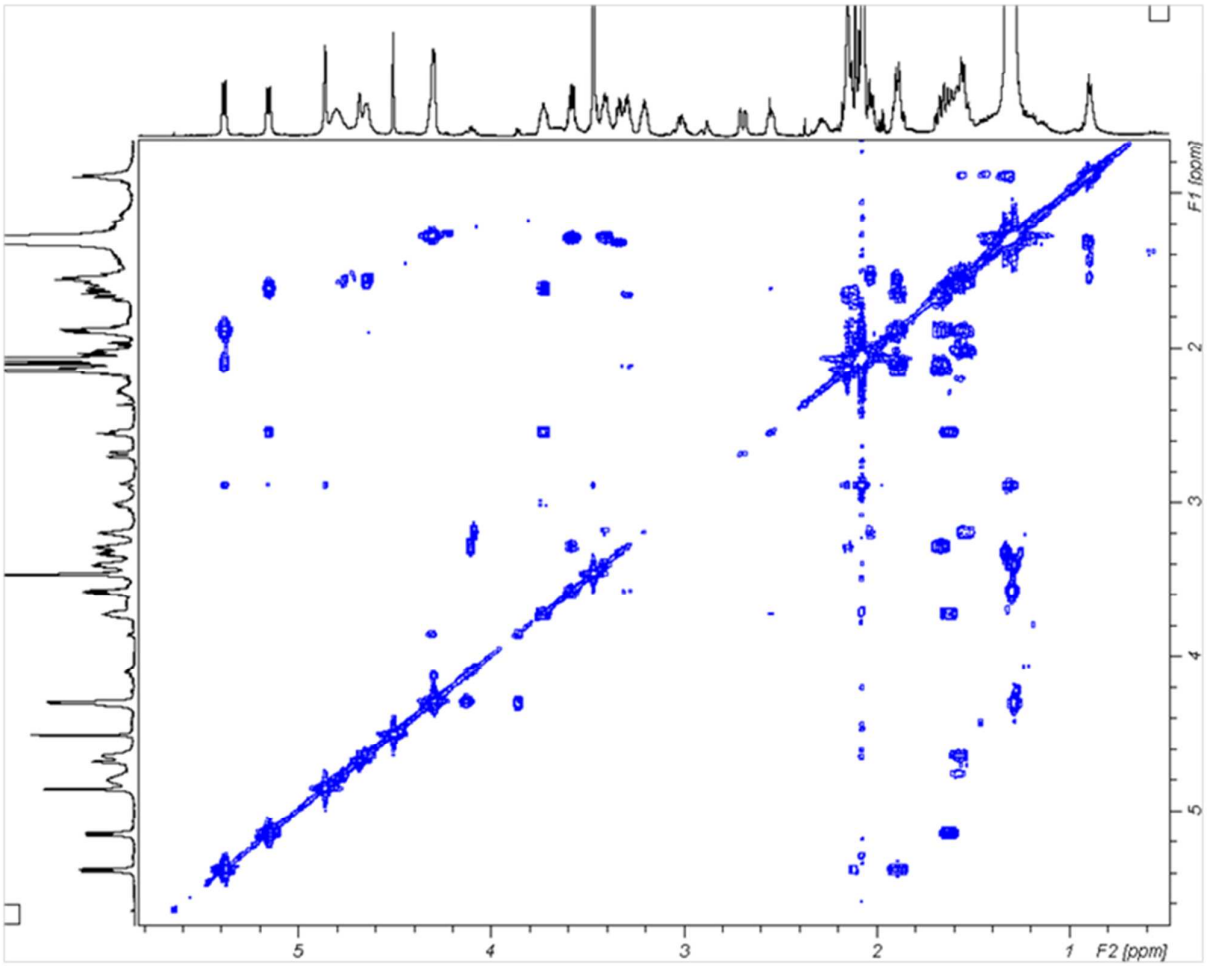


Figure S17: COSY chart for **8** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

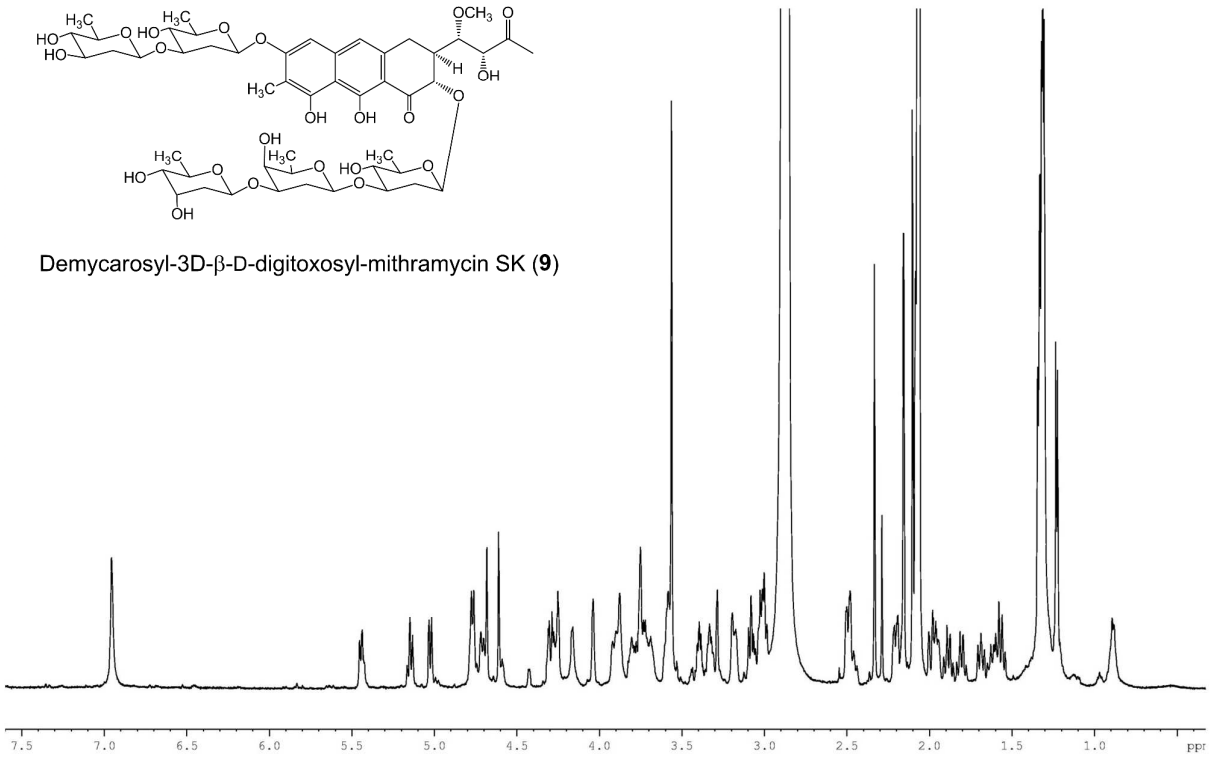


Figure S18: ^{1}H-NMR expanded chart for **9** in acetone-*d*6 (600 MHz)

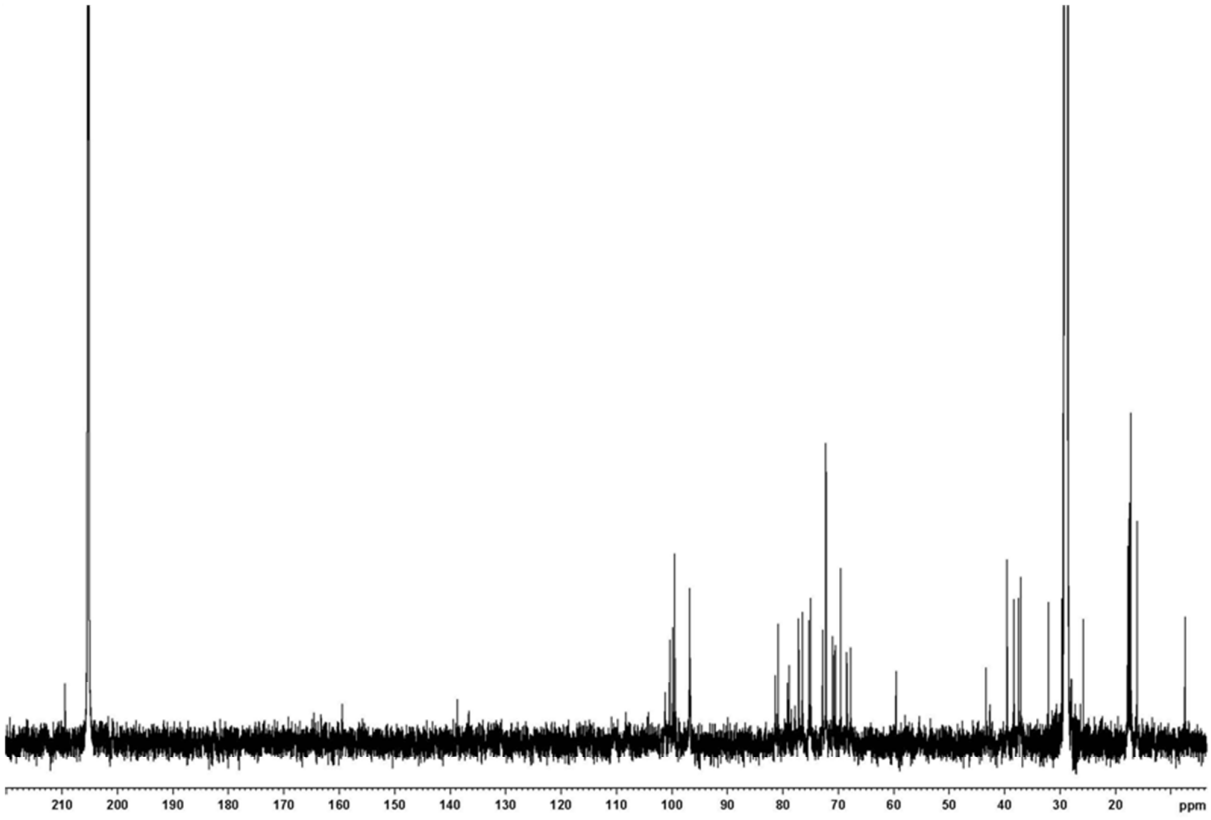


Figure S19: ^{13}C-NMR chart for **9** in acetone-*d*6 (150 MHz)

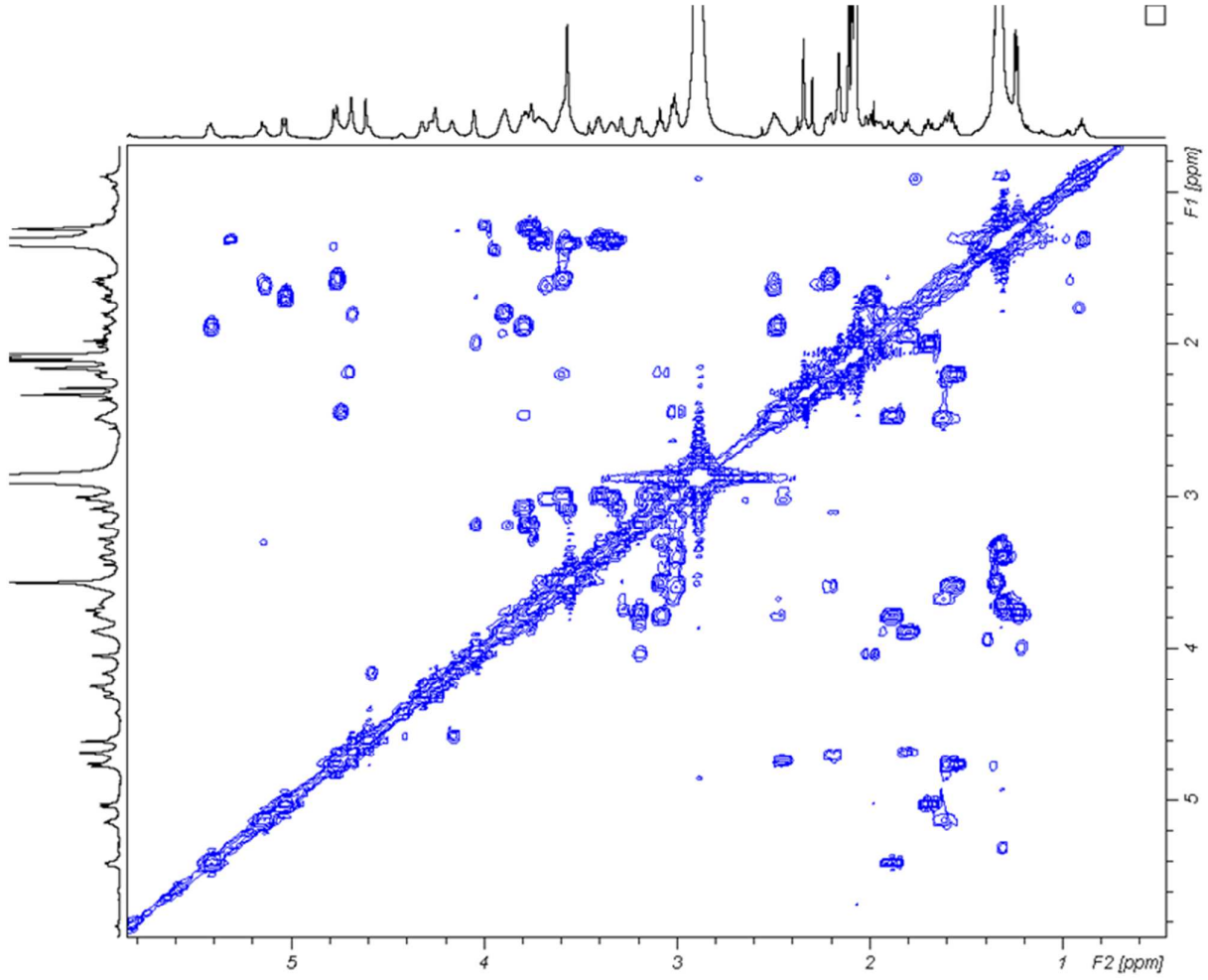


Figure S20: COSY chart for **9** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

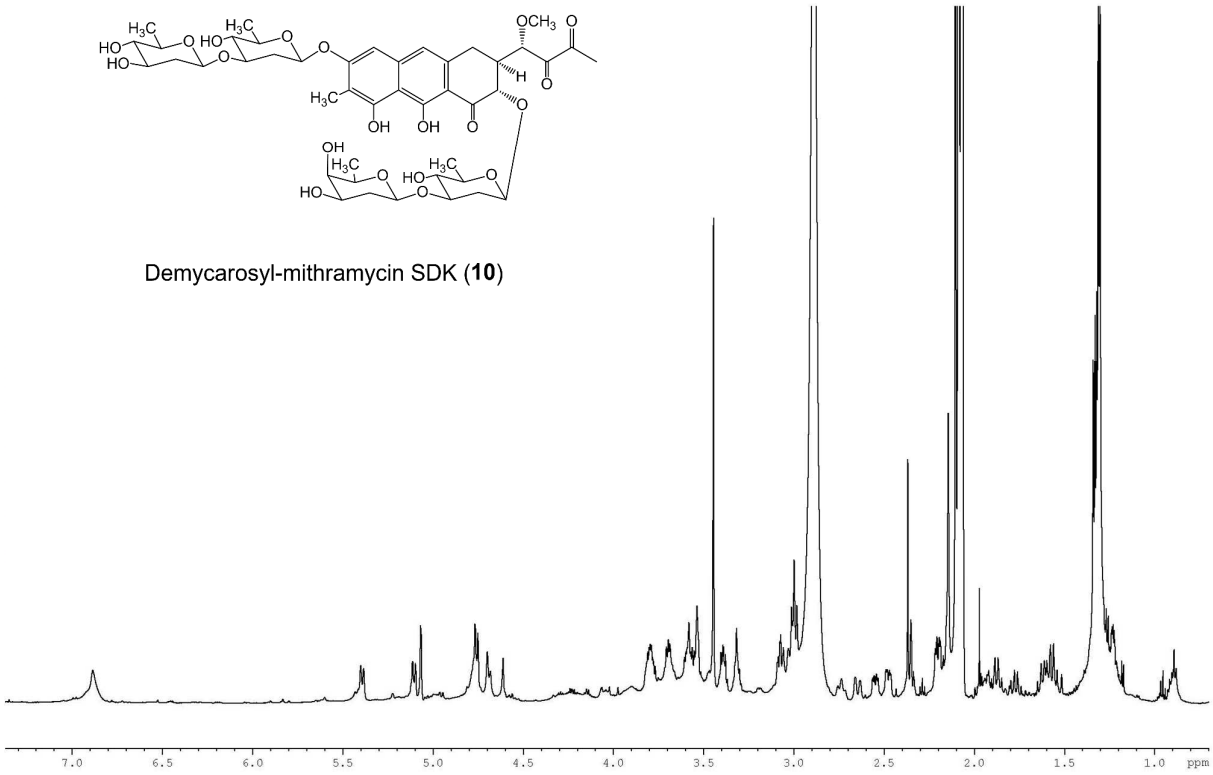


Figure S21: ^{1}H-NMR expanded chart for **10** in acetone-*d*6 (600 MHz)

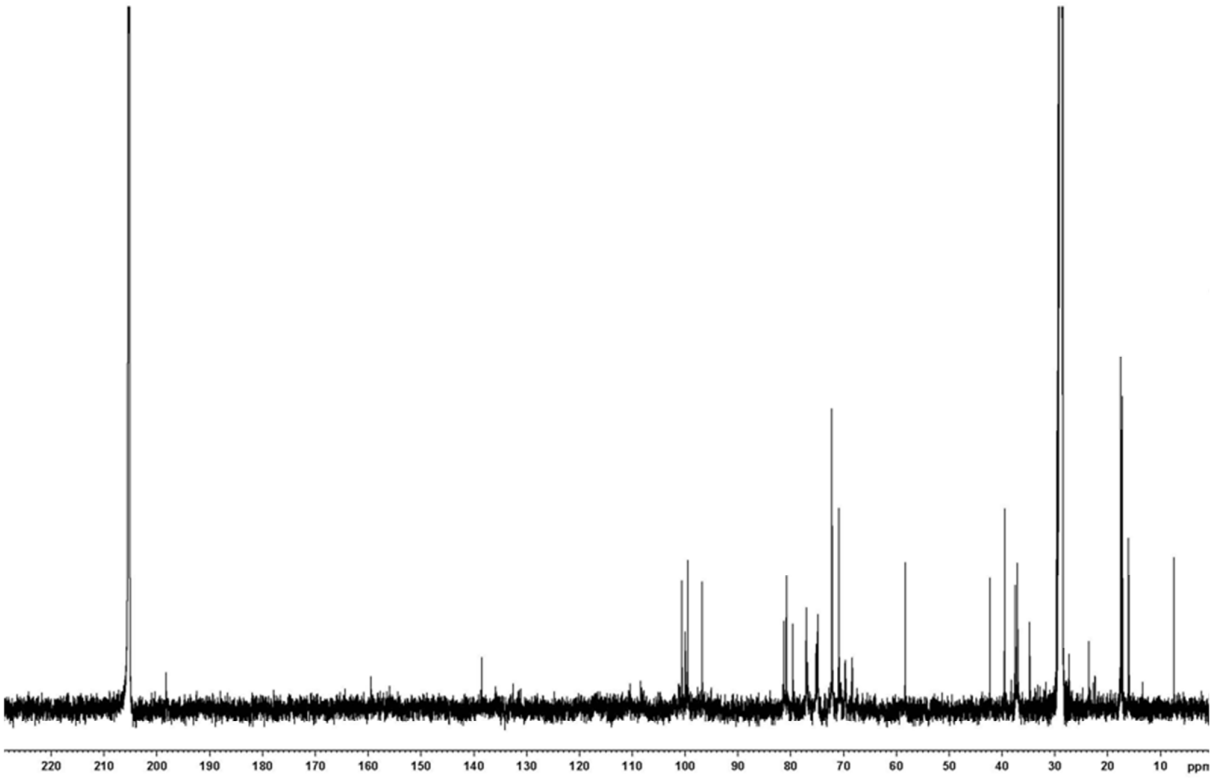


Figure S22: ^{13}C-NMR chart for **10** in acetone-*d*6 (150 MHz)

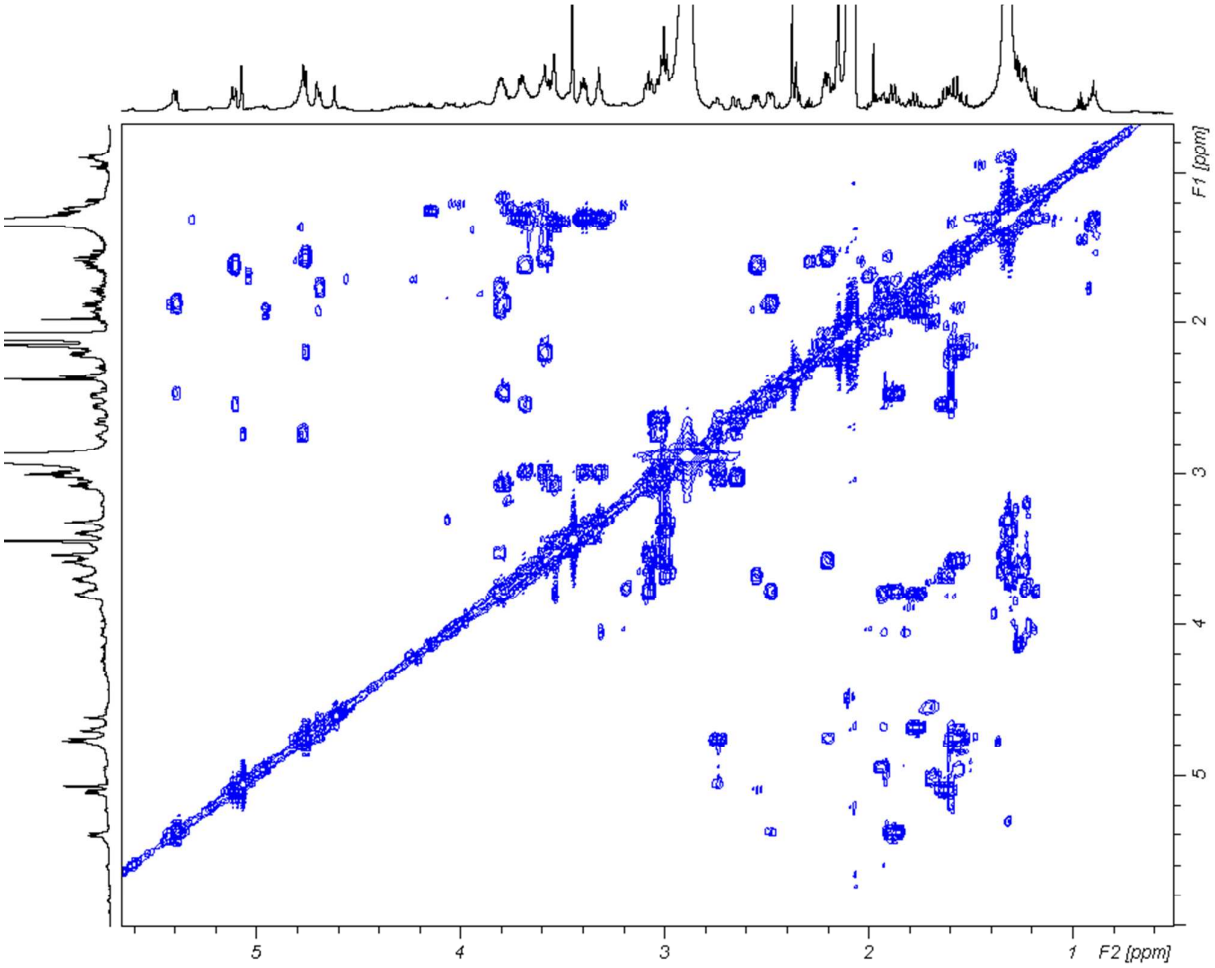


Figure S23: COSY chart for **10** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

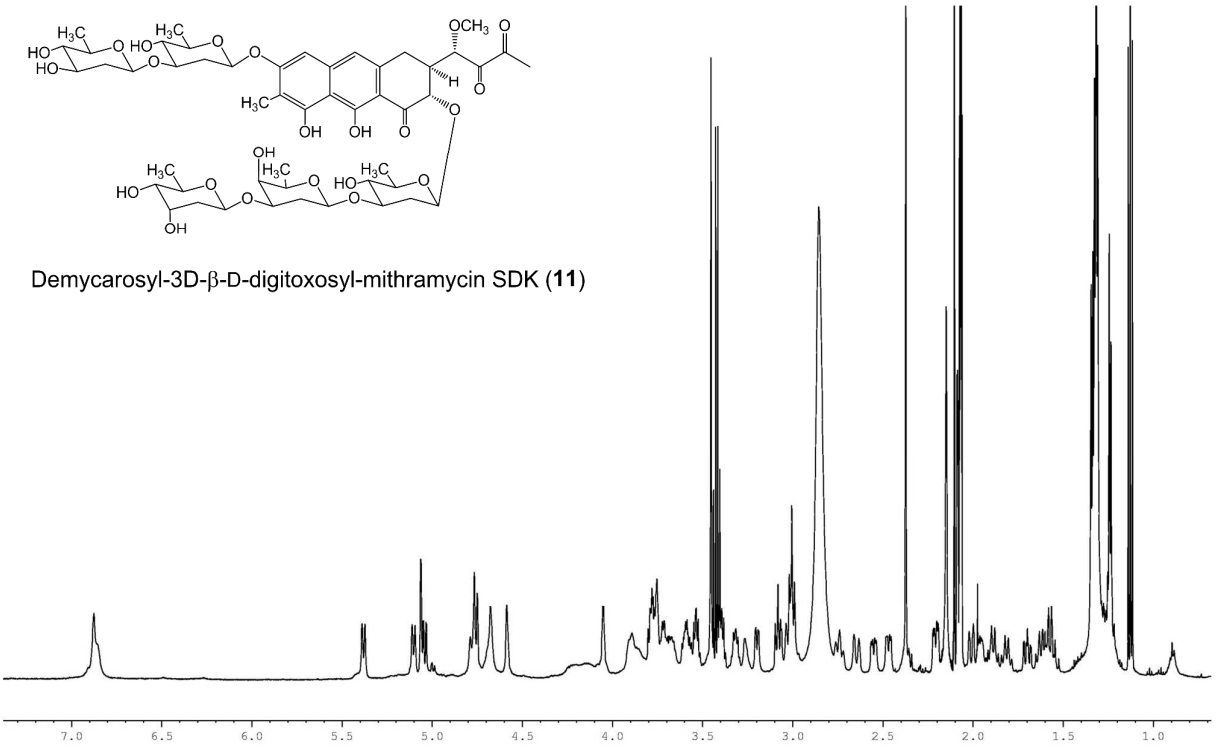


Figure S24: ^{1}H-NMR expanded chart for **11** in acetone-*d*6 (600 MHz)

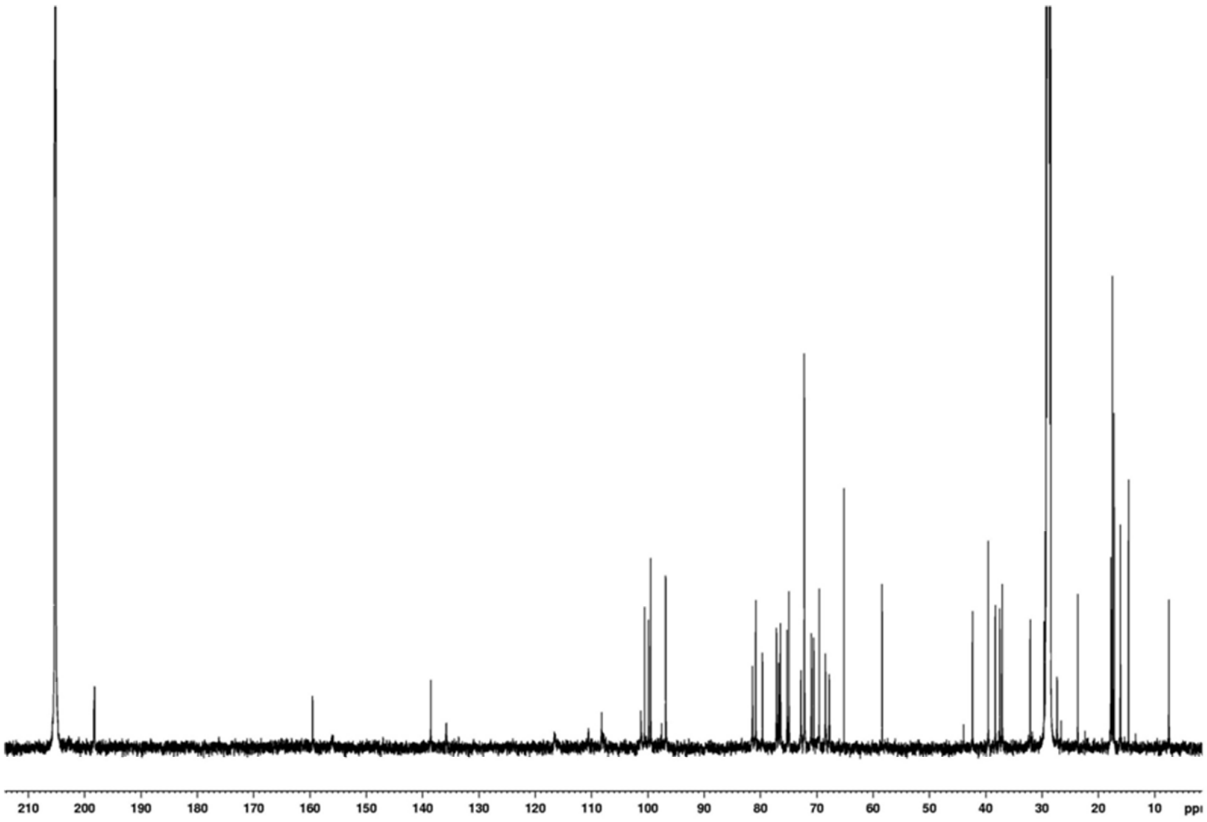


Figure S25: ^{13}C-NMR chart for **11** in acetone-*d*6 (150 MHz)

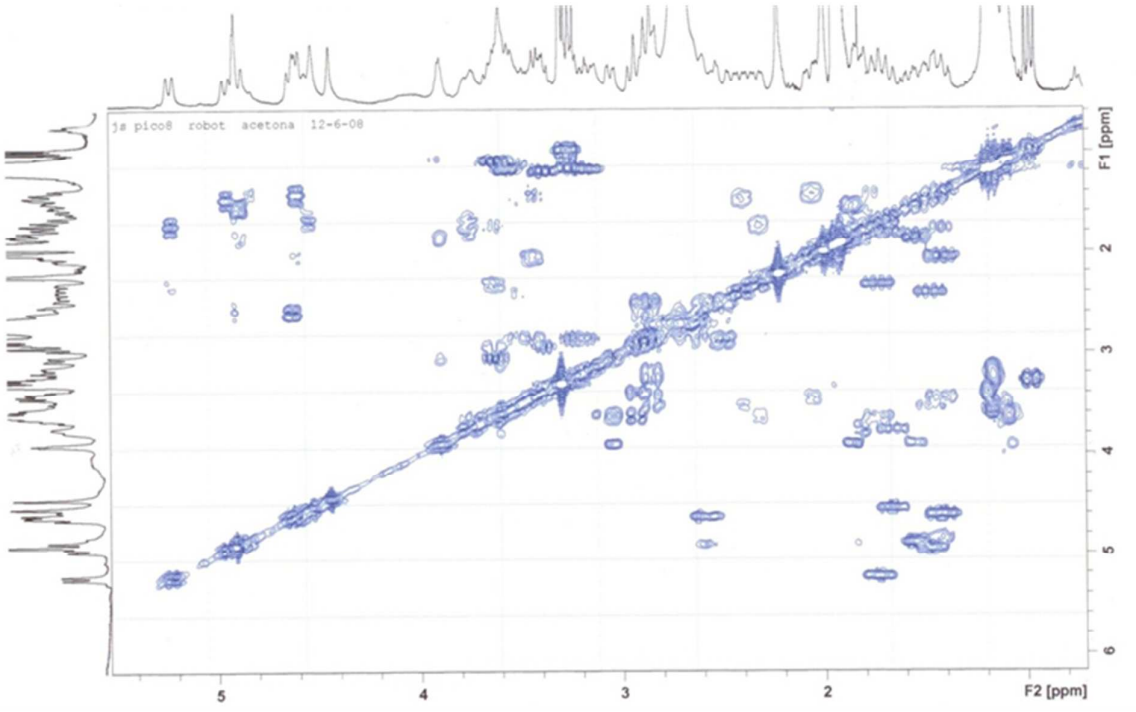


Figure S26: COSY chart for **11** in acetone-*d*6 (256 points for F1 and F2, 48 transients)

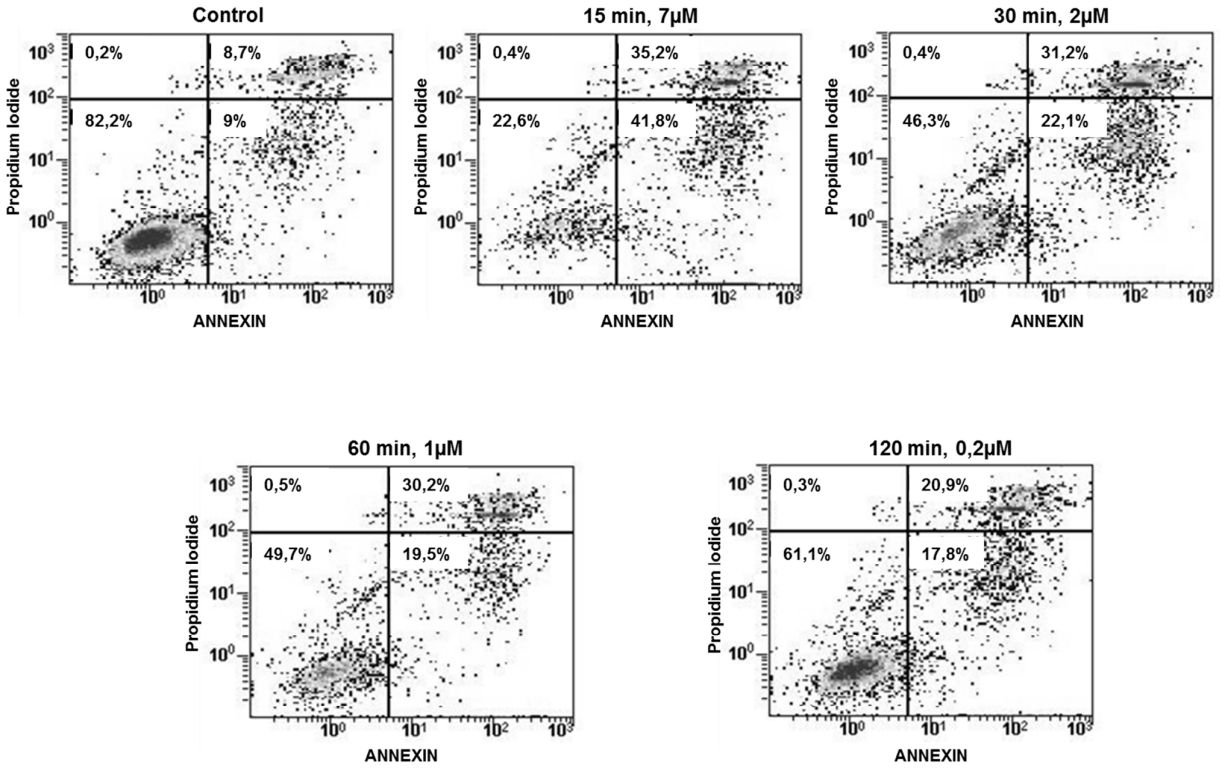


Figure S27. Flow cytometry analysis of Colo205 cells exposed to compound **9** at concentration and time indicated and stained with Annexin-V-fluorescein and Propidium Iodide after 48h.

Abbreviations used:

cox2, cyclooxygenase-2
ESFTs, Ewing sarcoma family of tumors
EWS-FLI1, Ewing sarcoma breakpoint region 1 and Friend leukemia virus integration 1
GI_{50}, 50% growth inhibition
IP, intraperitoneal
IV, intravenous
MDR-1, multidrug resistant gene 1
NDP, nucleoside diphosphate
NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells
p21cip1/waf1 , cyclin-dependent kinase inhibitor 1
p53, protein 53
q2dx10, a single dose every 2 days times 10
q3dx10, a single dose every three days times 10
R5-A, modified R5 medium
siRNA, small interfering RNA
Sp1, Specificity Protein 1
STAT3, Signal Transducer and Activator of Transcription 3
TF, Transcription Factor
TGF-β, transforming growth factor-beta