Na^+/K^+-ATPase-Targeted Cytotoxicity of (+)-Digoxin and Several Semi-synthetic Derivatives

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Content of Supplementary Data

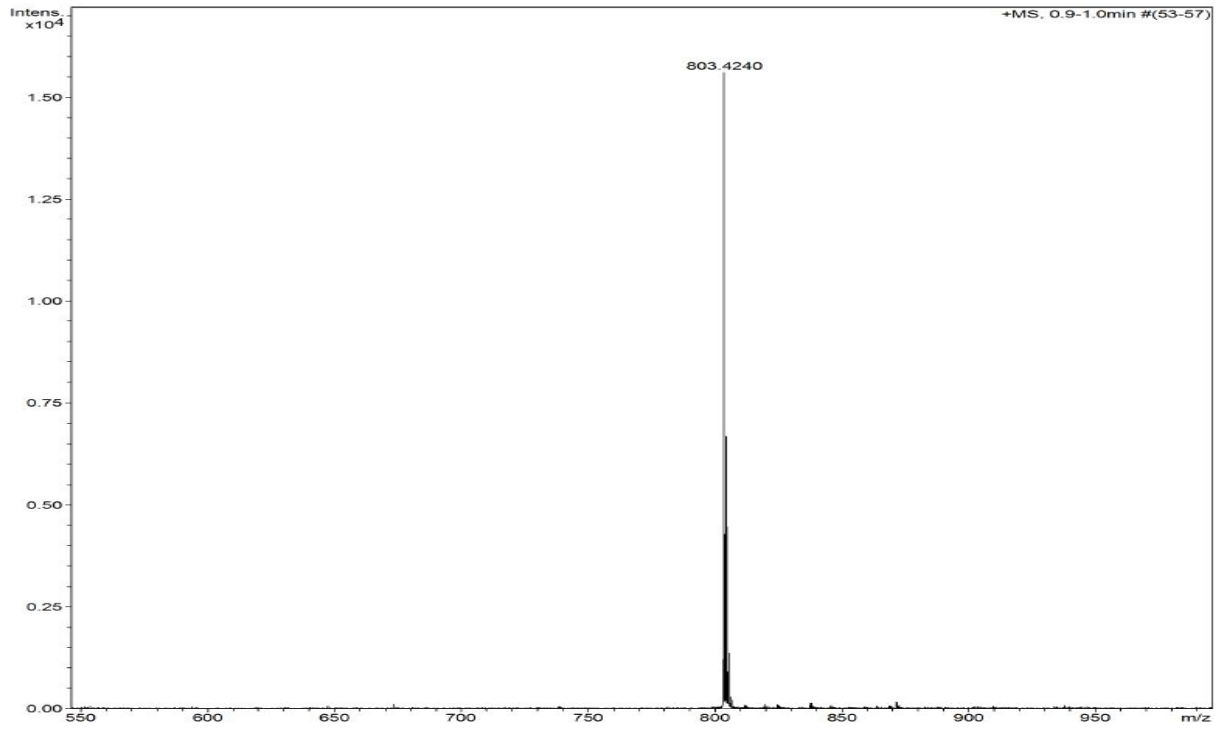
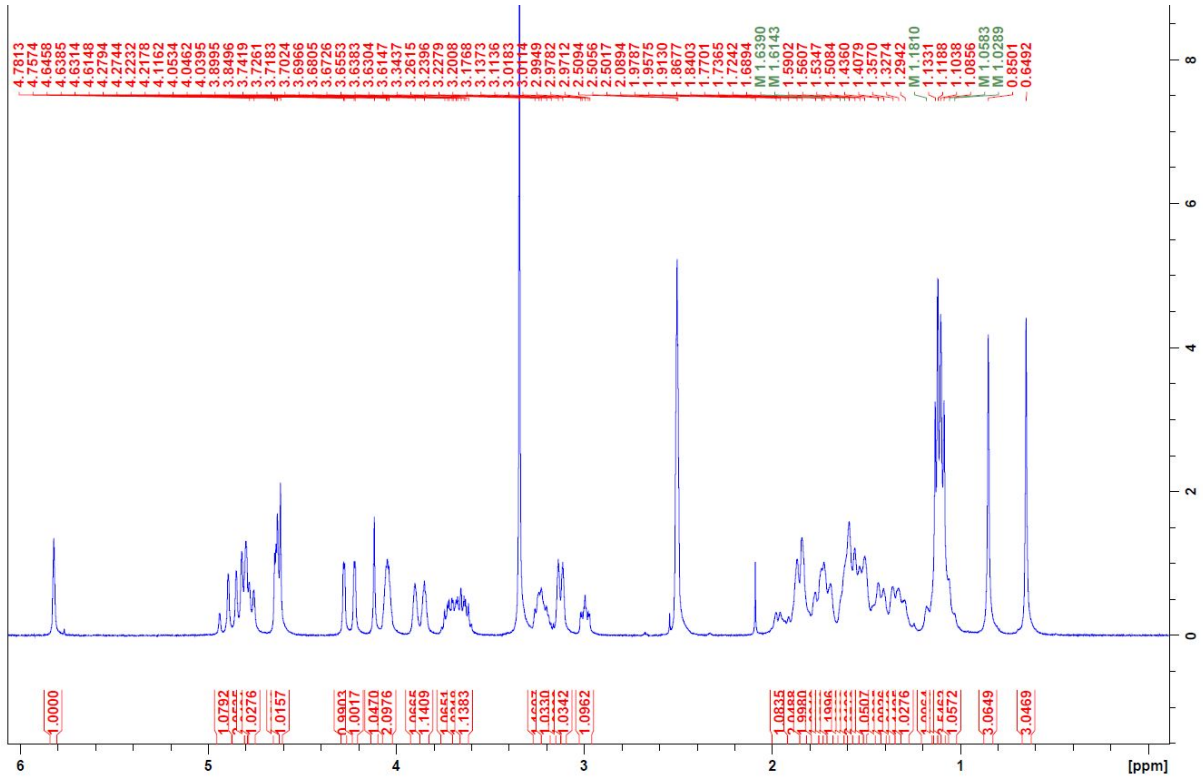


Figure S1a. Mass spectrum of (+)-digoxin (**1**).



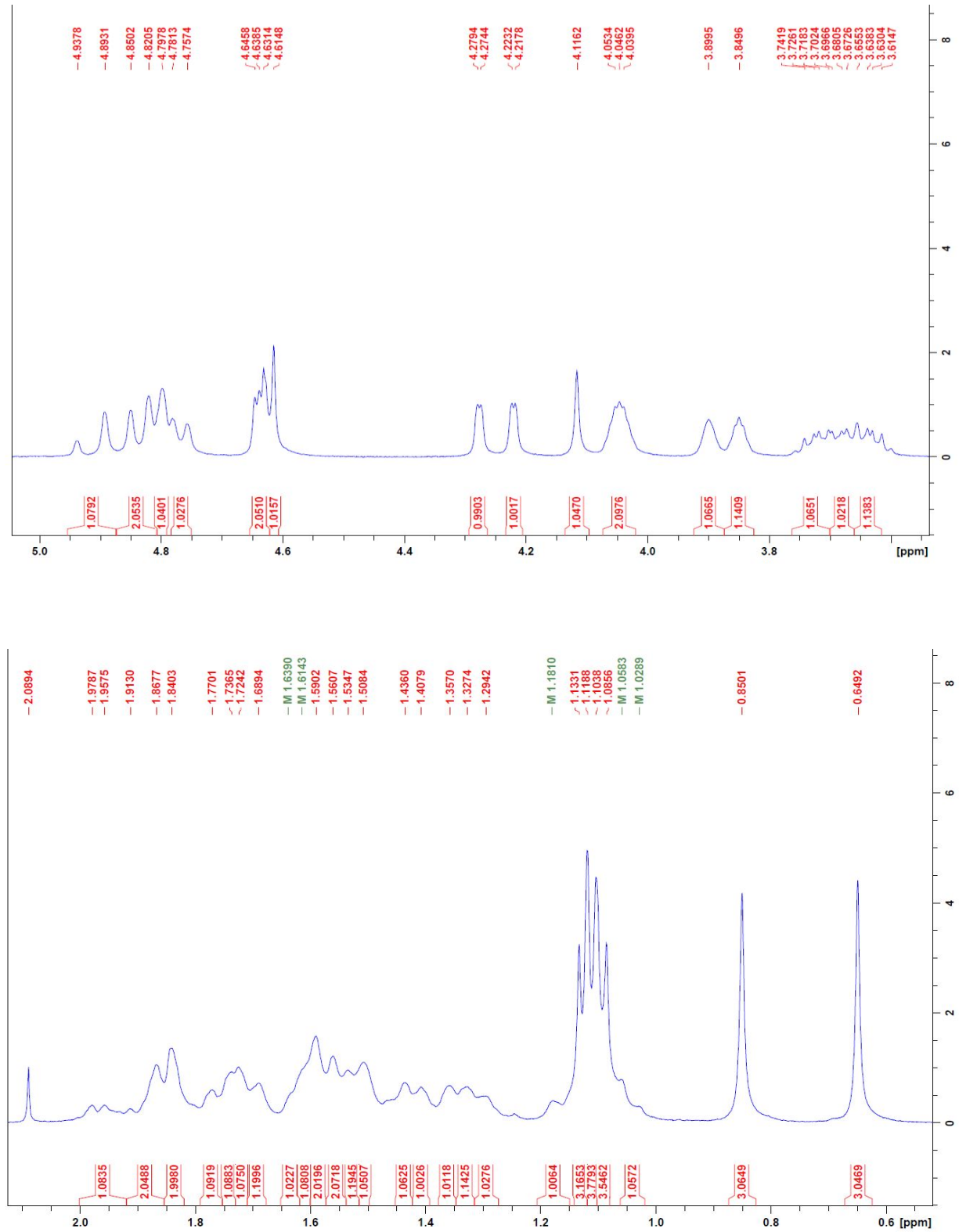


Figure S1b. ^{1}H NMR spectrum of (+)-digoxin (**1**).

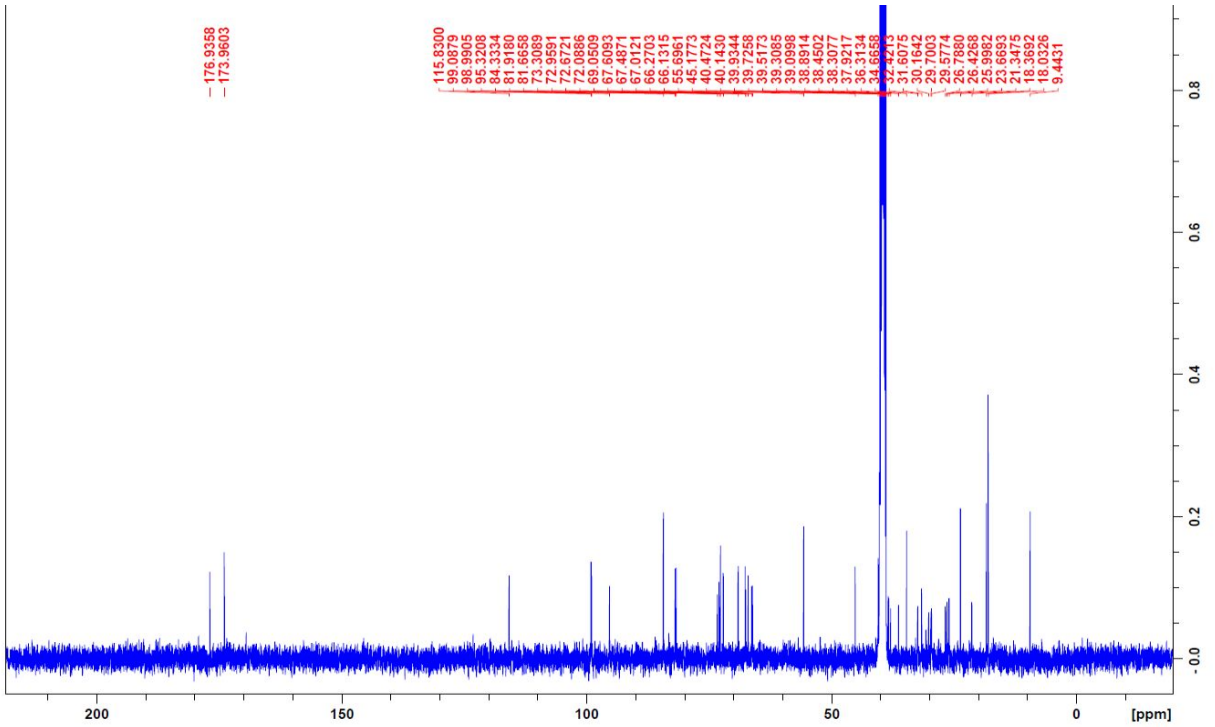


Figure S1c. ^{13}C NMR spectrum of (+)-digoxin (**1**).

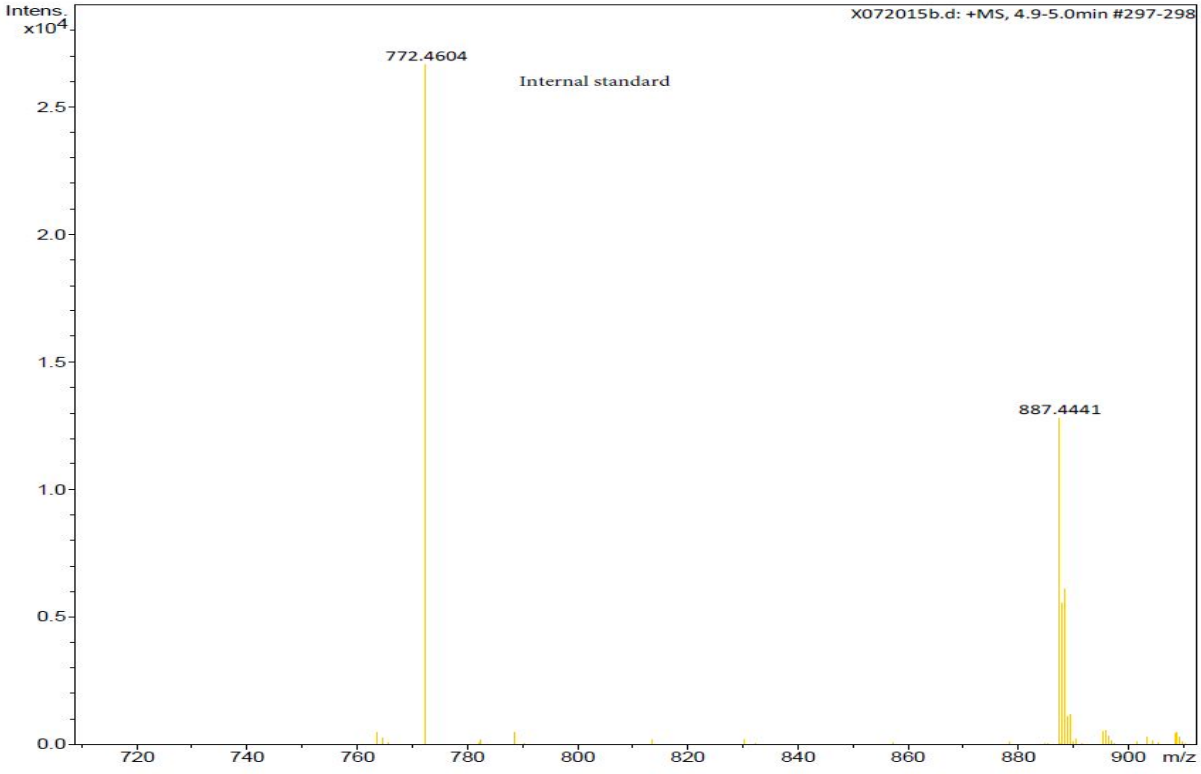
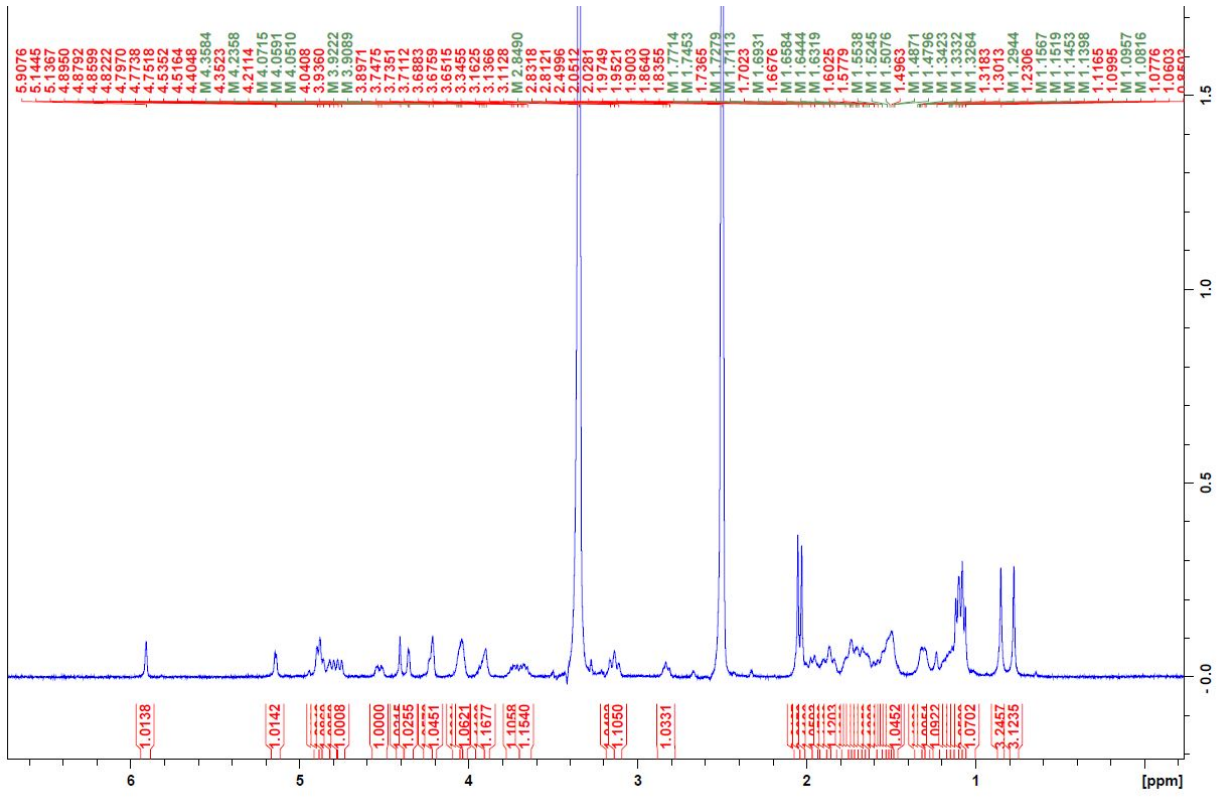
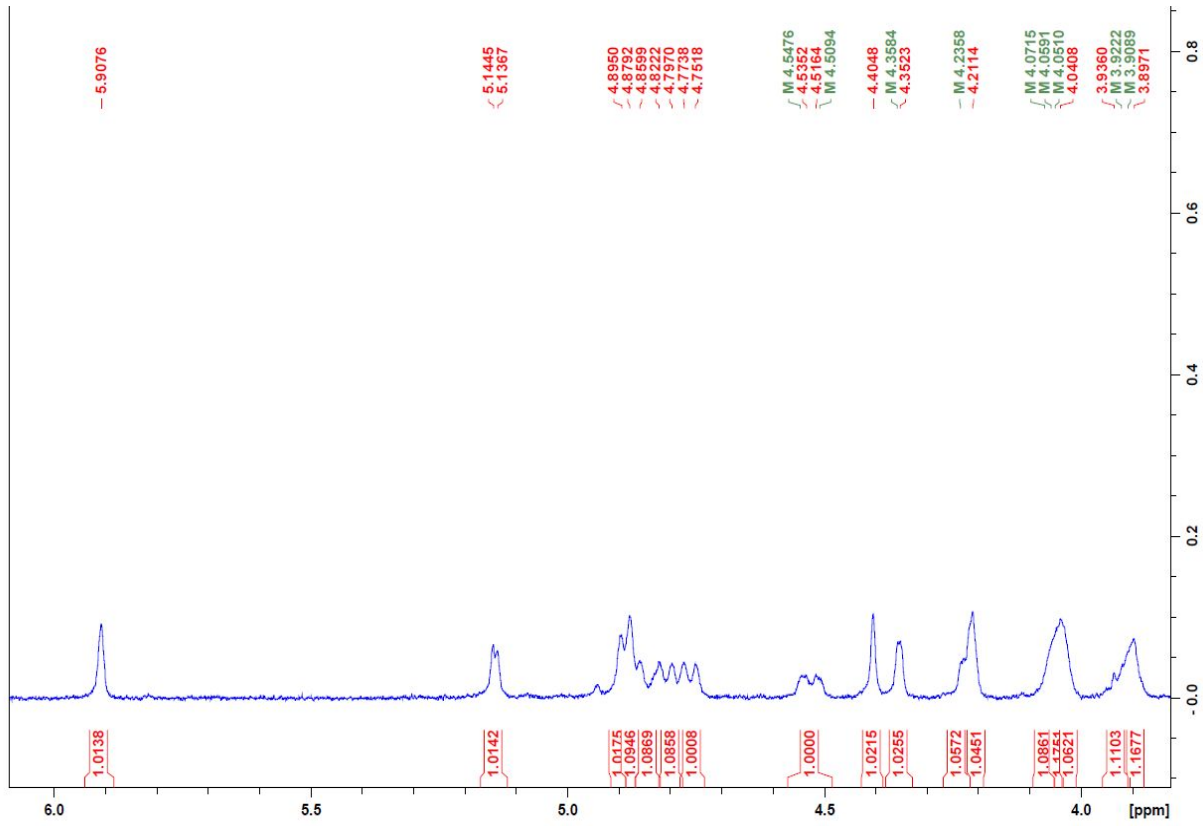


Figure S2a. Mass spectrum of (+)-12,4'c-di-*O*-acetyldigoxin (**2**).





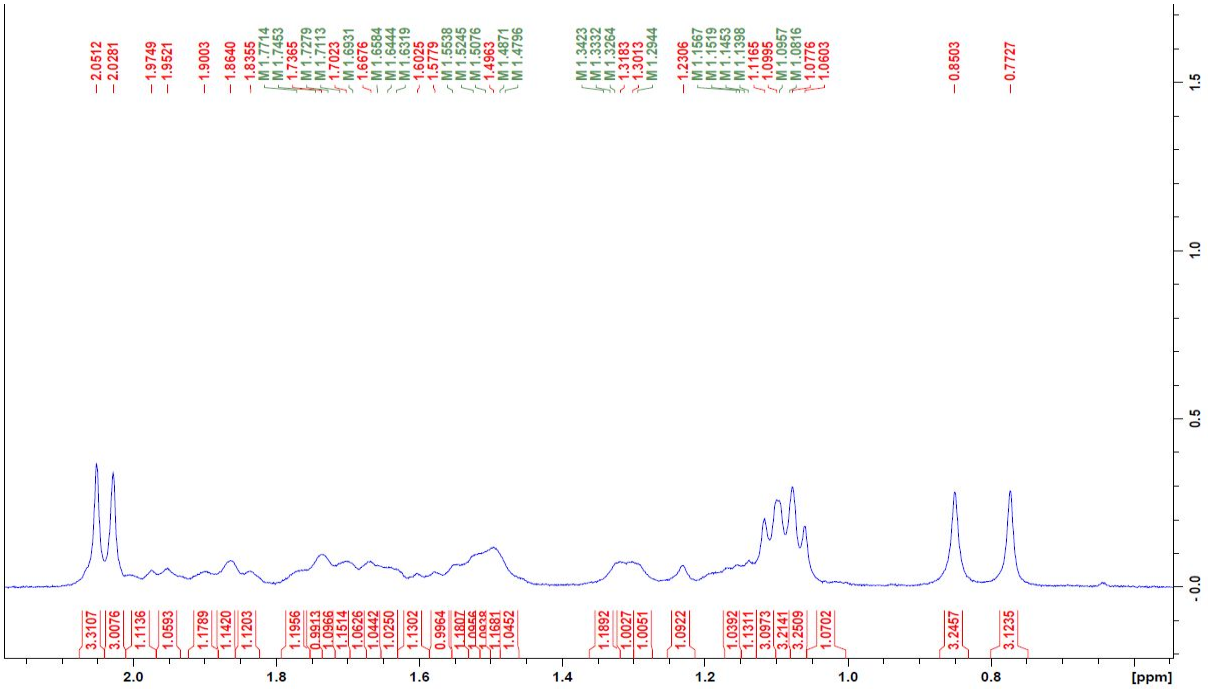


Figure S2b. ^{1}H NMR spectrum of (+)-12,4'c-di-*O*-acetyldigoxin (**2**).

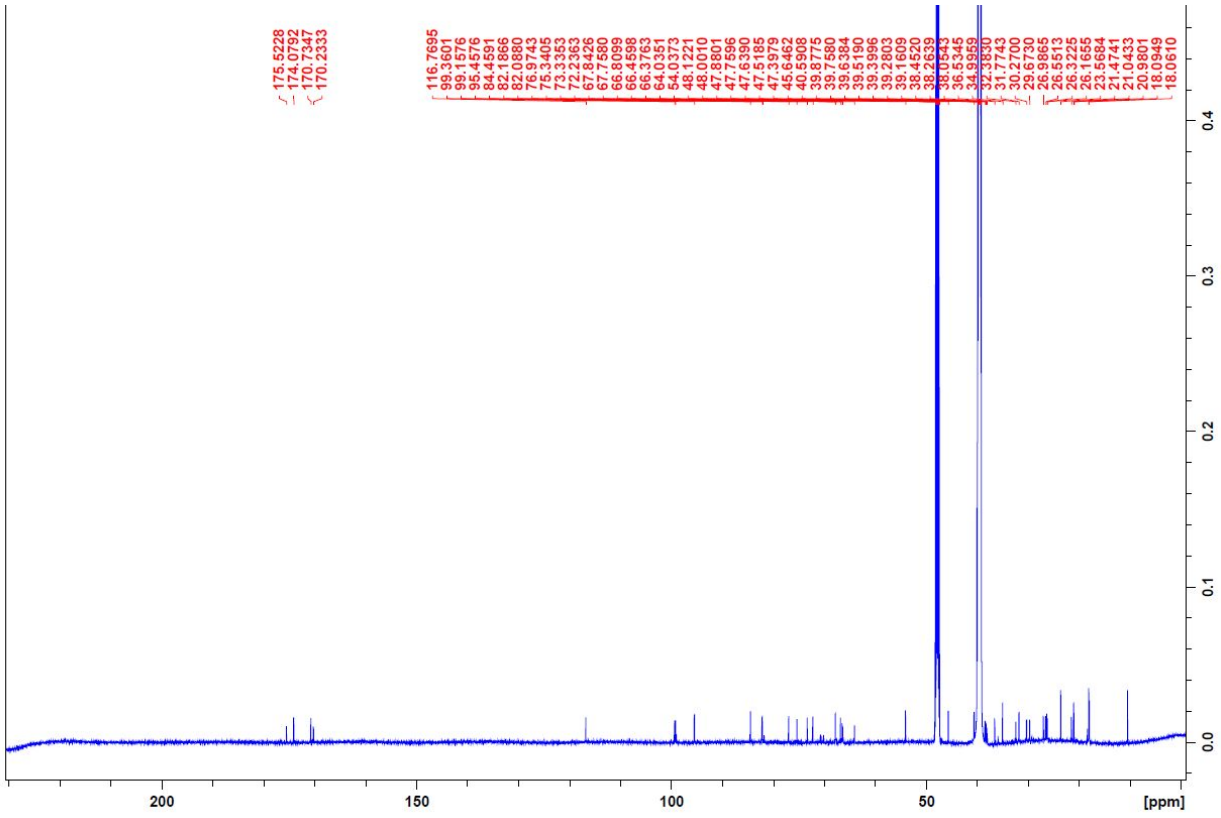


Figure S2c. ^{13}C NMR spectrum of (+)-12,4'c-di-*O*-acetyldigoxin (**2**).

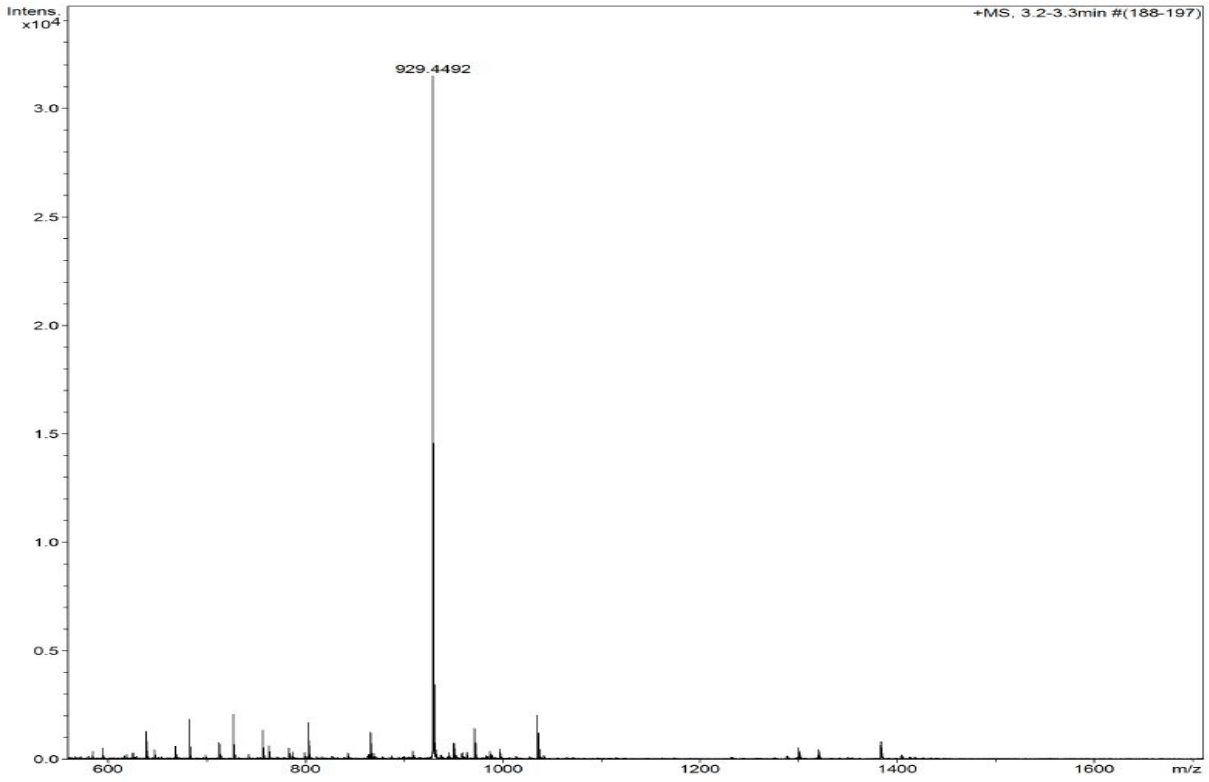
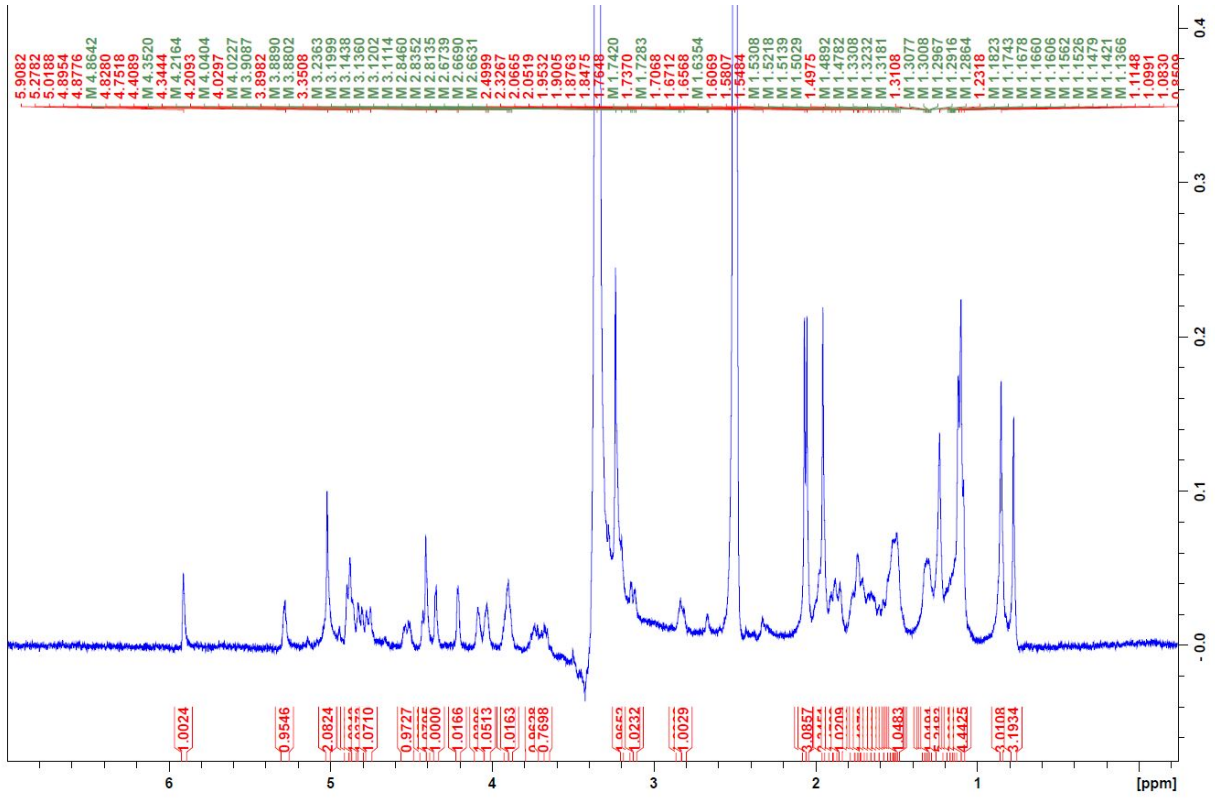


Figure S3a. Mass spectrum of (+)-12,3'c,4'c-tri-*O*-acetyldigoxin (**3**).



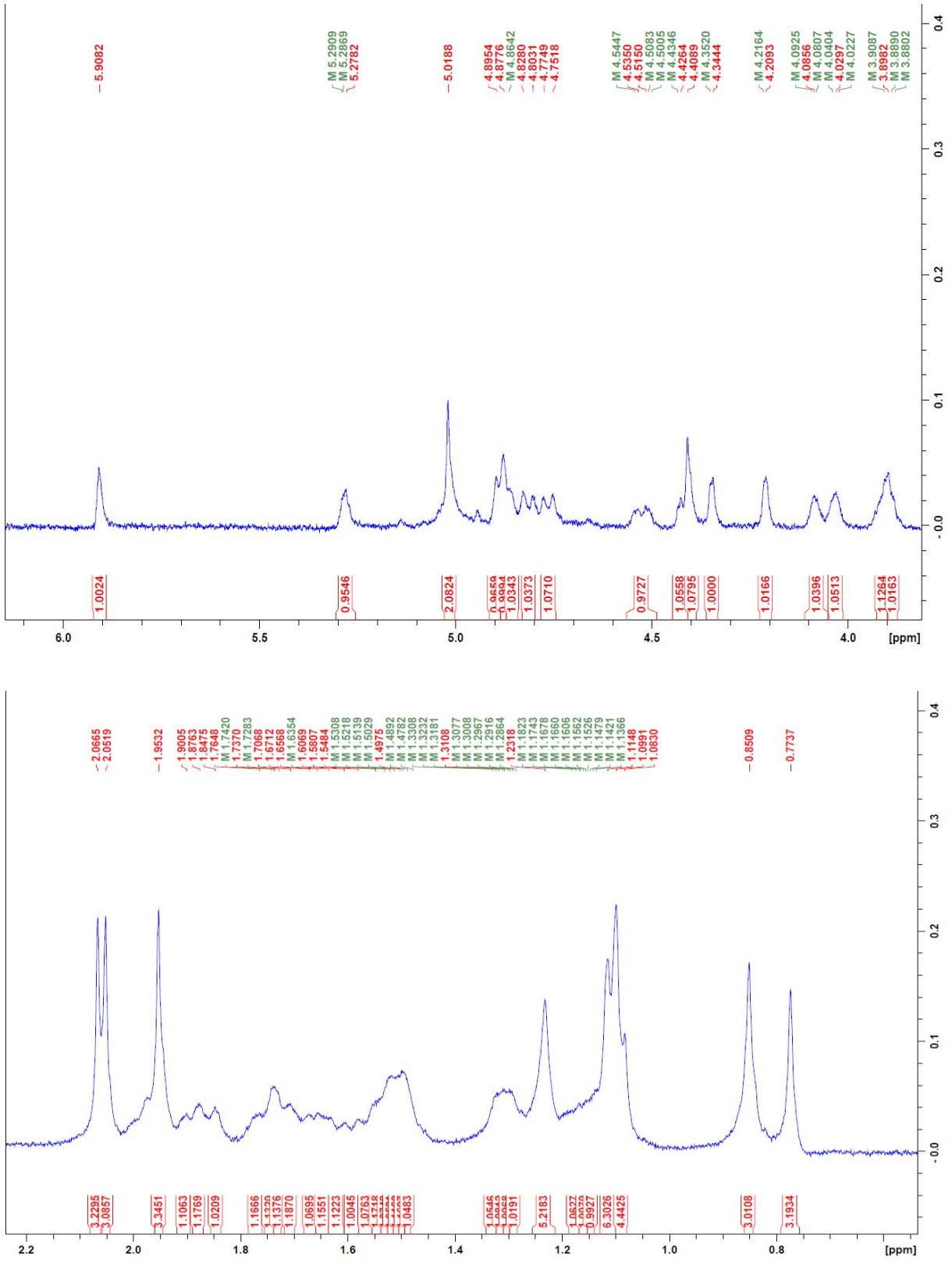


Figure S3b. ^{1}H NMR spectrum of (+)-12,3'c,4'c-tri-*O*-acetyldigoxin (**3**).

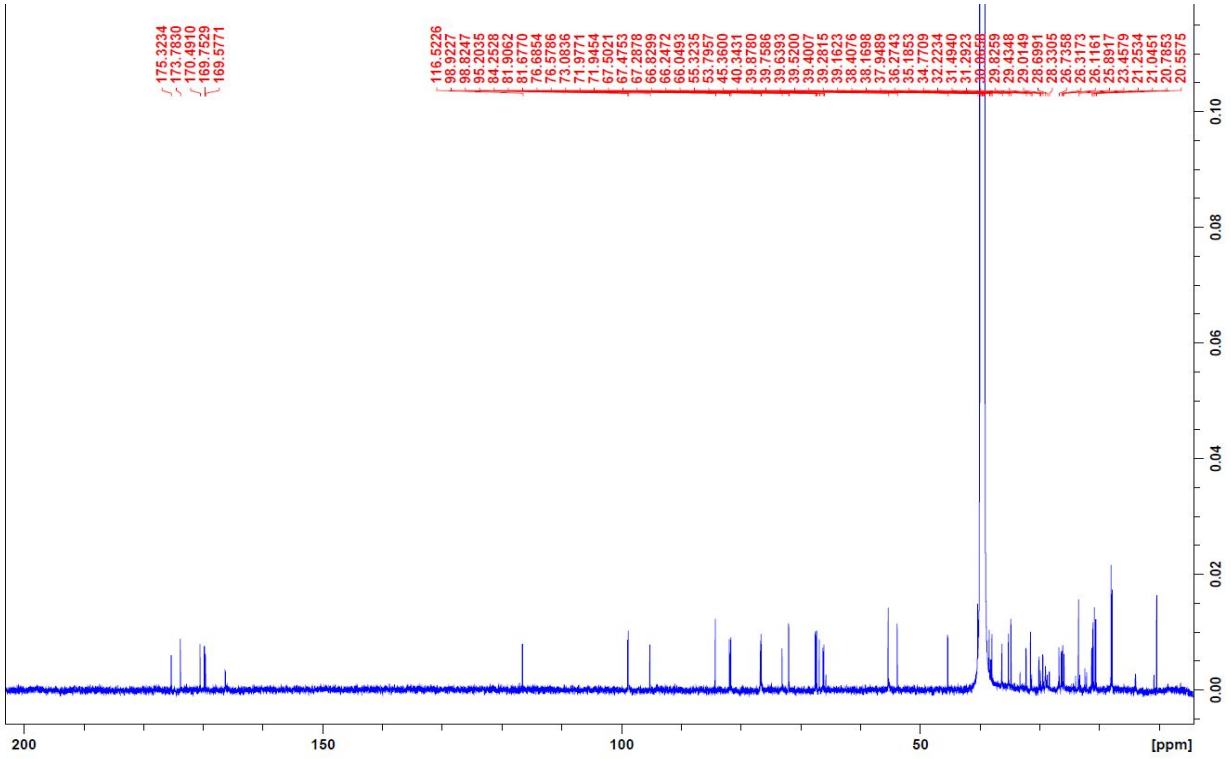


Figure S3c. ^{13}C NMR spectrum of (+)-12,3'c,4'c-tri-*O*-acetyldigoxin (**3**).

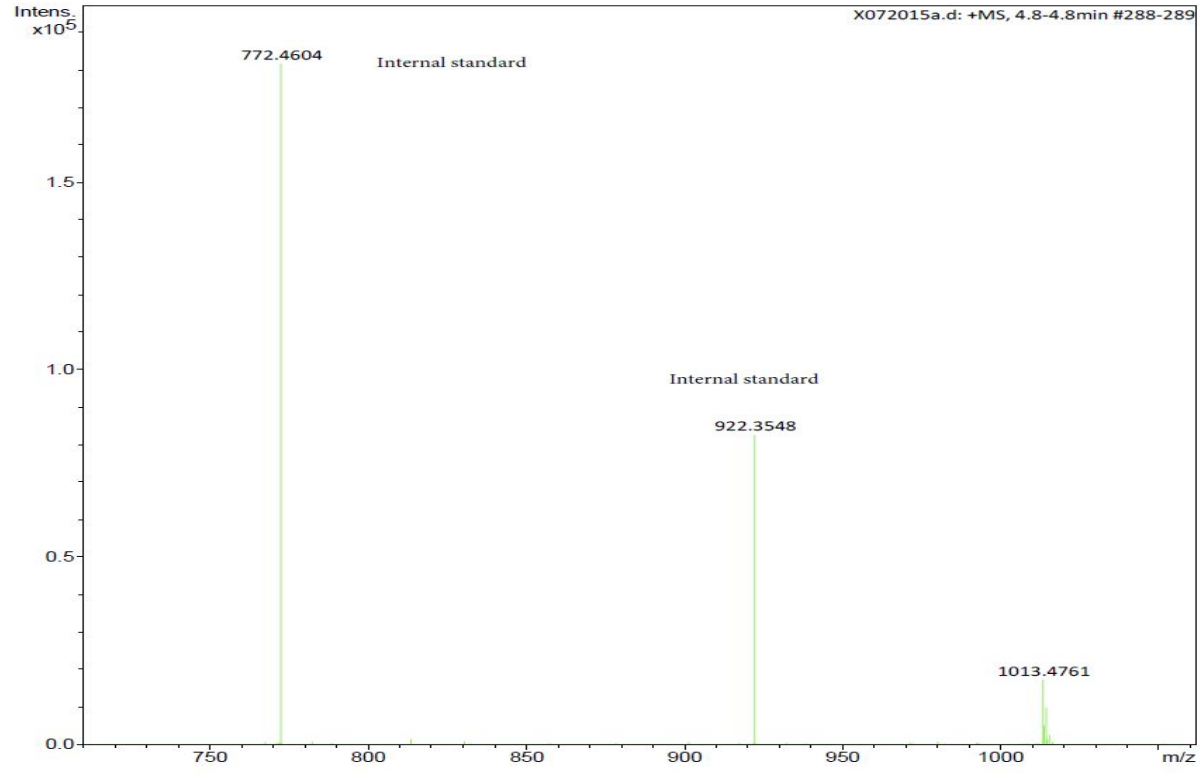
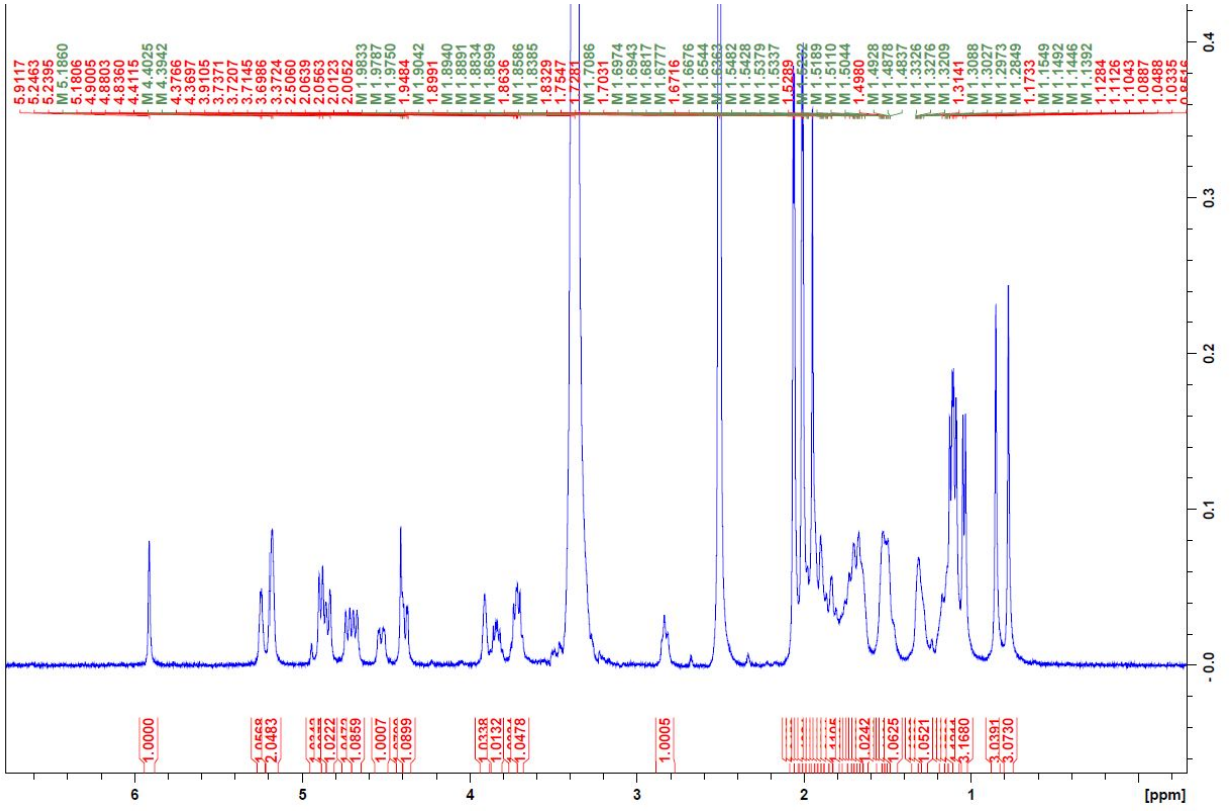
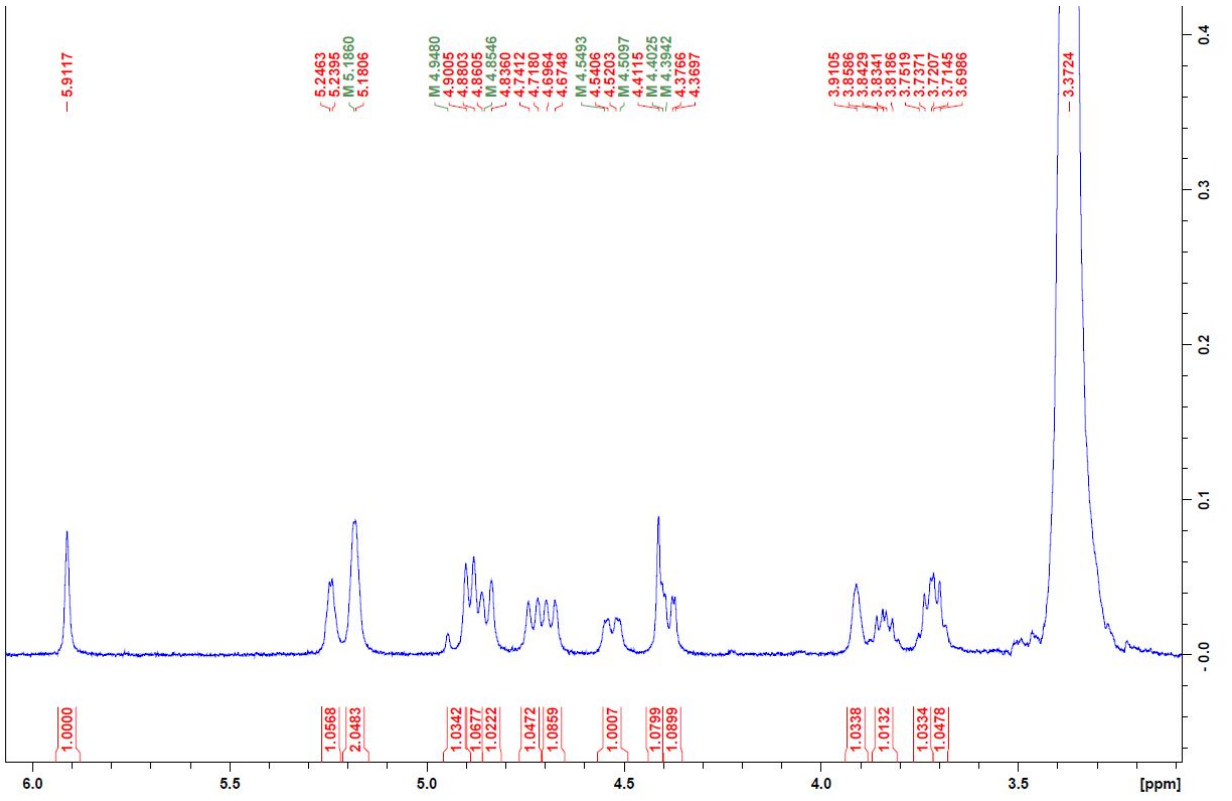


Figure S4a. Mass spectrum of (+)-12,3'a,3'b,3'c,4'c-penta-*O*-acetyldigoxin (**4**).





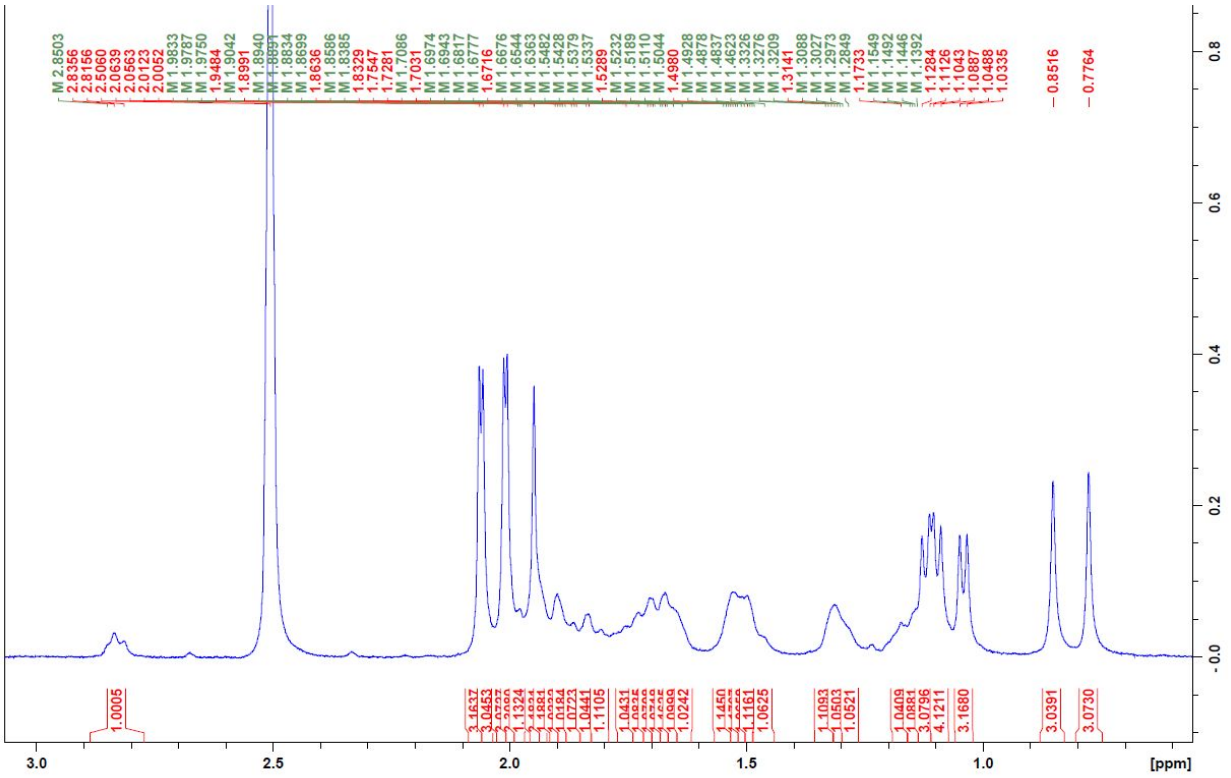


Figure S4b. [1]H NMR spectrum of (+)-12,3'a,3'b,3'c,4'c-penta-*O*-acetyldigoxin (**4**).

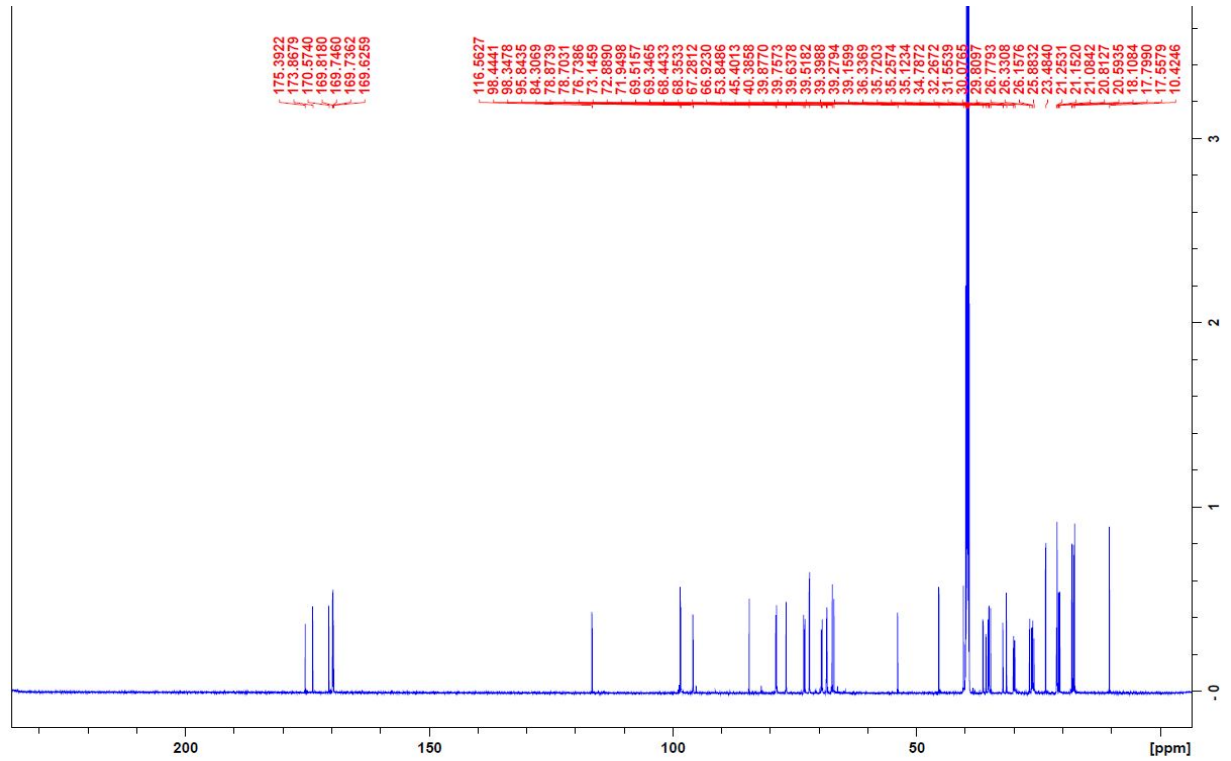


Figure S4c. [13]C NMR spectrum of (+)-12,3'a,3'b,3'c,4'c-penta-*O*-acetyldigoxin (**4**).

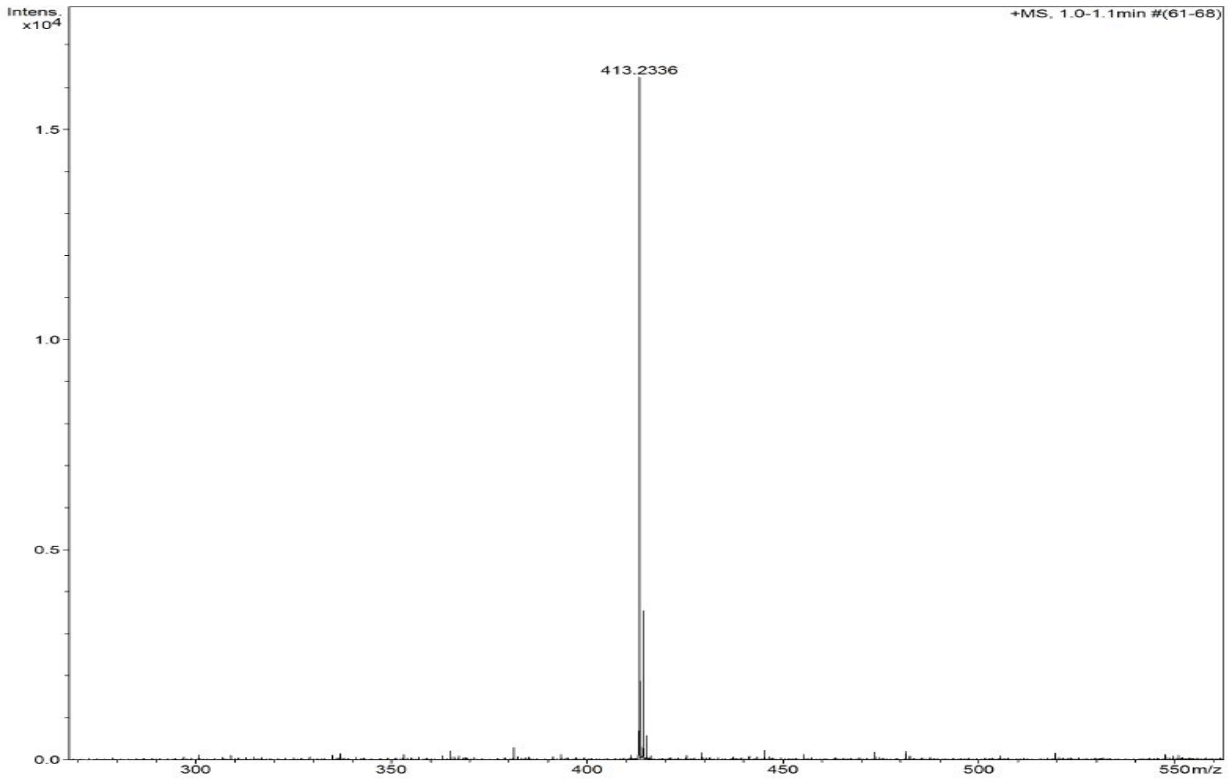


Figure S5a. Mass spectrum of (+)-digoxigenin (**5**).

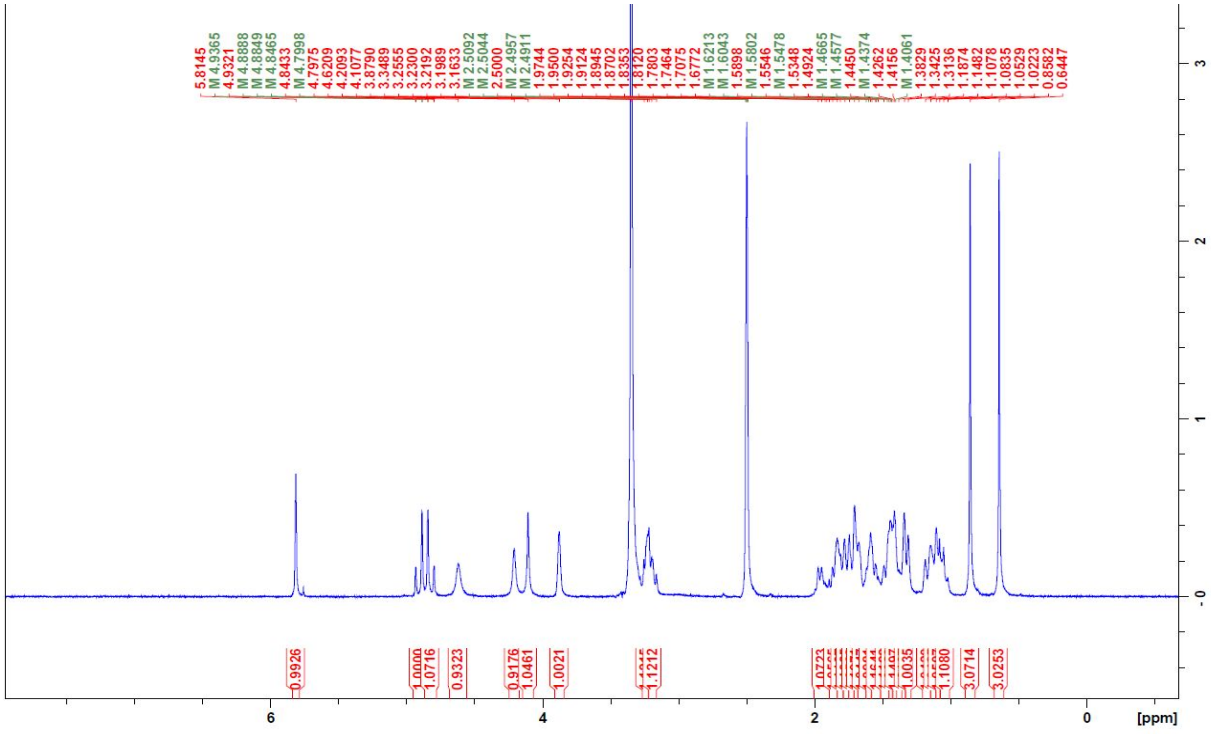


Figure S5b. ^{1}H NMR spectrum of (+)-digoxigenin (**5**).

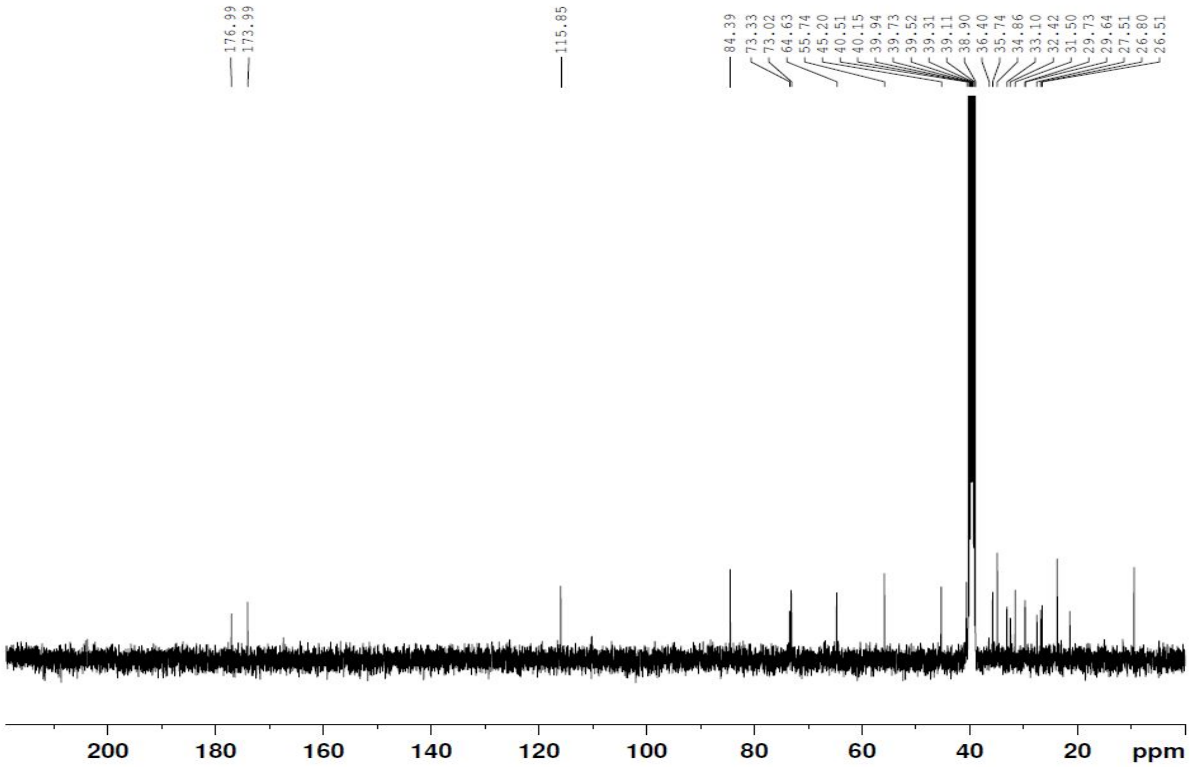


Figure S5c. ^{13}C NMR spectrum of (+)-digoxigenin (**5**).

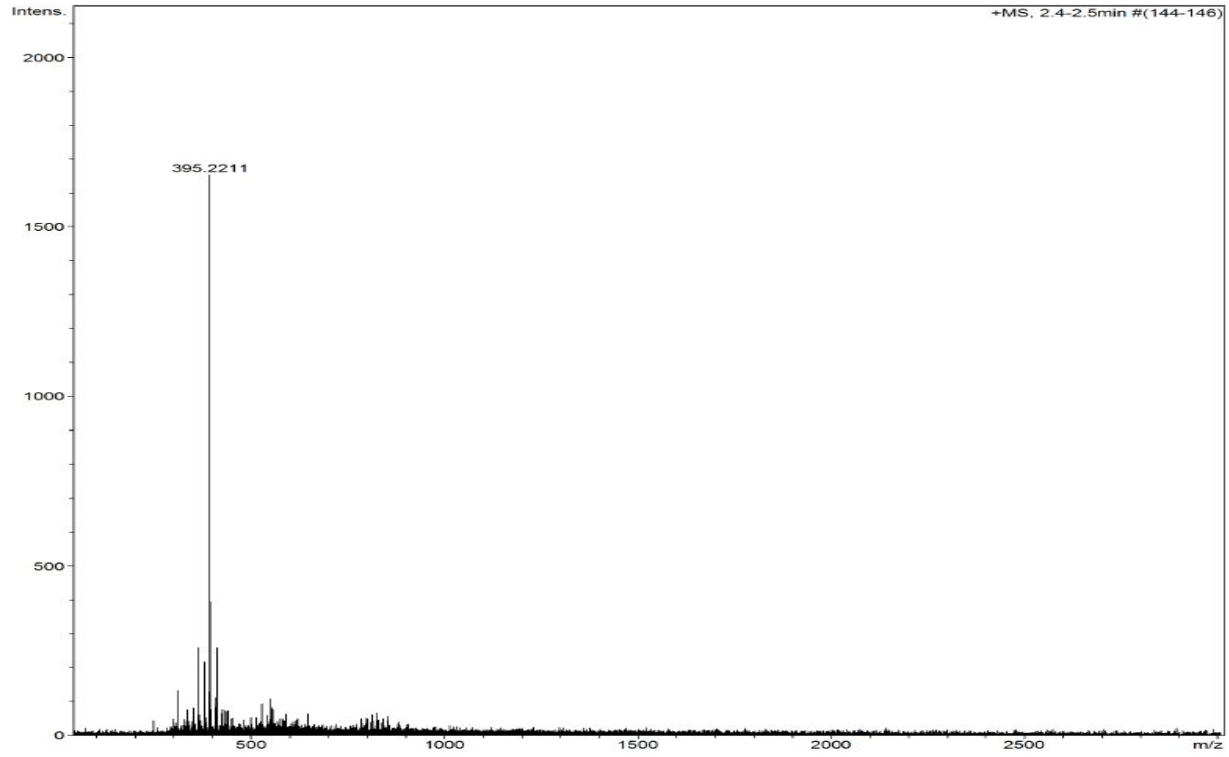


Figure S6a. Mass spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

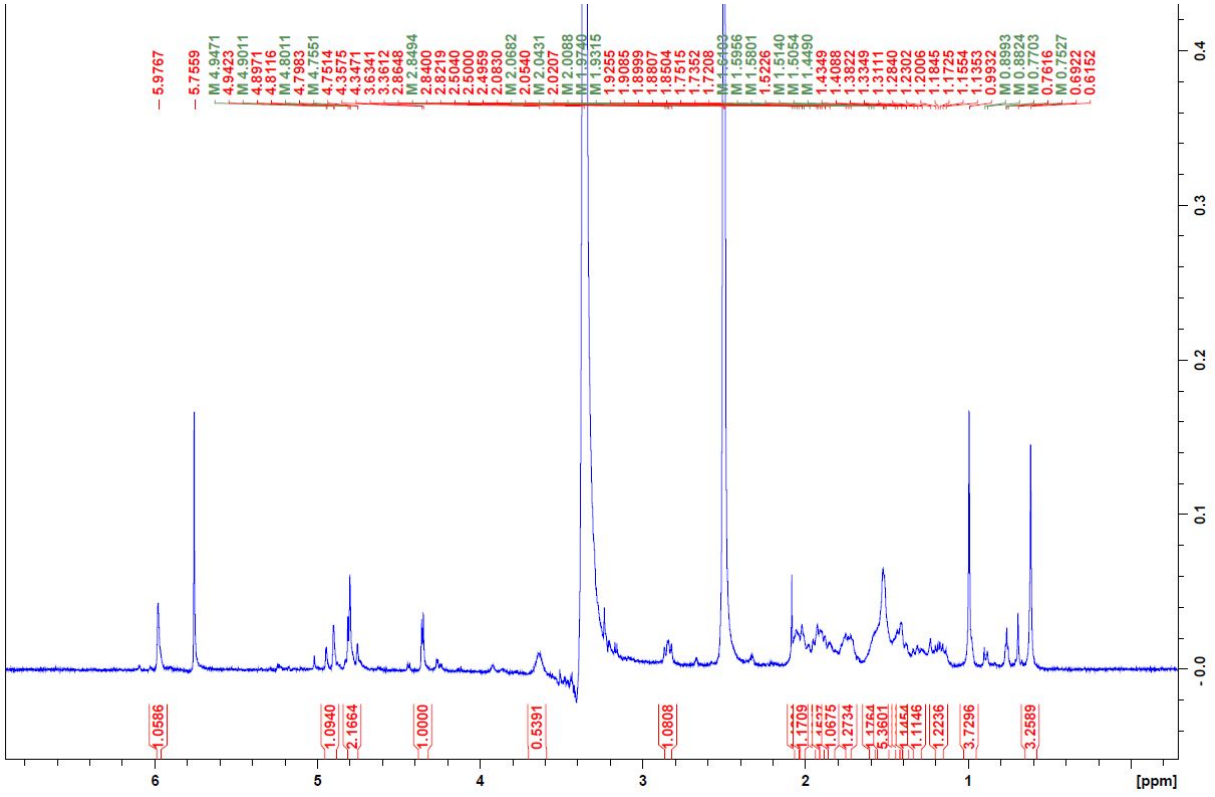


Figure S6b. [1]H NMR spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

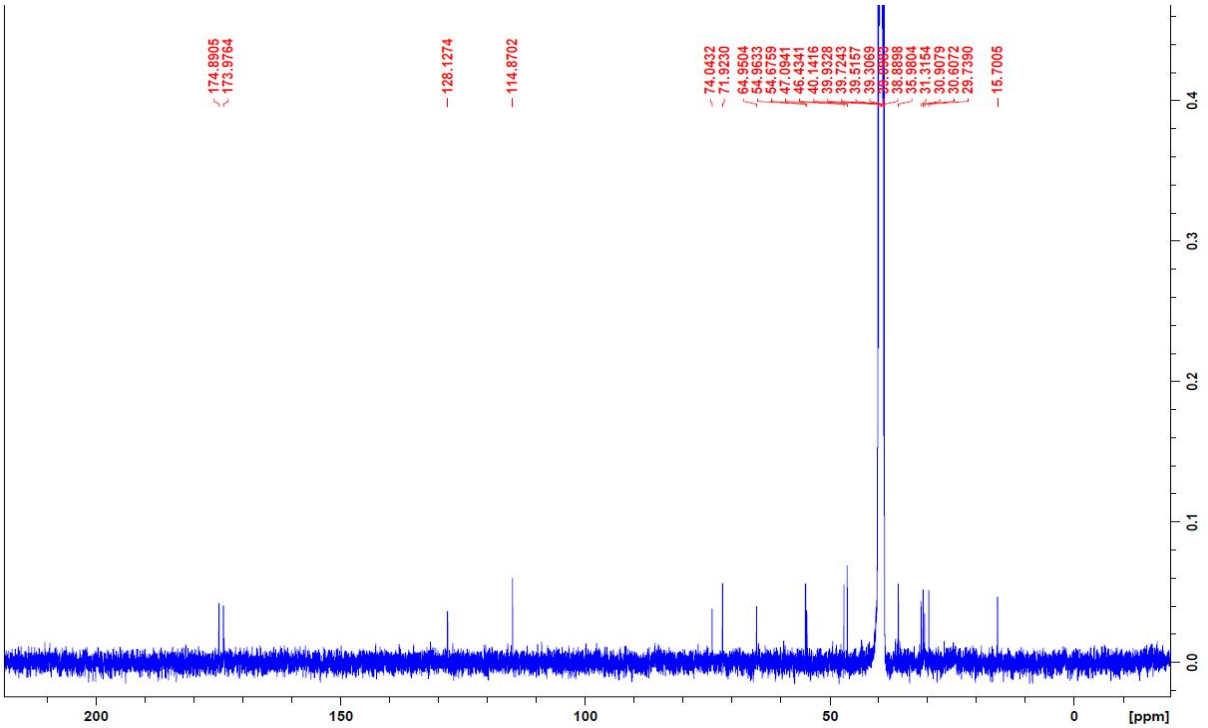


Figure S6c. [13]C NMR spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

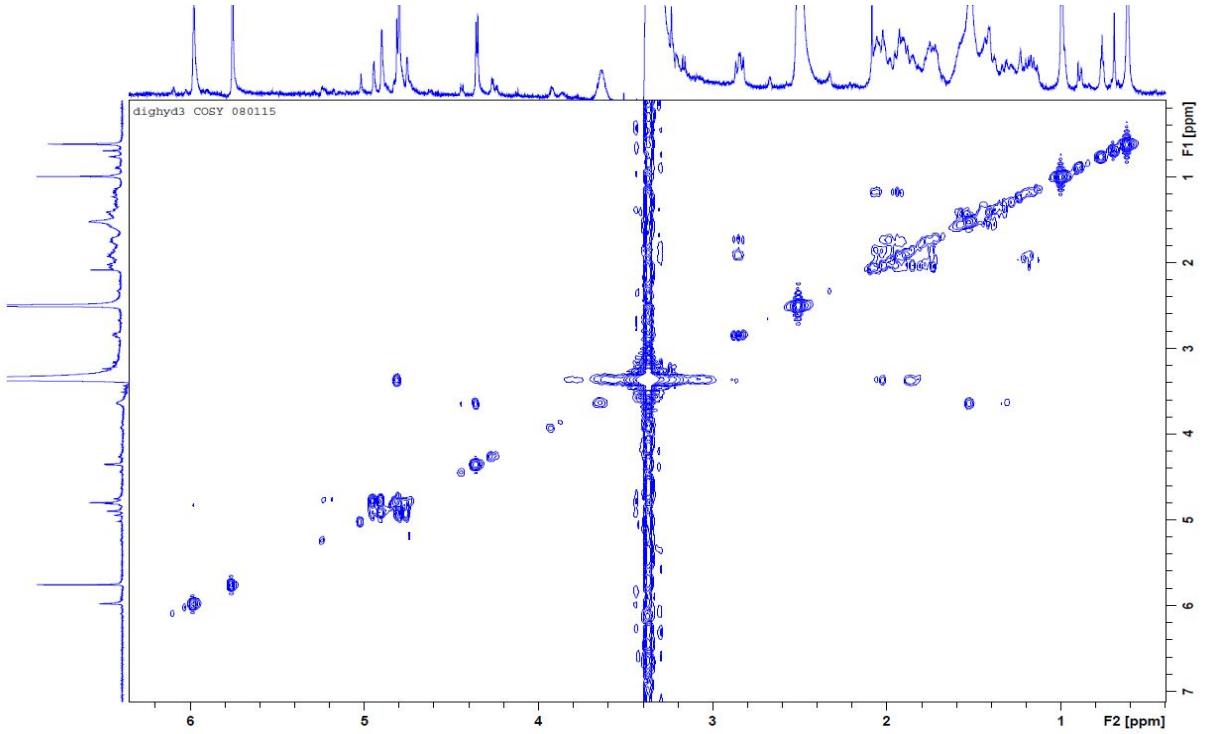


Figure S6d. COSY NMR spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

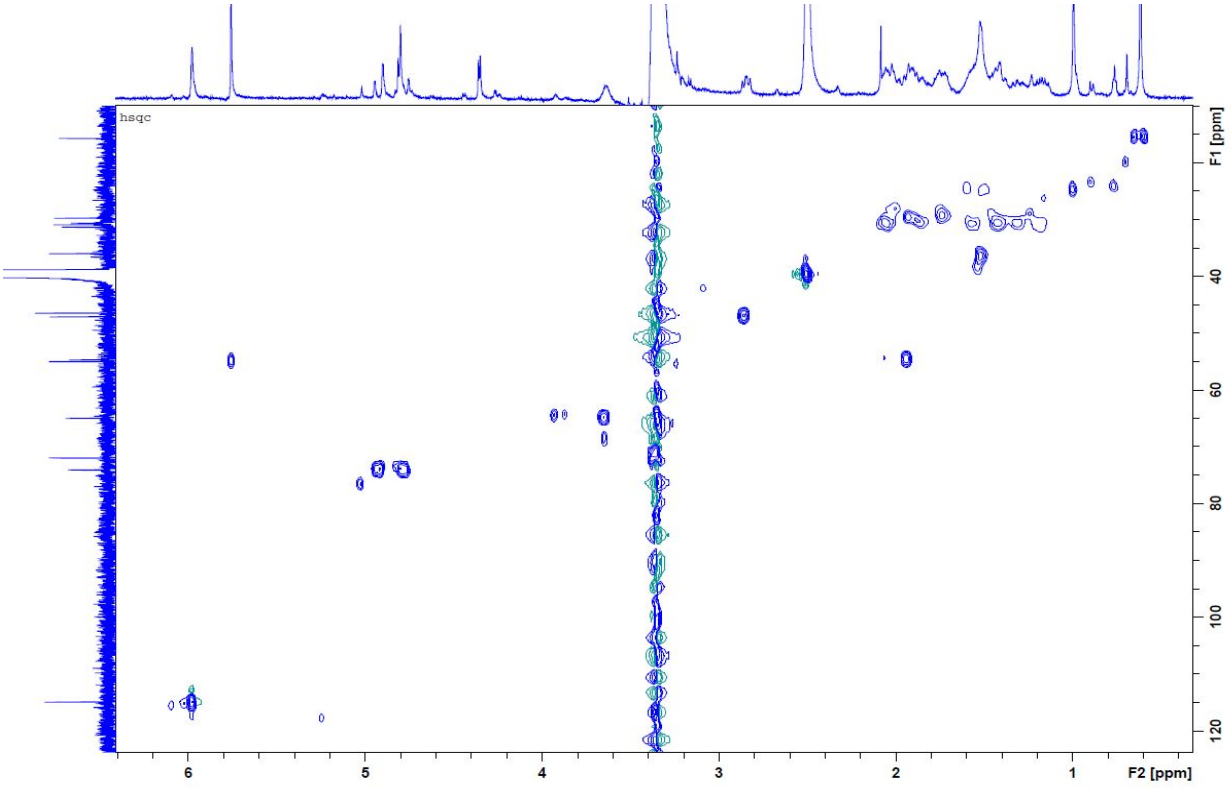


Figure S6e. HSQC spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

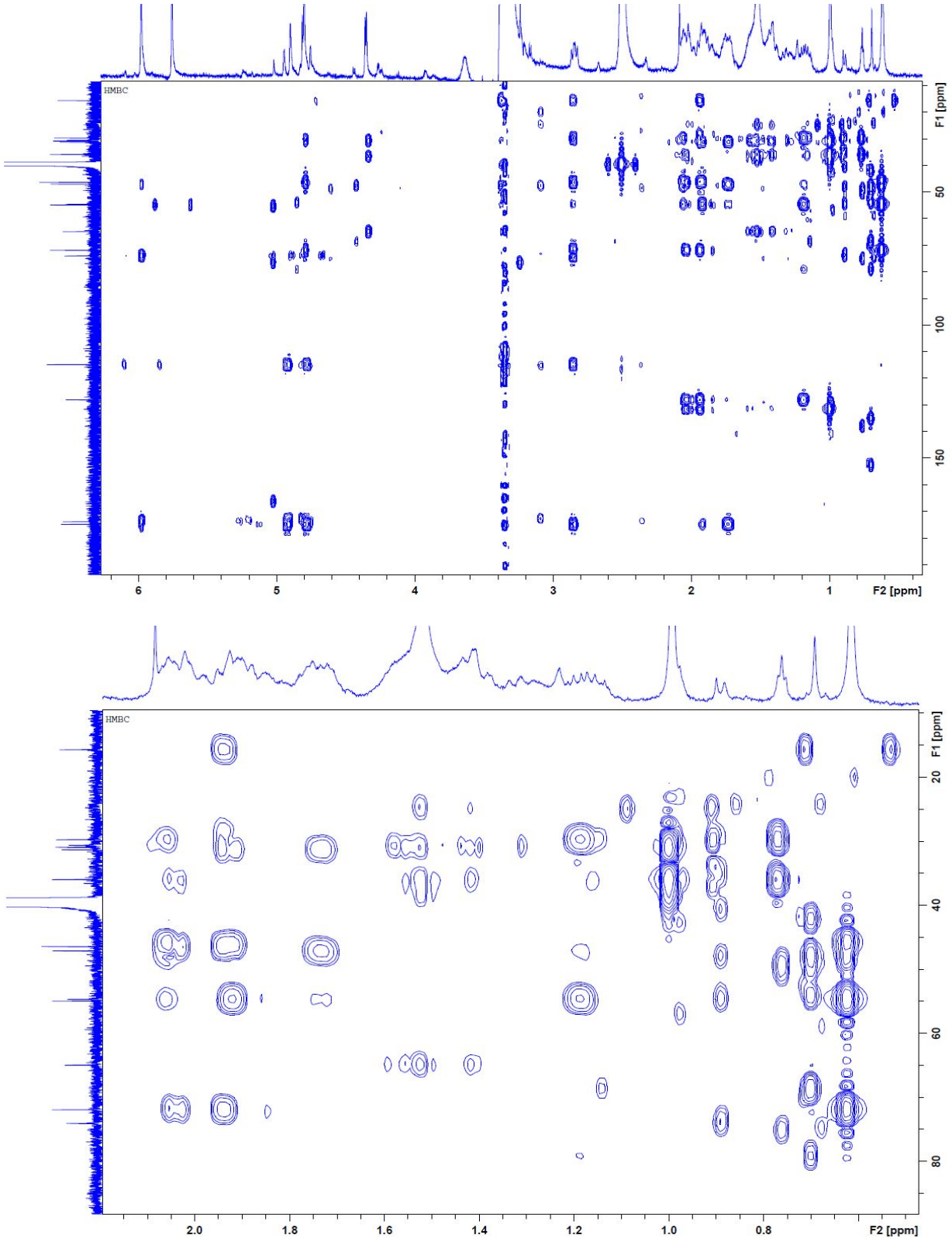


Figure S6f. HMBC NMR spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

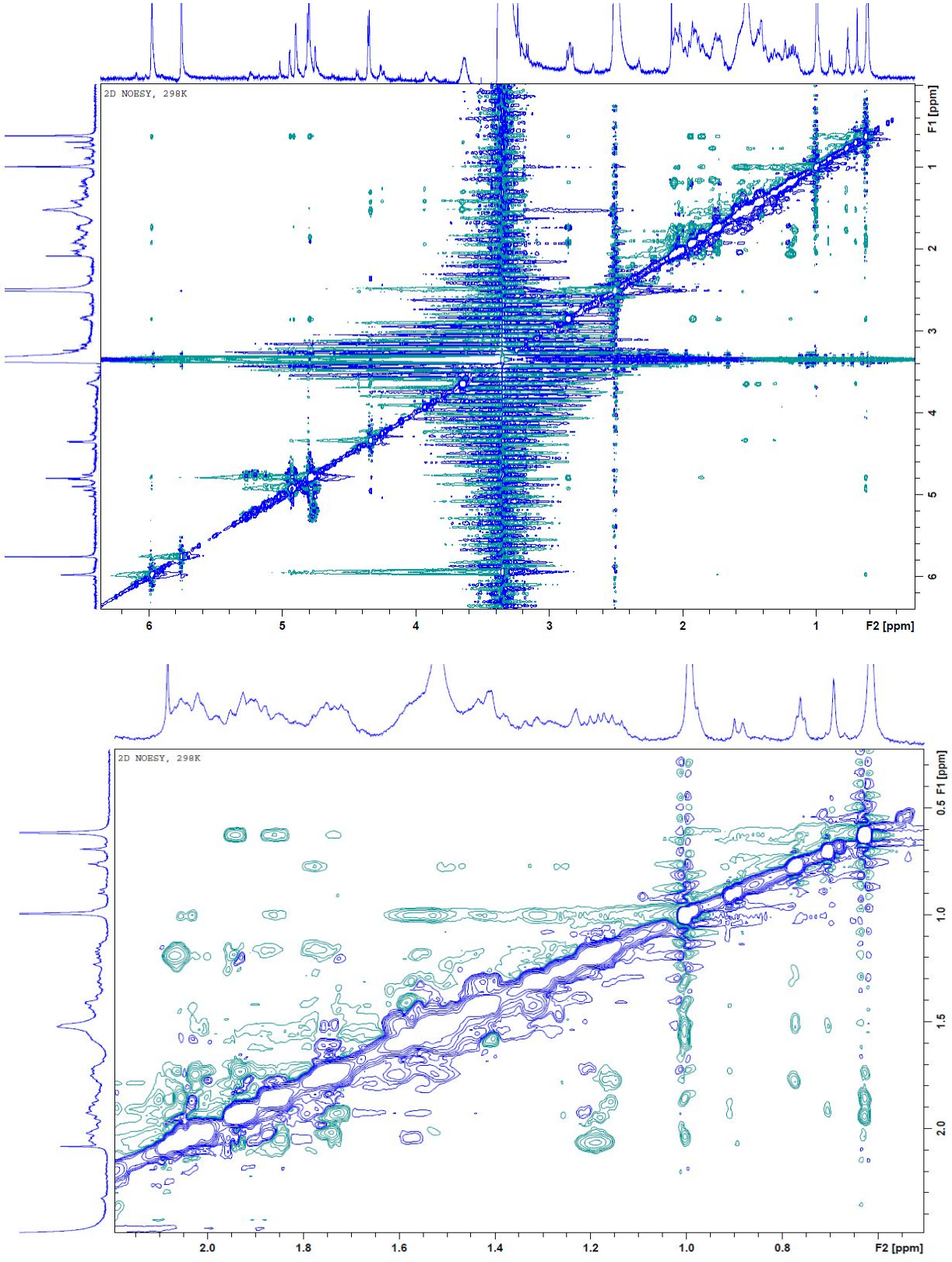


Figure S6g. NOESY NMR spectrum of (+)-8(9)-β-anhydrodigoxigenin (**6**).

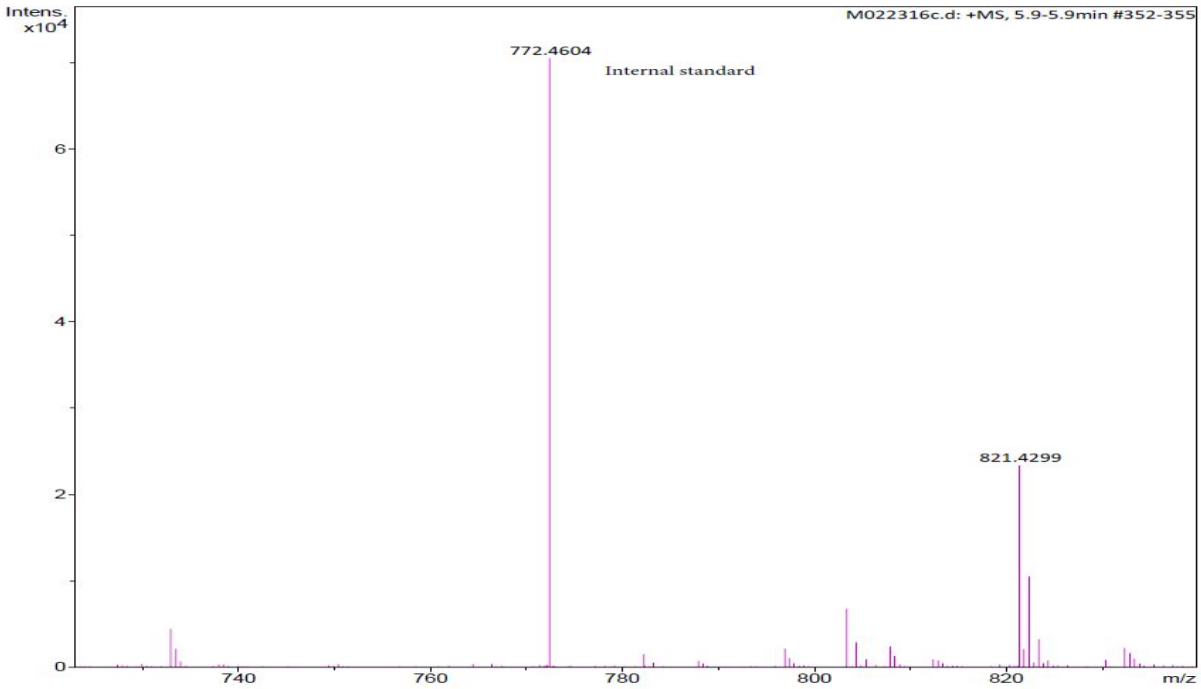
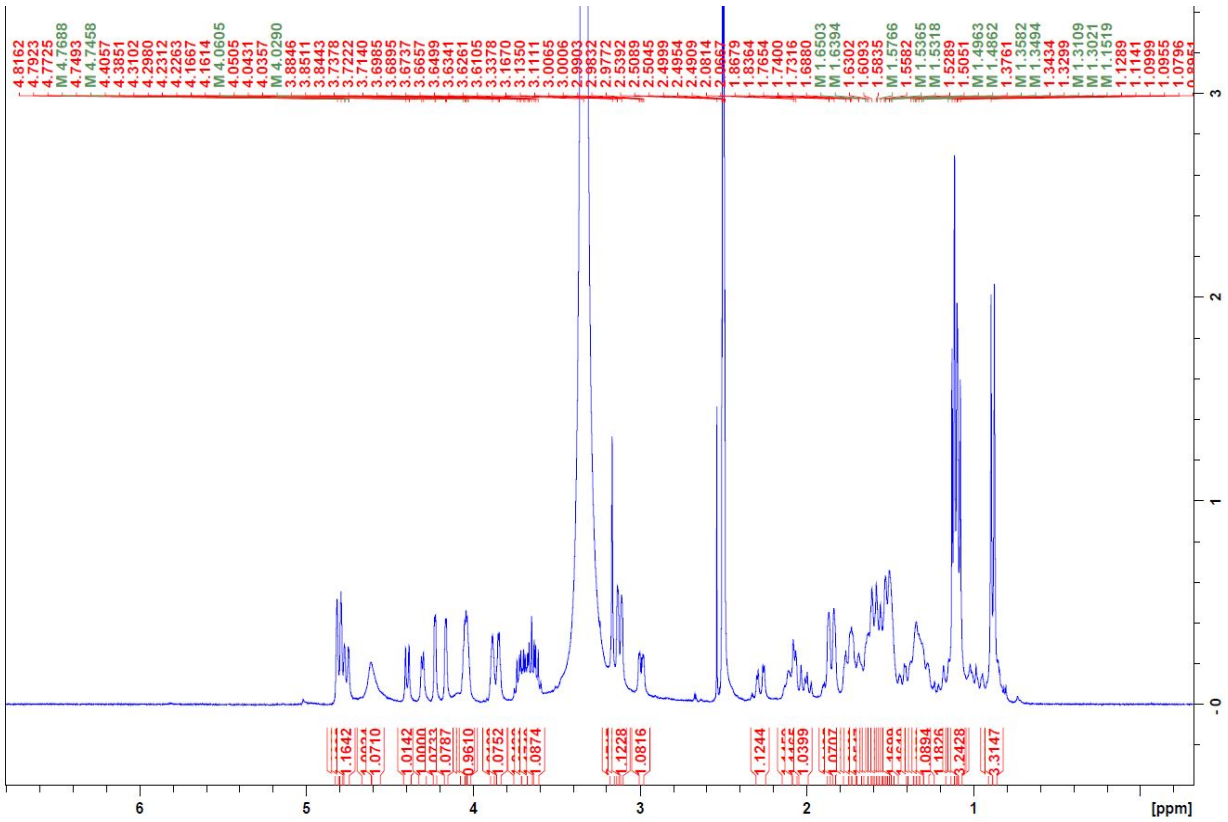


Figure S7a. Mass spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).



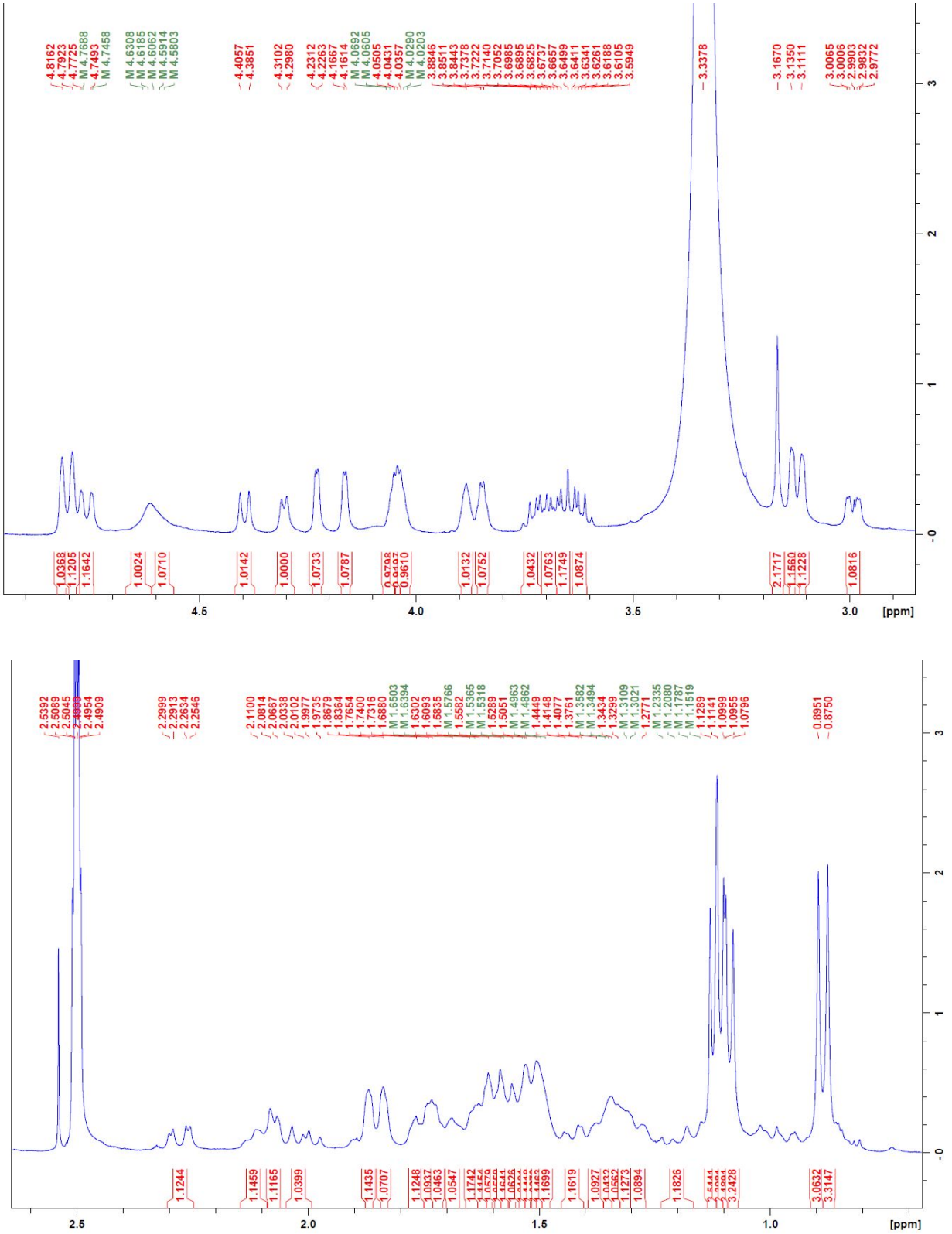


Figure S7b. ^{1}H NMR spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).

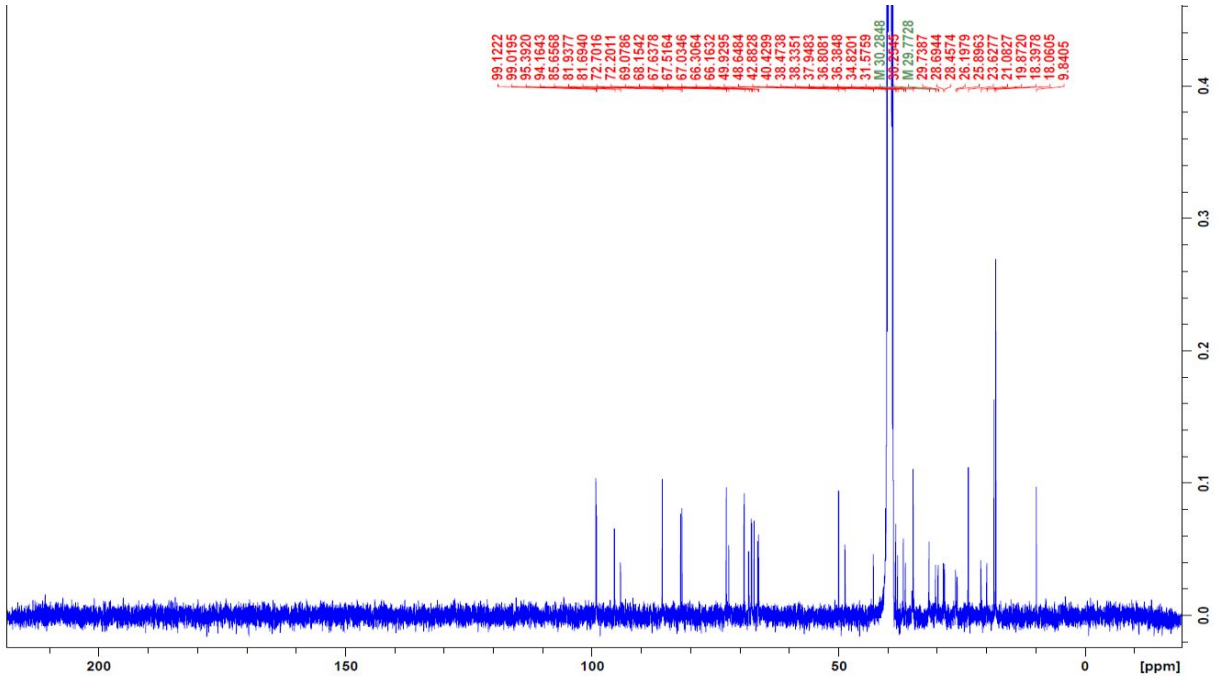


Figure S7c. ^{13}C NMR spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).

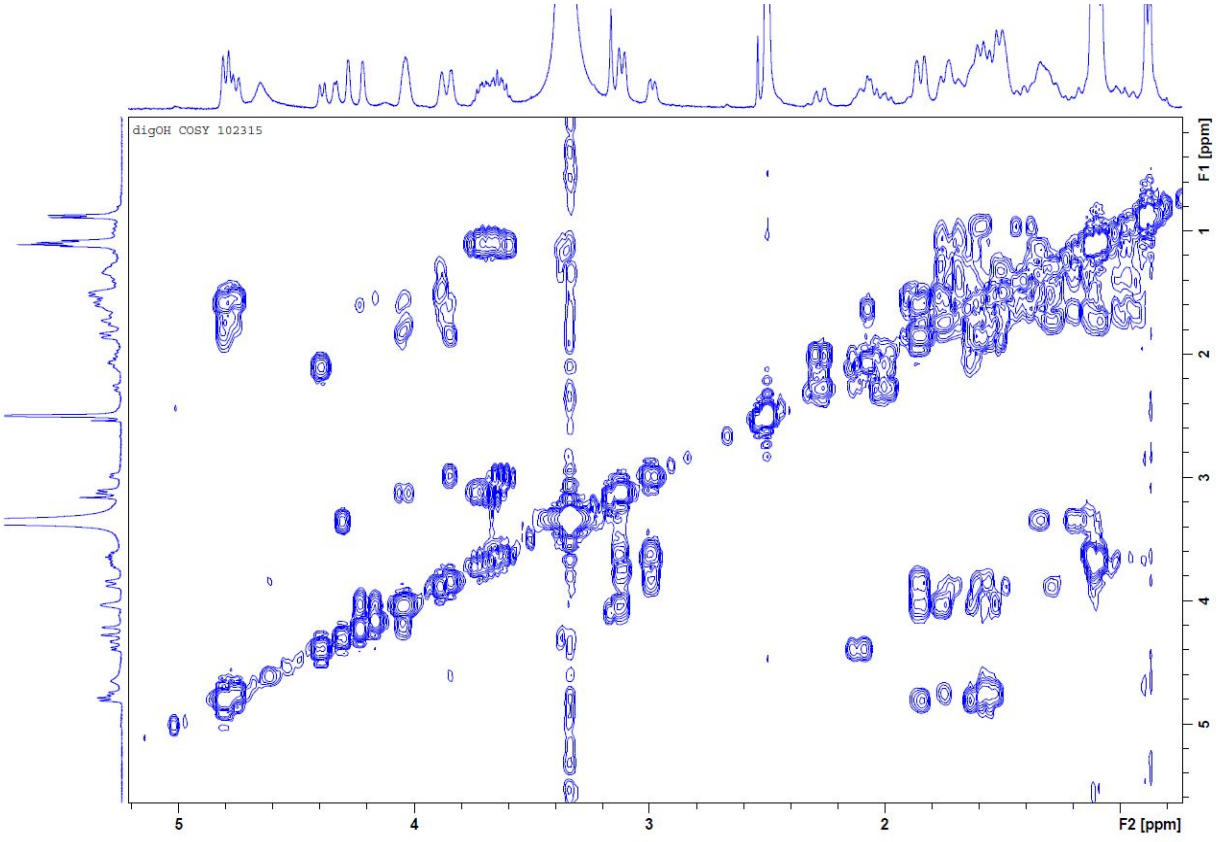


Figure S7d. COSY NMR spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).

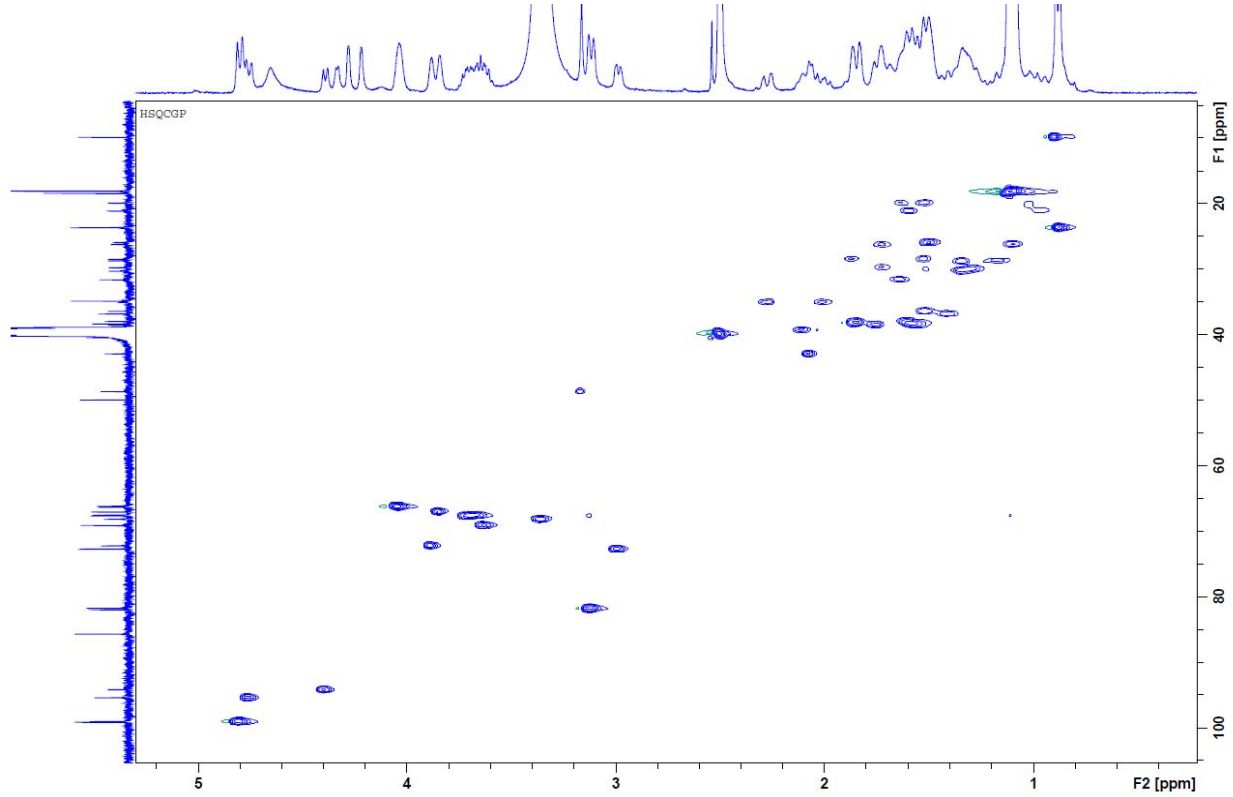


Figure S7e. HSQC NMR spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).

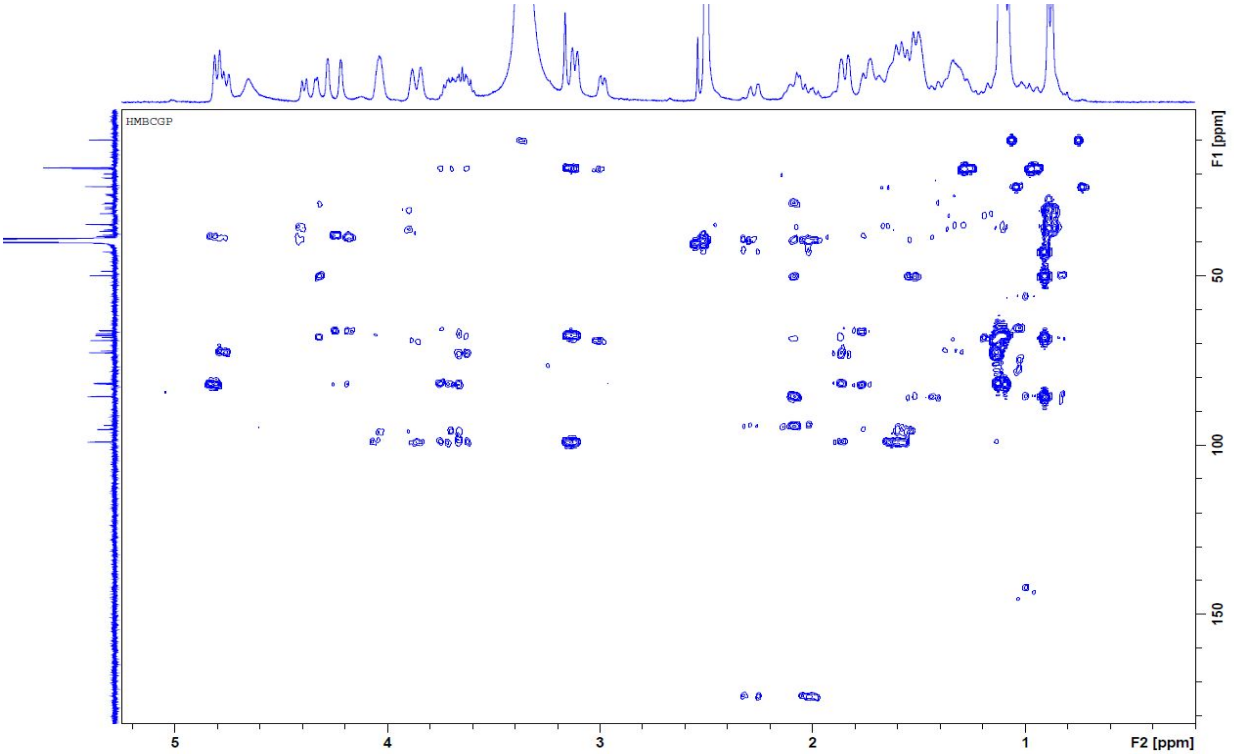


Figure S7f. HMBC NMR spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).

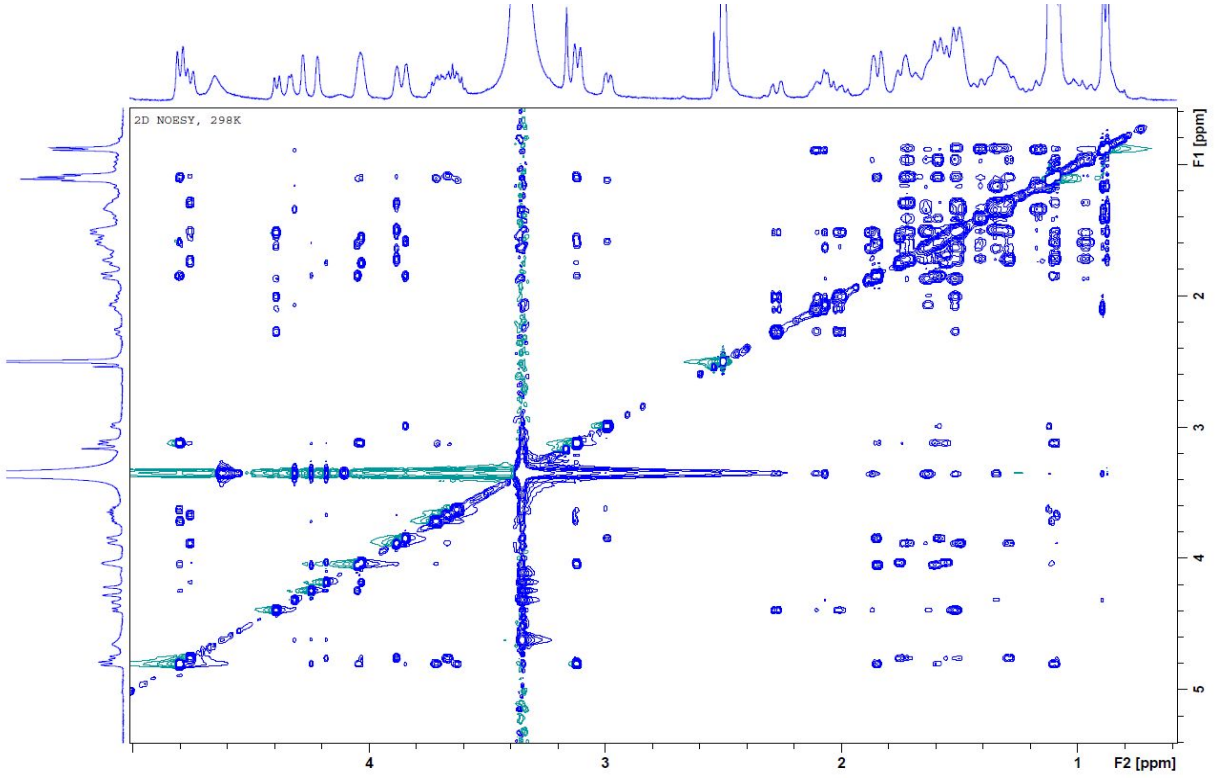


Figure S7g. NOESY NMR spectrum of (+)-17-*epi*-20,22-dihydro-21α-hydroxydigoxin (**7**).

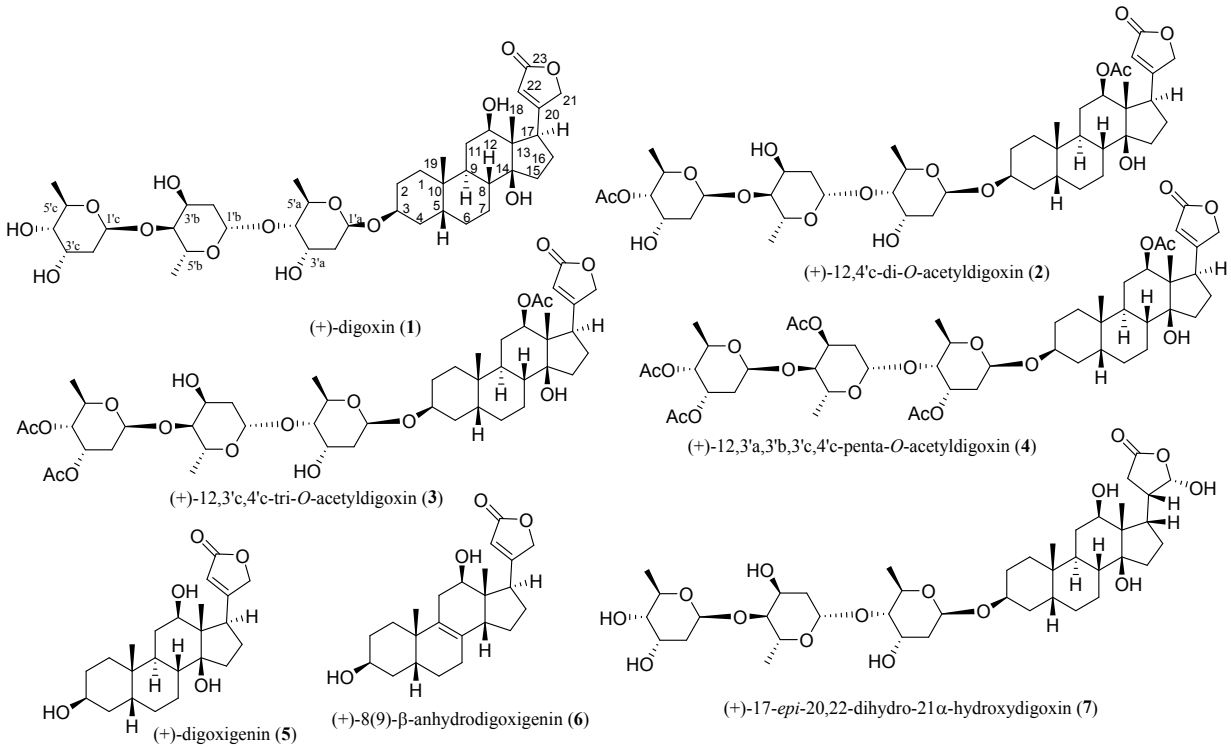


Figure S8. Structures of digoxin (**1**) and its synthetic derivatives **1**–**7**.

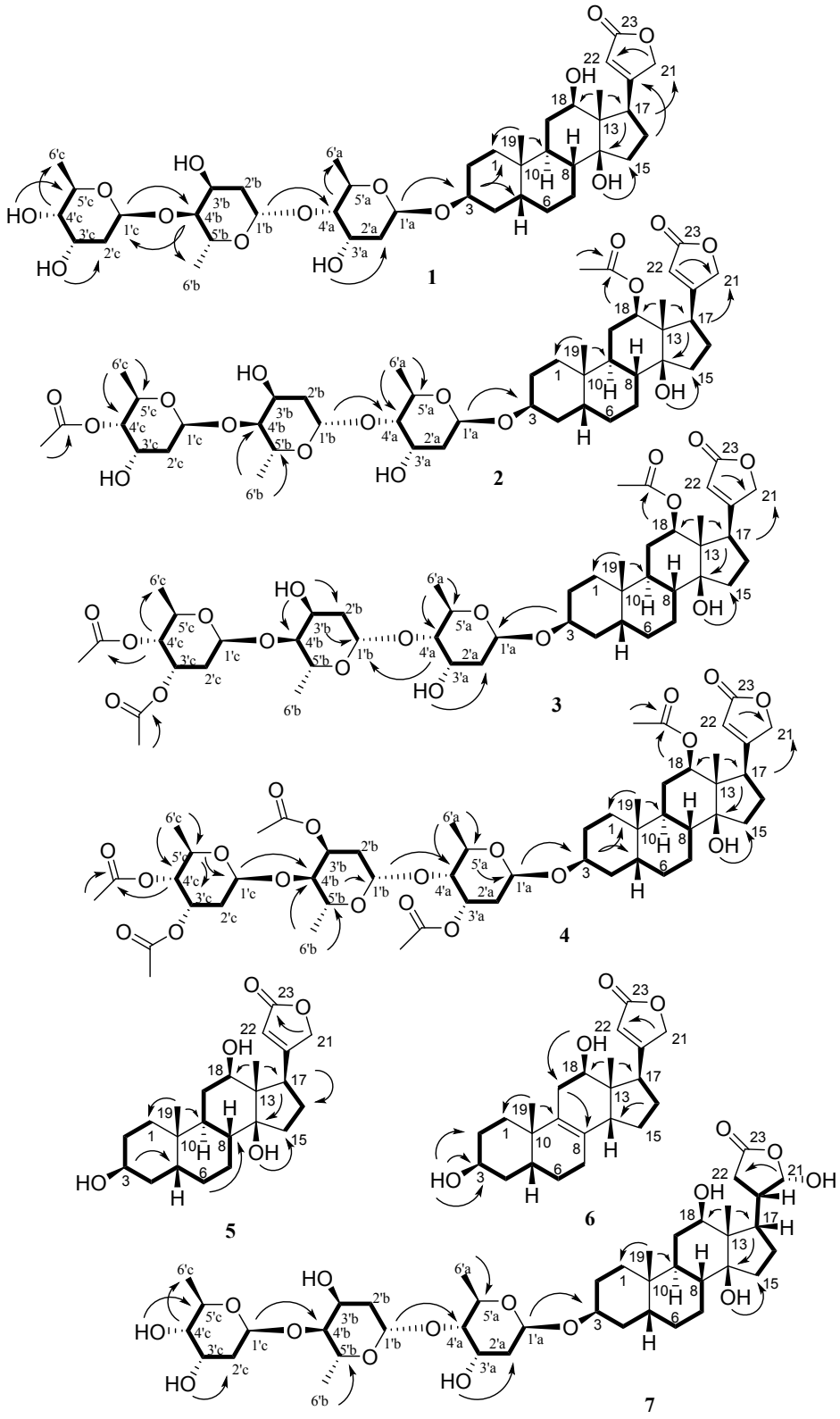


Figure S9. COSY and key HMBC correlations of the isolates **1**–**7**.

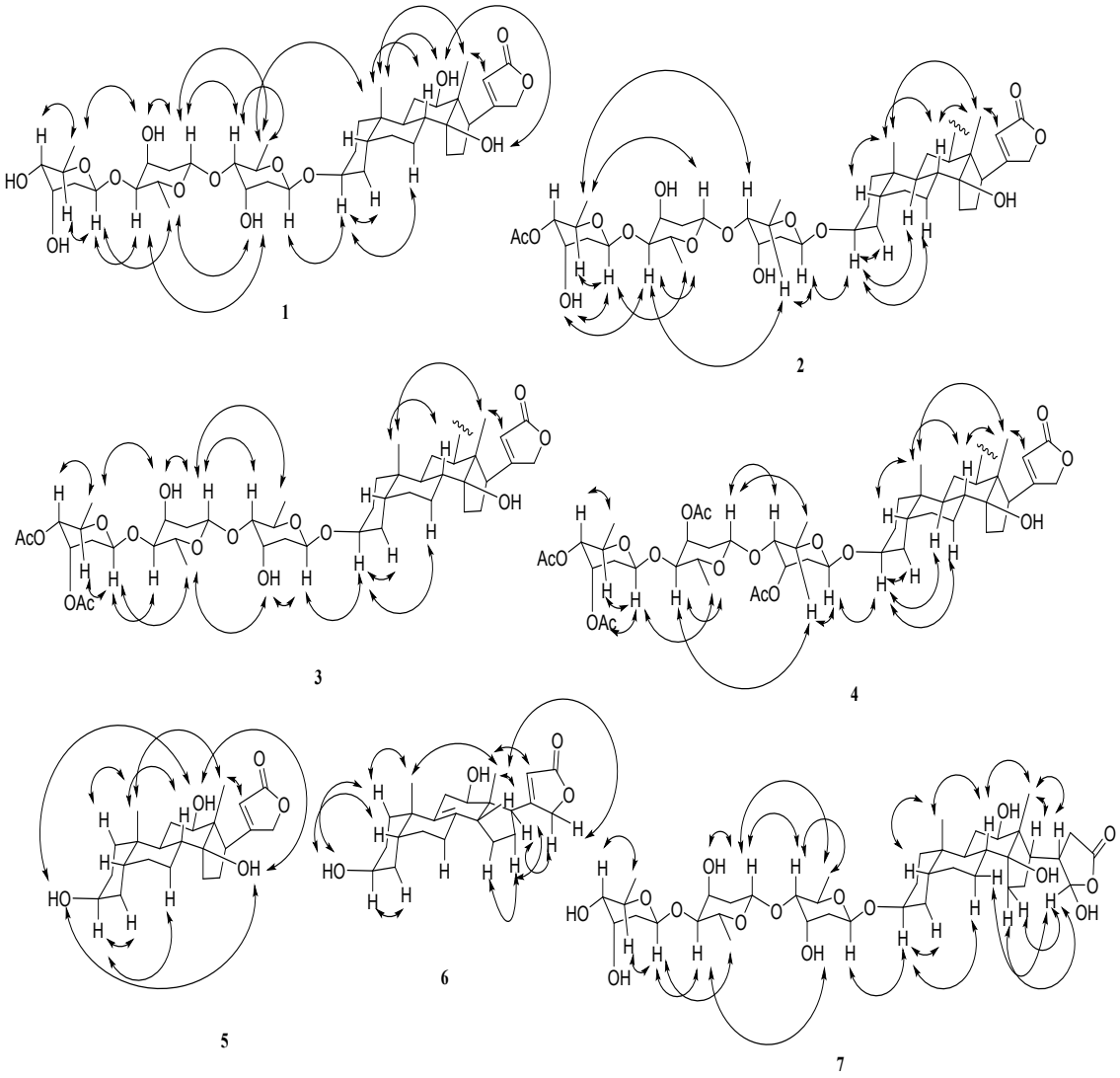


Figure S10. Selected NOESY correlations of **1**–**7**.

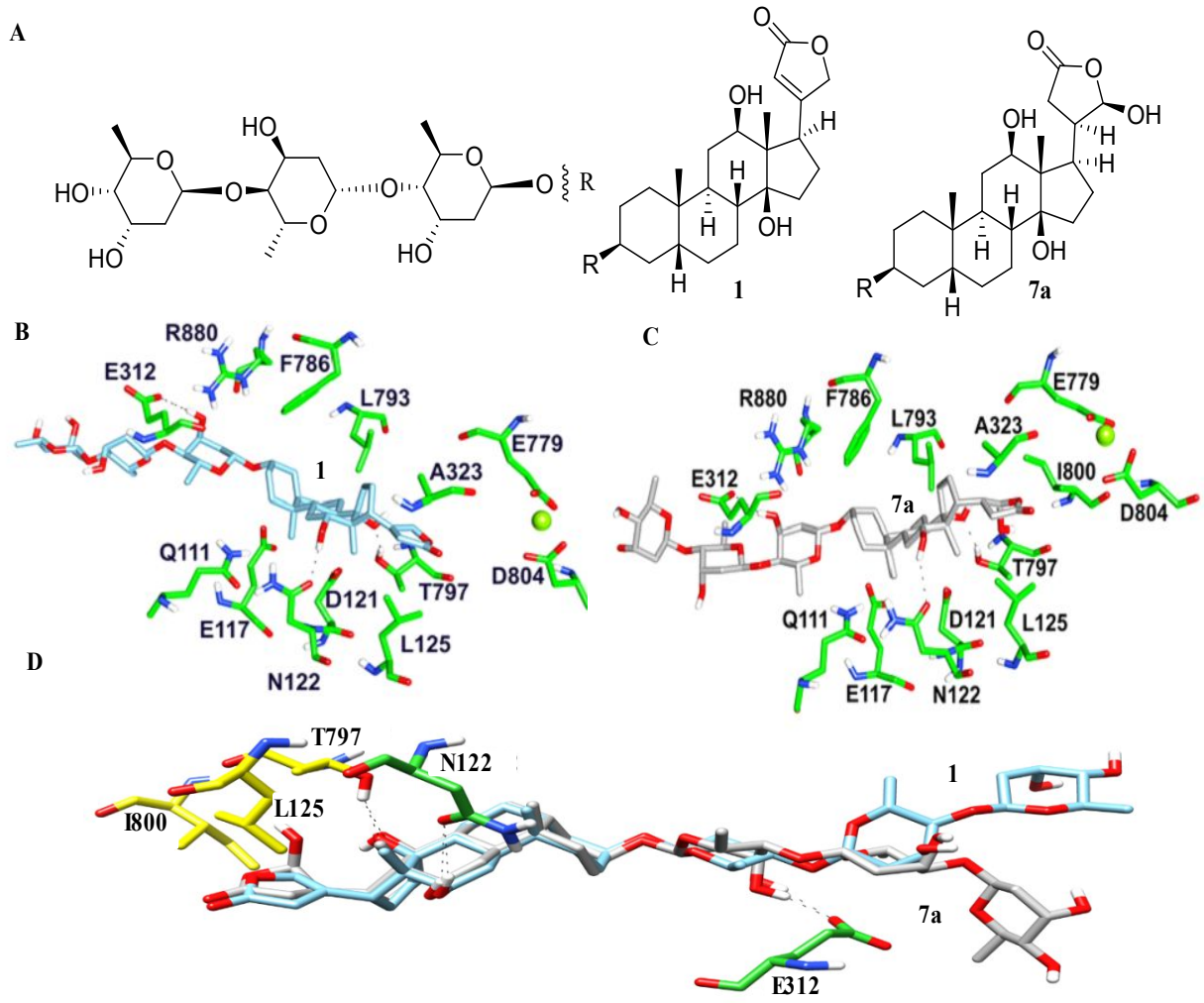


Figure S11. Structures of **1** and 20,22-dihydro-21β-hydroxydigoxin (**7a**) (A). Docking profiles for **1** (cyan) (B) and **7a** (gray) (C) and Na^+/K^+-ATPase. The overlaid docking profile of (+)-digoxin (**1**, cyan) in the Na^+/K^+-ATPase crystal complex and the docking result for **7a** (gray) shown by using Chimera1.10.2 (residues within 5 Å around OH-21 of **7a** are colored in yellow) (D).

Table S1. ^{1}H NMR Spectroscopic Data of **2**–**6**[a]

position	**2**	**3**	**4**	**5**	**6**
1	α 1.29 m	α 1.31 m	α 1.33 m	α 1.34 m	α 1.41 m
	β 1.34 m	β 1.31 m	β 1.33 m	β 1.46 m	β 1.52 m
2	α 1.51 m	α 1.50 m	α 1.53 m	α 1.59 m	α 1.52 m
	β 1.51 m	β 1.50 m	β 1.50 m	β 1.31 m	β 1.33 m
3	α 3.91 br s	α 3.89 br s	α 3.91 br s	α 3.88 m	α 3.63 m
4	α 1.77 m	α 1.74 m	α 1.75 m	α 1.81 m	α 1.52 m
	β 1.32 m	β 1.33 m	β 1.30 m	β 1.19 m	β 1.52 m
5	β 1.55 m	β 1.52 m	β 1.55 m	β 1.71 m	β 1.52 m
6	α 1.17 m	α 1.17 m	α 1.17 m	α 1.15 m	α 1.45 m
	β 1.74 m	β 1.76 m	β 1.73 m	β 1.78 m	β 1.58 m
7	α 1.06 m	α 1.10 m	α 1.11 m	α 1.11 m	α 1.38 m
	β 1.73 m	β 1.67 m	β 1.71 m	β 1.75 m	β 1.61 m
8	β 1.49 m	β 1.49 m	β 1.48 m	β 1.43 m	
9	α 1.64 m	α 1.61 m	α 1.64 m	α 1.60 m	
11	α 1.52 m	α 1.51 m	α 1.53 m	α 1.49 m	α 2.02 m
	β 1.15 m	β 1.16 m	β 1.15 m	β 1.08 m	β 1.90 m
12	α 4.55 m	α 4.54 m	α 4.55 m	α 3.22 m	α 3.35 m
14					β 1.93 m
15	α 1.95 m	α 1.94 m	α 1.95 m	α 1.89 m	α 2.07 m
	β 1.70 m	β 1.64 m	β 1.67 m	β 1.68 m	β 1.20 m
16	α 1.97 m	α 1.95 m	α 1.98 m	α 1.97 m	α 1.91 m
	β 1.90 m	β 1.90 m	β 1.88 m	β 1.84 m	β 1.75 m
17	α 2.85 m	α 2.85 m	α 2.85 m	α 3.26 m	α 2.86 m
18	β 0.77 s	β 0.77 s	β 0.78 s	β 0.64 s	β 0.62 s
19	β 0.85 s	β 0.85 s	β 0.85 s	β 0.86 s	β 0.99 s
21	β 4.82	β 4.86	β 4.90 d	β 4.85 dd	β 4.80 dd
	overlapped	overlapped	(18.4)	(19.1, 1.3)	(18.4, 1.5)
	α 4.90	α 4.90	α 4.95 d	α 4.94 dd	α 4.94 dd
	overlapped	overlapped	(19.0)	(19.1, 1.8)	(18.4, 1.9)
22	5.91 br s	5.91 br s	5.91 br s	5.81 br s	5.98 br s
1'a	α 4.77 m	α 4.77 m	α 4.70 m		
2'a	β 1.58 m	β 1.55 m	β 1.70 m		
	α 1.75 m	α 1.67 m	α 1.86 m		
3'a	β 4.07 m	β 4.04 m	β 5.19 m		
4'a	β 3.14 m	β 3.12 m	β 3.37 m		
5'a	α 3.69 m	α 3.70 m	α 3.75 m		
6'a	β 1.08 d (6.9)	β 1.10 d (6.4)	β 1.13 d (6.3)		
1'b	β 4.86 m	β 4.83 m	β 4.74 m		
2'b	β 1.86 m	β 1.85 m	β 1.90 m		
	α 1.67 m	α 1.76 m	α 1.68 m		
3'b	α 4.05 m	α 4.09 m	α 5.19 m		
4'b	α 3.16 m	α 3.21 m	α 3.39 m		
5'b	β 3.75 m	β 3.77 m	β 3.75 m		
6'b	α 1.12 d (6.0)	α 1.11 d (6.3)	α 1.10 d (6.4)		
1'c	α 4.88 m	α 4.88 m	α 4.88 m		
2'c	β 1.60 m	β 1.88 m	β 1.84 m		
	α 1.84 m	α 1.97 m	α 1.94 m		
3'c	β 4.04 m	β 5.29 m	β 5.25 m		
4'c	β 4.24 m	β 4.43 m	β 4.40 m		
5'c	α 3.94 m	α 3.91 m	α 3.86 m		
6'c	β 1.10 d (7.2)	β 1.11 d (6.3)	β 1.05 d (6.1)		
3-OH				β 4.21 br s	β 4.36 br s

12-OH				β 4.62 br s	β 4.81 br s
14-OH	β 4.40 s	β 4.41 s	β 4.41 s	β 4.11 s	
21-OH					
3'a-OH	α 4.21 s	α 4.22 s			
3'b-OH	β 4.36 s	β 4.35 s			
3'c-OH	α 5.14 s				
4'c-OH					
12-OAc	2.05 s	2.05 s	2.06 s		
3'a-OAc			2.01 s		
3'b-OAc			2.01 s		
3'c-OAc		1.95 s	1.95 s		
4'c-OAc	2.03 s	2.07 s	2.06 s		

[a]Assignments of chemical shifts are based on the analysis of 1D- and 2D-NMR spectra. The overlapped signals were assigned from $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectra without designating multiplicity. Data (δ) were measured in DMSO-d_6 at 400.13 MHz and referenced to the solvent residual peak at δ 2.50.[31]

Table S2. ^{13}C NMR Spectroscopic Data of **2**–**6**[a]

position	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]
1	30.27 CH_2	30.07 CH_2	30.08 CH_2	29.64 CH_2	30.91 CH_2
2	26.17 CH_2	25.89 CH_2	25.88 CH_2	27.51 CH_2	31.32 CH_2
3	72.24 CH	71.95 CH	72.89 CH	64.63 CH	64.85 CH
4	29.67 CH_2	29.43 CH_2	29.81 CH_2	33.10 CH_2	36.67 CH_2
5	36.53 CH	36.27 CH	36.34 CH	35.74 CH	35.98 CH
6	26.55 CH_2	26.12 CH_2	26.33 CH_2	26.51 CH_2	24.85 CH_2
7	21.47 CH_2	21.25 CH_2	21.25 CH_2	21.38 CH_2	29.74 CH_2
8	40.59 CH	40.34 CH	40.39 CH	40.51 CH	128.13 C
9	31.77 CH	31.49 CH	31.55 CH	31.50 CH	[c]131.57 C
10	35.00 C	34.77 C	34.79 C	34.86 C	35.98 C
11	26.32 CH_2	26.74 CH_2	26.16 CH_2	29.73 CH_2	30.61 CH_2
12	76.97 CH	76.69 CH	76.74 CH	73.02 CH	71.92 CH
13	54.04 C	53.80 C	53.85 C	55.74 C	46.43 C
14	84.46 C	84.25 C	84.31 C	84.39 C	54.68 CH
15	32.38 CH_2	32.22 CH_2	32.27 CH_2	32.42 CH_2	31.32 CH_2
16	26.99 CH_2	26.32 CH_2	26.78 CH_2	26.80 CH_2	29.74 CH_2
17	45.65 CH	45.36 CH	45.40 CH	45.20 CH	47.09 CH
18	10.48 CH_3	10.39 CH_3	10.42 CH_3	9.46 CH_3	15.70 CH_3
19	23.57 CH_3	23.46 CH_3	23.48 CH_3	23.75 CH_3	[c]24.85 CH_3
20	175.52 C	175.32 C	175.39 C	176.99 C	174.89 C
21	73.34 CH_2	73.08 CH_2	73.15 CH_2	73.33 CH_2	74.04 CH_2
22	116.77 CH	116.52 CH	116.56 CH	115.85 CH	114.87 CH
23	174.08 C	173.78 C	173.87 C	173.99 C	173.98 C
1'a	95.46 CH	95.20 CH	95.84 CH		
2'a	38.45 CH_2	38.41 CH_2	35.72 CH_2		
3'a	66.46 CH	66.25 CH	69.35 CH		
4'a	82.09 CH	81.91 CH	78.87 CH		
5'a	67.76 CH	67.48 CH	68.44 CH		
6'a	17.95 CH_3	17.80 CH_3	18.11 CH_3		
1'b	99.16 CH	98.82 CH	98.35 CH		
2'b	38.05 CH_2	37.95 CH_2	35.26 CH_2		
3'b	66.38 CH	66.05 CH	69.52 CH		
4'b	82.19 CH	81.68 CH	78.70 CH		
5'b	67.84 CH	67.50 CH	68.35 CH		
6'b	18.09 CH_3	17.99 CH_3	17.80 CH_3		
1'c	99.36 CH	98.92 CH	98.44 CH		
2'c	38.26 CH_2	35.19 CH_2	35.12 CH_2		

3'c	64.04 CH	66.83 CH	66.92 CH
4'c	75.34 CH	71.98 CH	71.95 CH
5'c	66.81 CH	67.29 CH	67.28 CH
6'c	18.06 CH_3	18.00 CH_3	17.56 CH_3
12-OAc	170.73 C	170.49 C	170.57 C
	21.04 CH_3	21.05 CH_3	21.08 CH_3
3'a-OAc			169.82 C
			21.15 CH_3
3'b-OAc			169.74/75 C
			21.15 CH_3
3'c-OAc		169.58 C	169.74/75 C
		20.56 CH_3	20.59 CH_3
4'c-OAc	170.23 C	169.75 C	169.63 C
	20.98 CH_3	20.79 CH_3	20.81 CH_3

[a]Assignments of chemical shifts are based on the analysis of 1D- and 2D-NMR spectra. CH_3, CH_2, CH, and C multiplicities were determined by DEPT 90, DEPT 135, and HSQC experiments.
[a]Data (δ) were measured in DMSO-d_6 at 176.02 MHz and referenced to the solvent residual peak at δ 39.52.[31]
[b]Data (δ) were measured in DMSO-d_6 at 100.61 MHz and referenced to the solvent residual peak at δ 39.52.[31]
[c]Data (δ) were shown in the 2D HSQC and HMBC NMR spectra.

Table S3. Cytotoxicity of **1**–**7**[a]

compound	HT-29[b]	MDA-MB-231[c]	OVCAR3[d]	MDA-MB-435[e]
1	0.28	0.31	0.10	0.17
2	5.1	8.2	2.5	5.4
3	12.4	12.8	6.2	15.6
4	25.2	14.9	8.2	22.8
5	3.6	3.2	2.4	0.9
6	>67.2	>67.2	>67.2	>67.2
7	>31.3	>31.3	>31.3	>31.3
Digitoxin[f]	0.068	0.48	0.12	0.043
Paclitaxel[g]	0.0008	0.0027	0.0033	0.0002

[a]IC_{50} values are the concentration (μM) required for 50% inhibition of cell viability for a given test compound with a 72 h treatment and were calculated using nonlinear regression analysis with measurements performed in triplicate and representative of three independent experiments, where the values generally agreed within 10%.
[b]IC_{50} value toward the HT-29 human colon cancer cell line.
[c]IC_{50} value toward the MDA-MB-231 human breast cancer cell line.
[d]IC_{50} value toward the OVCAR3 human ovarian cancer cell line.
[e]IC_{50} value toward the MDA-MB-435 human melanoma cell line.
[f]Data reported previously.[28]
[g]Positive control.

Analytical data of (+)-digoxin (**1**)

*(+)-Digoxin (**1**).* Amorphous colorless powder; $[\alpha]^{20}_D$ +23 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.30) nm; ECD (MeOH, nm) λ_{max} (Δε) 241.2 (+7.51); IR (dried film) ν_{max} 3445, 1740, 1623, 1590, 1441, 1369, 1068, 868 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive-ion HRESIMS *m*/*z* 803.4240, calcd for $C_{41}H_{64}O_{14}Na^+$, 803.4188.