Supplementary Information for:

Glycosylated 18β-Glycyrrhetinic Acid Derivatives as Promising Inhibitors of the SARS-CoV-2 Main Protease

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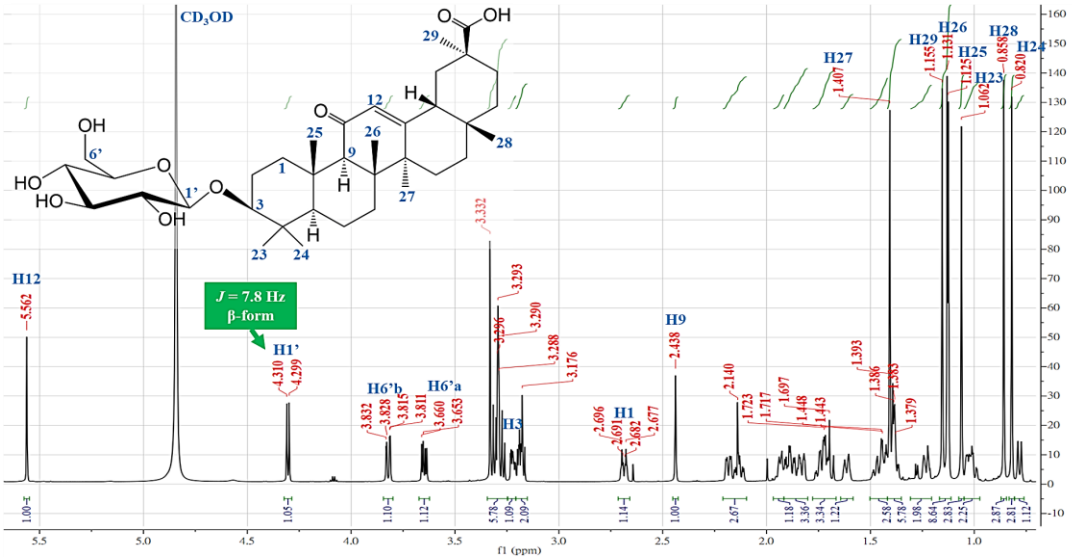
Figures

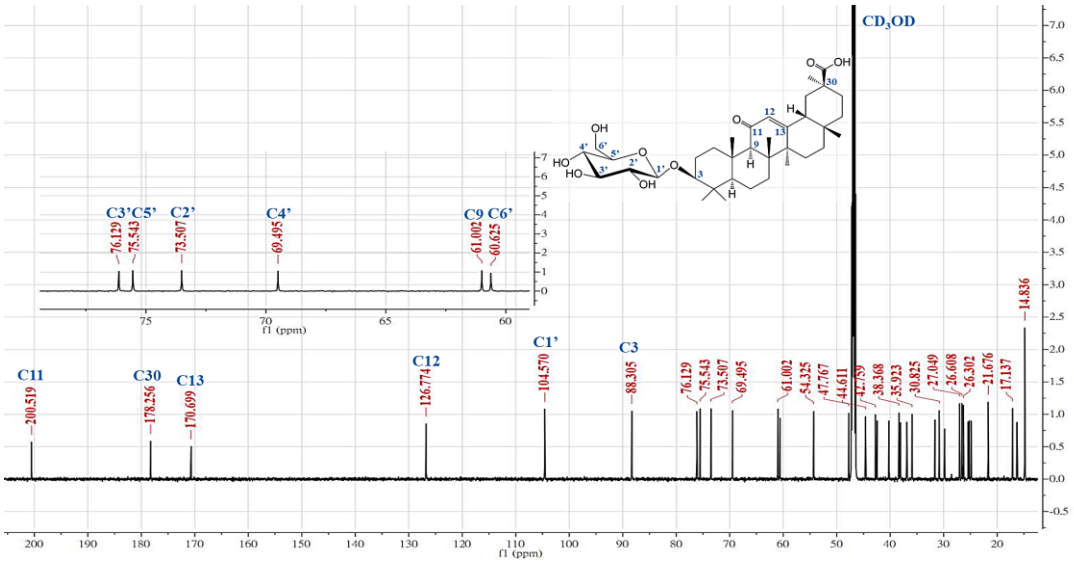
Tables

Fig. S1 NMR spectra of 18β-GA-3-O-β-Glc (**3**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) HSQC, and (F) HMBC.

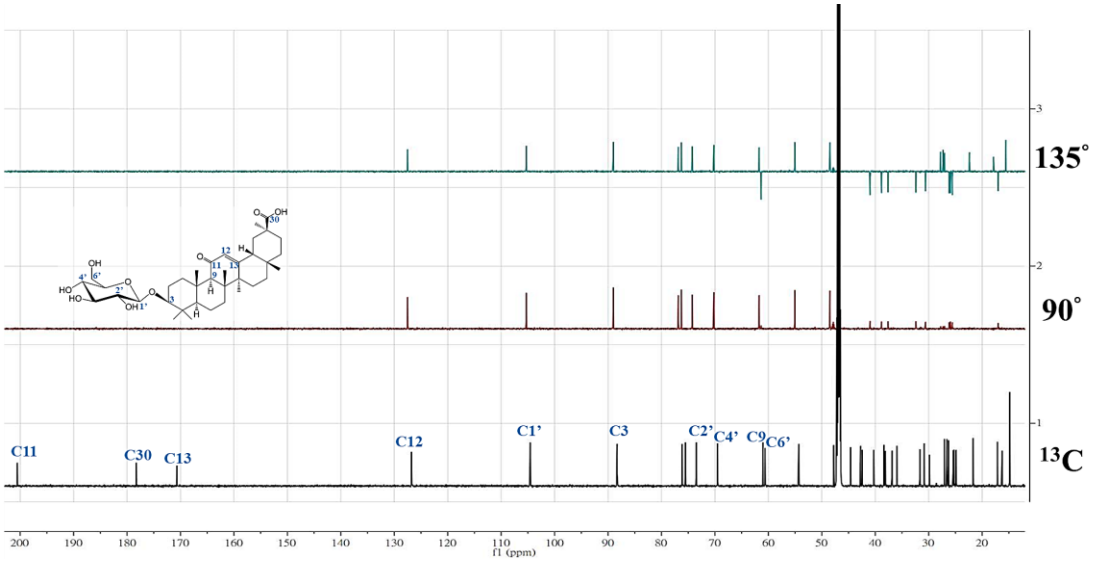
(A) ^{1}H NMR



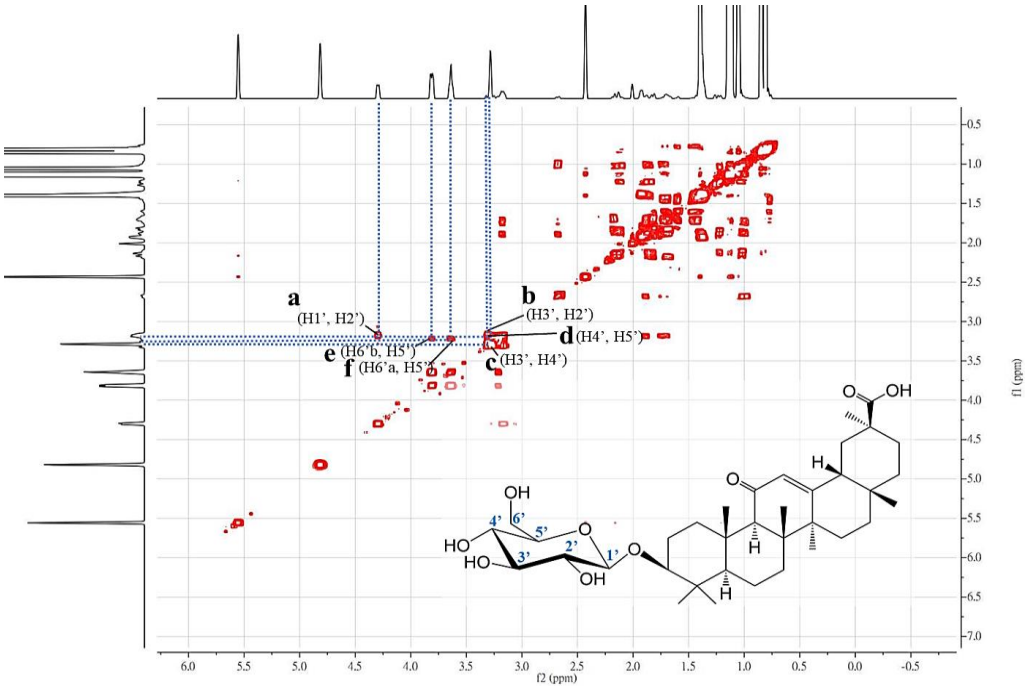
(B) ^{13}C NMR



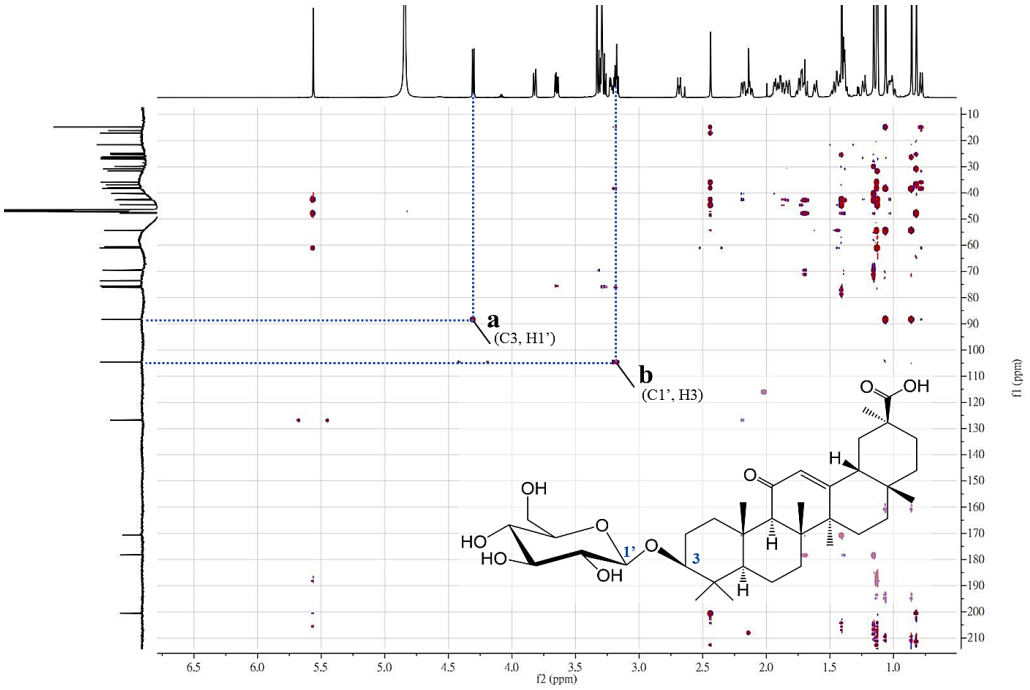
(C) DEPT



(D) ^{1}H-^{1}H COSY



(E) HSQC


a
(C3, H1')
b
(C1', H3)
f2 (ppm)
f1 (ppm)

(F) HMBC

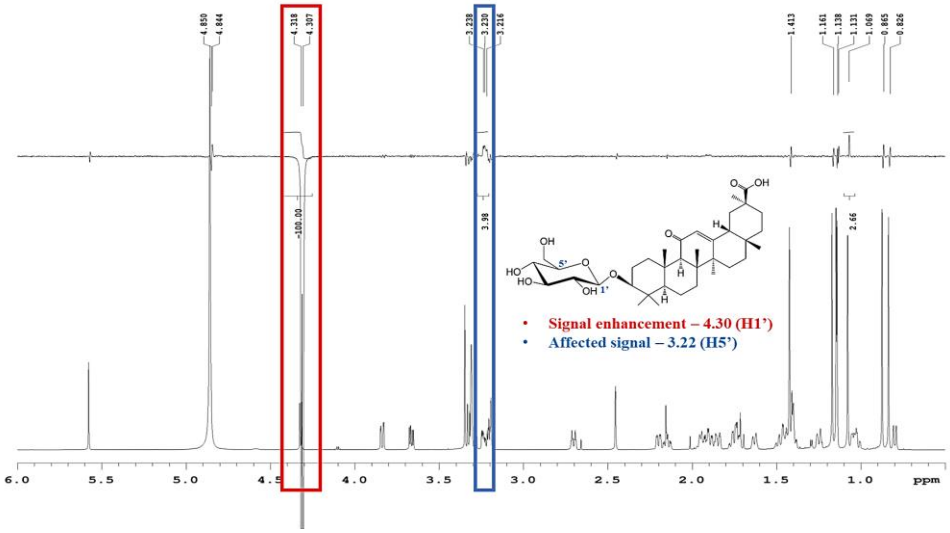
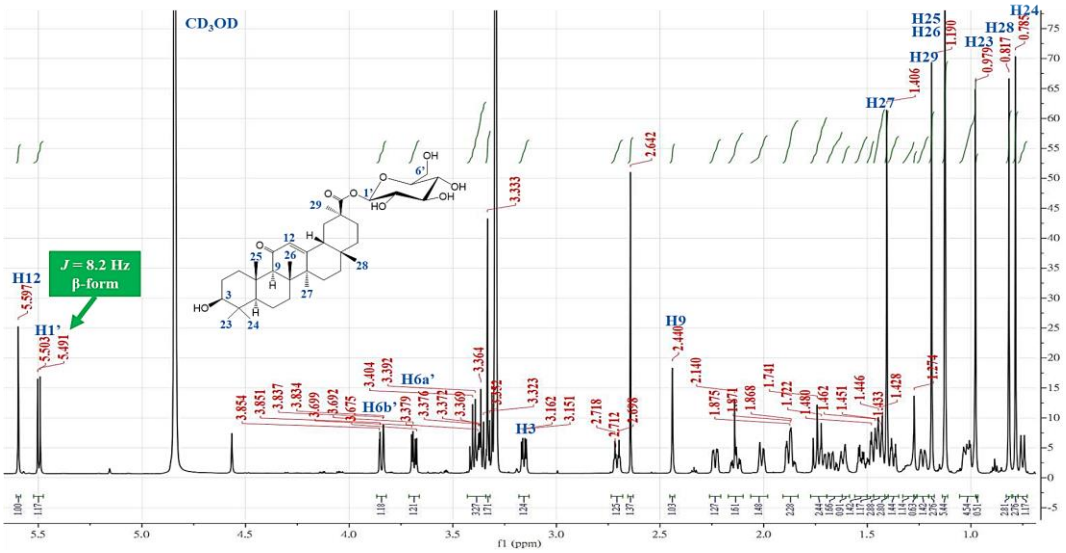
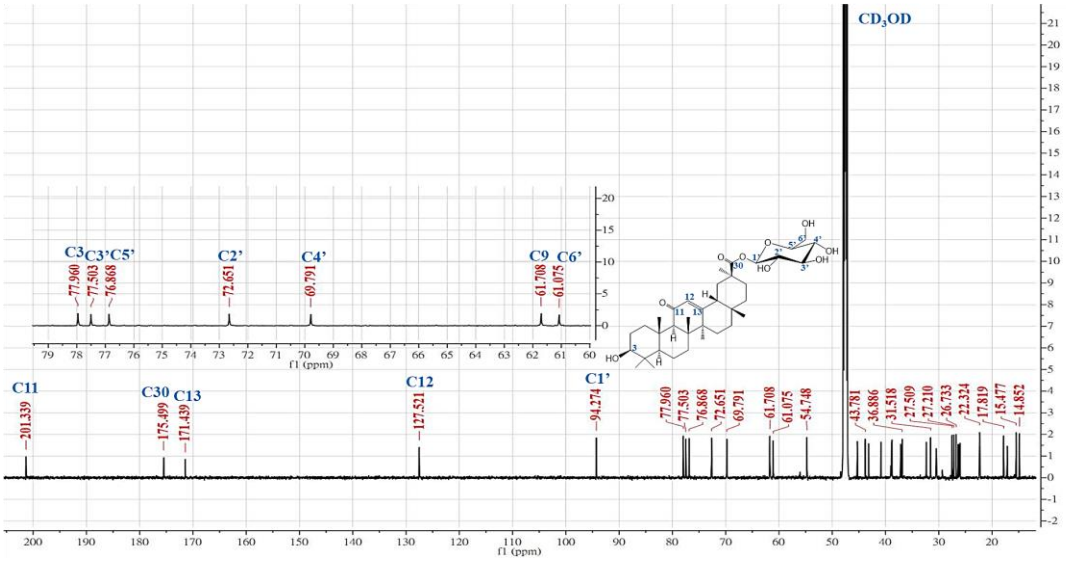

Signal enhancement – 4.30 (H1')
Affected signal – 3.22 (H5')
ppm

Fig. S2 High–resolution UPLC/ESI MS and NMR of 18β-GA-30-O-β-Glc (**4**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, and (E) NOE.

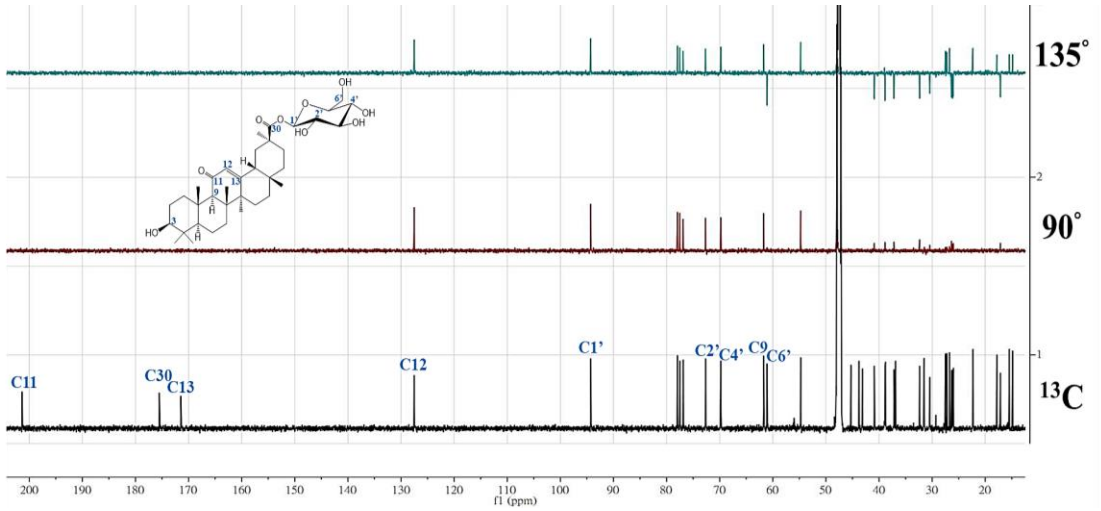
(A) ^{1}H NMR



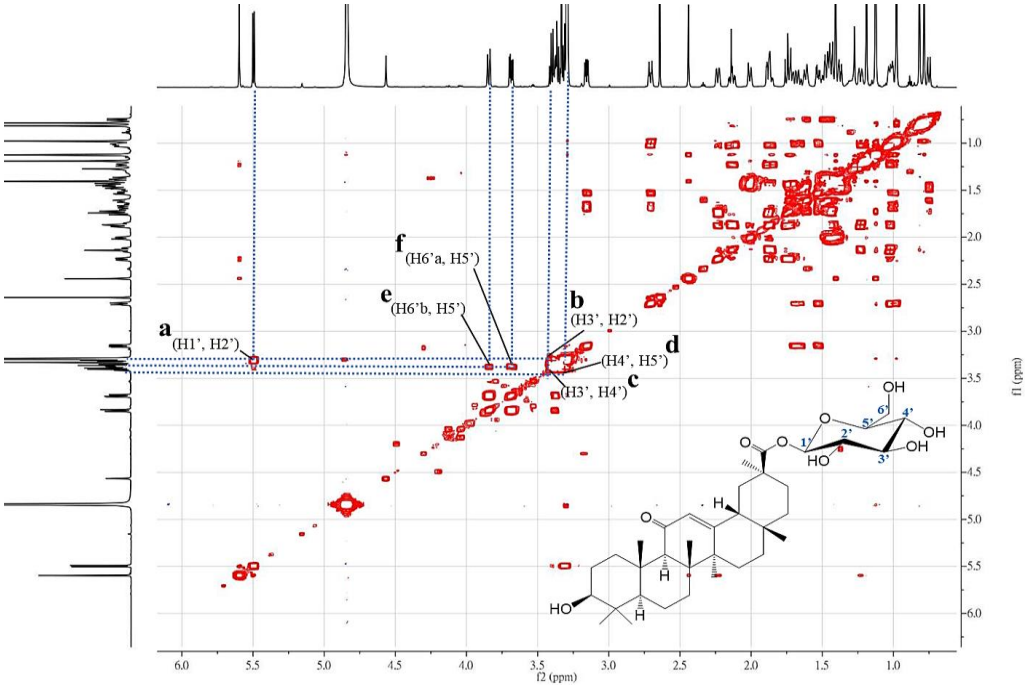
(B) ^{13}C NMR



(C) DEPT



(D) ^{1}H-^{1}H COSY



(E) NOE

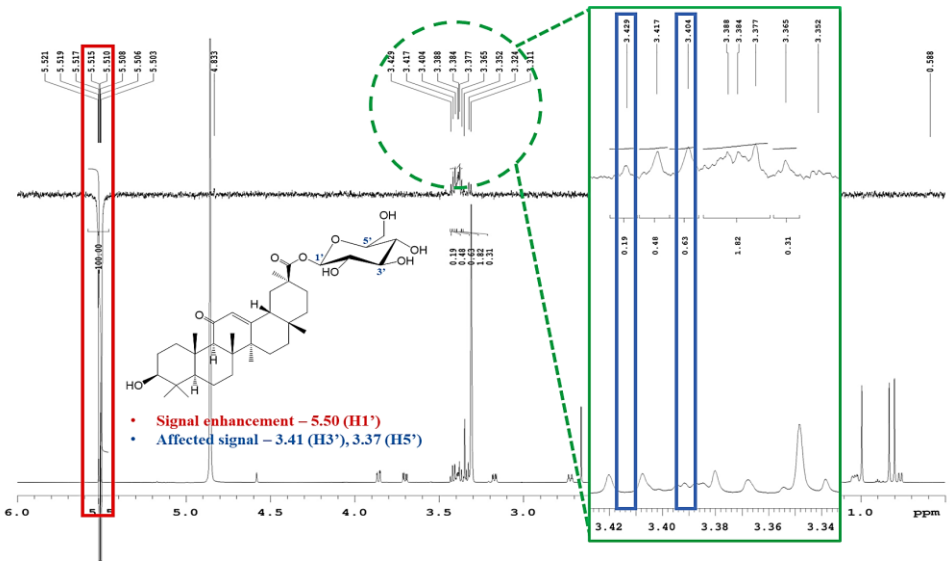
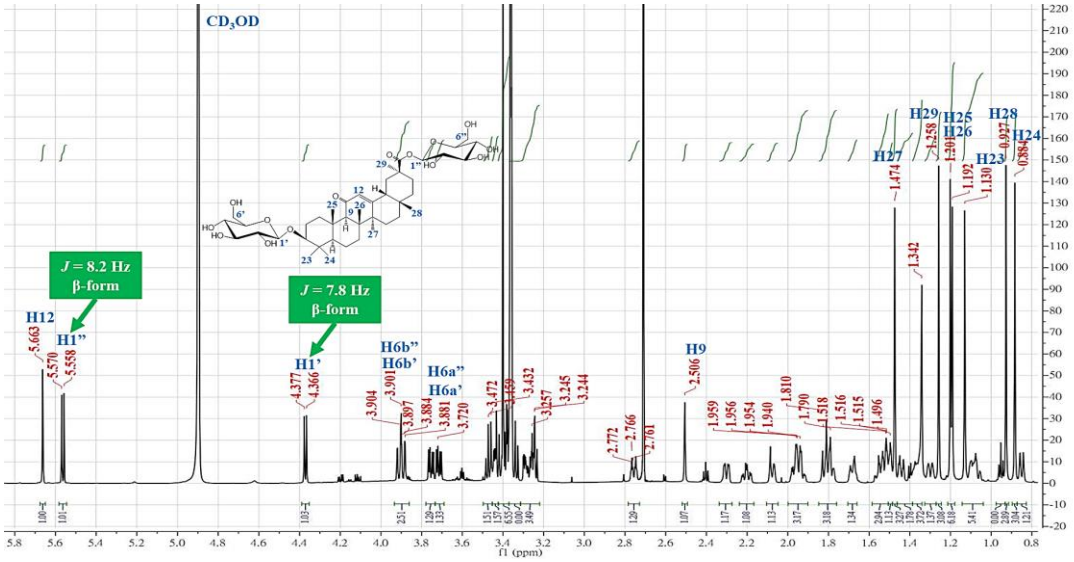
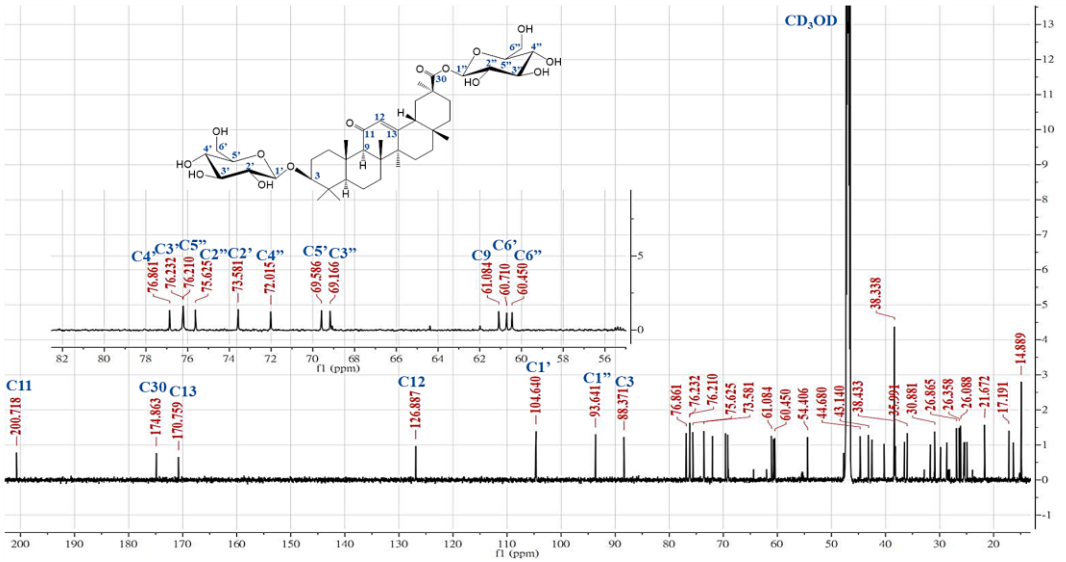

• Signal enhancement – 5.50 (H1')
• Affected signal – 3.41 (H3'), 3.37 (H5')
6.0
5.0
4.5
4.0
3.5
3.0
1.0
ppm
3.42
3.40
3.38
3.36
3.34

Fig. S3 High–resolution UPLC/ESI MS and NMR of 18β-GA-3,30-O-β-bis-Glc (**5**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) HSQC, (F) HMBC, and (G) NOE.

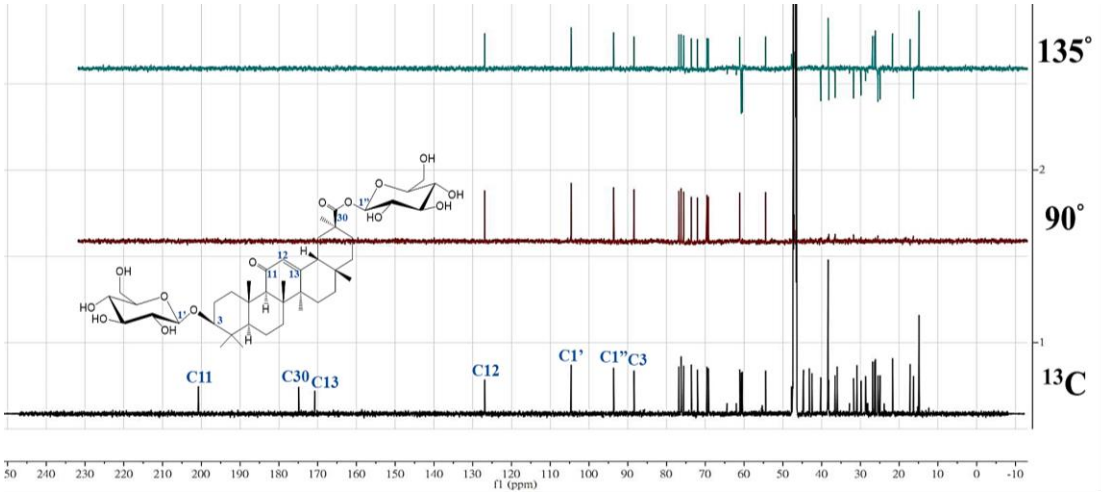
(A) ^{1}H NMR



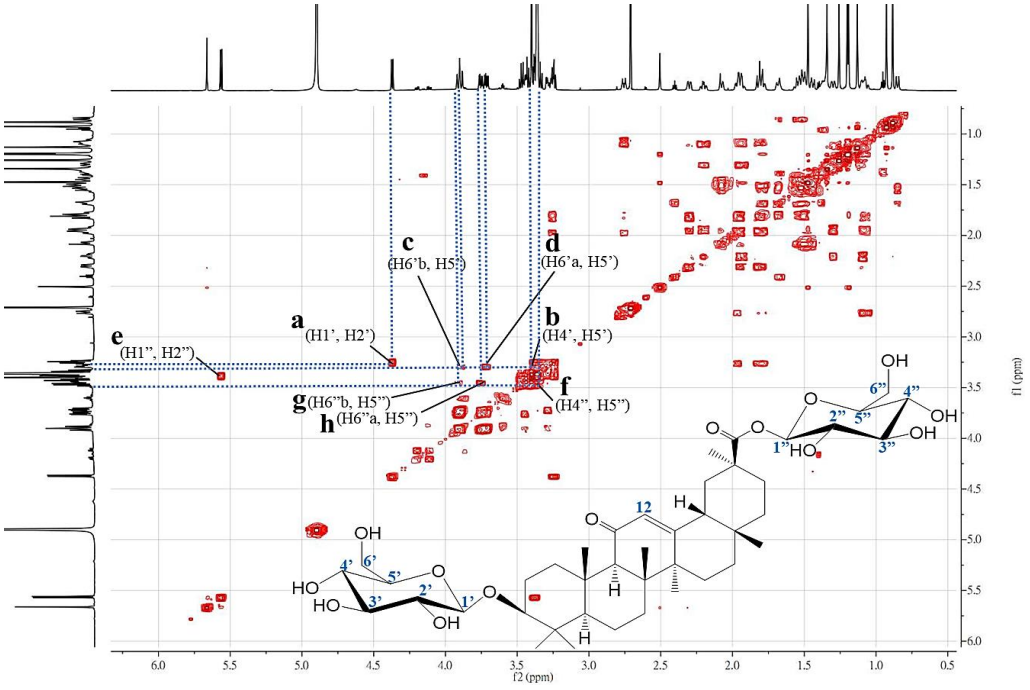
(B) ^{13}C NMR



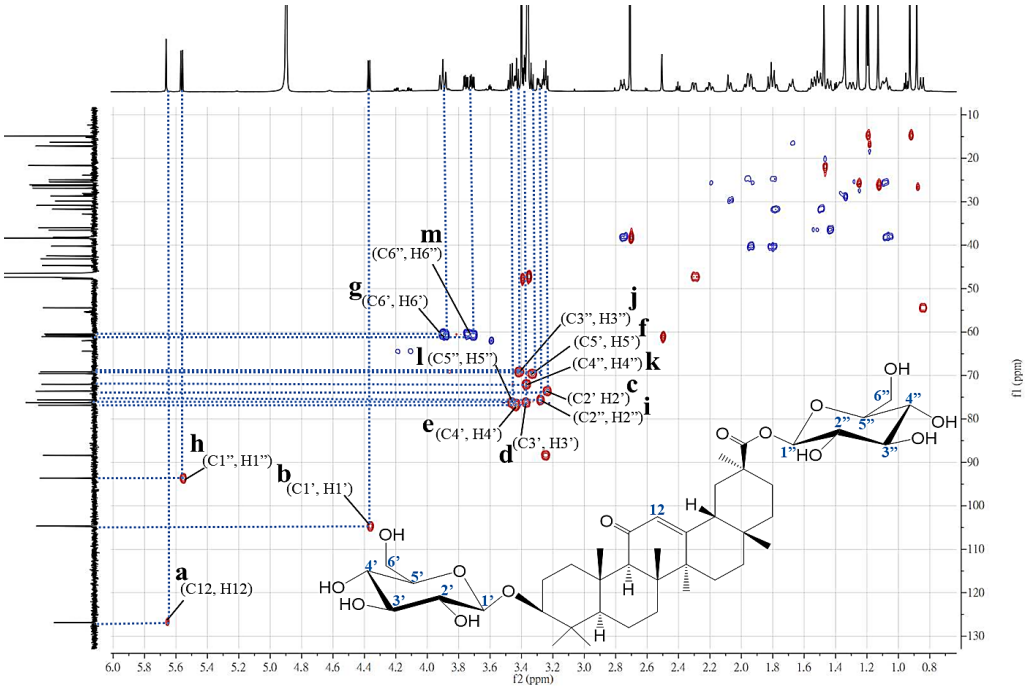
(C) DEPT



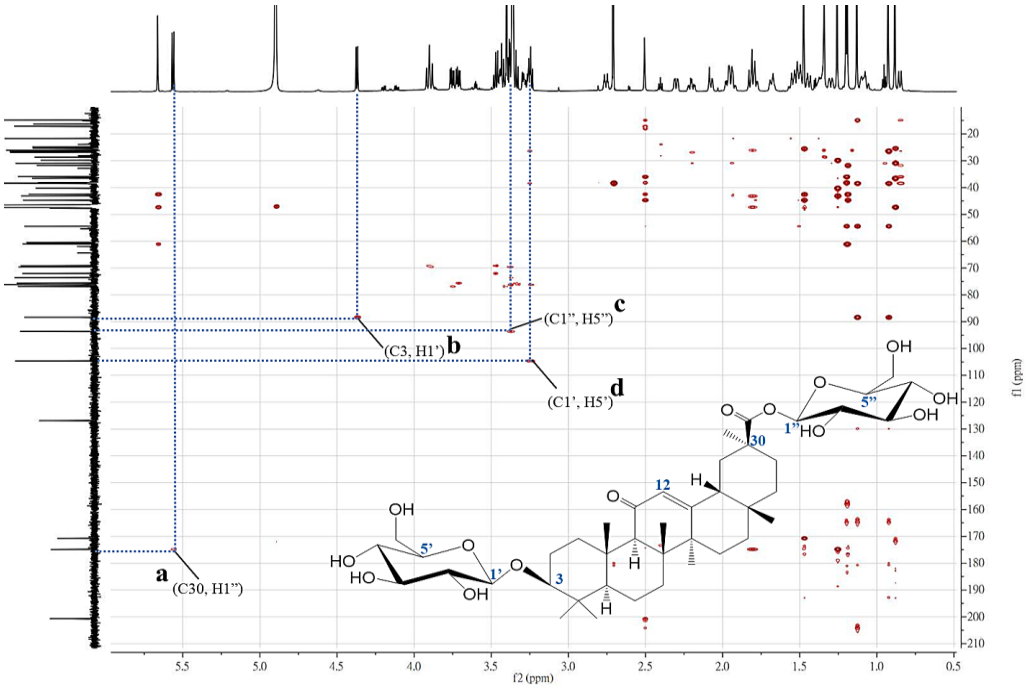
(D) ^{1}H-^{1}H COSY



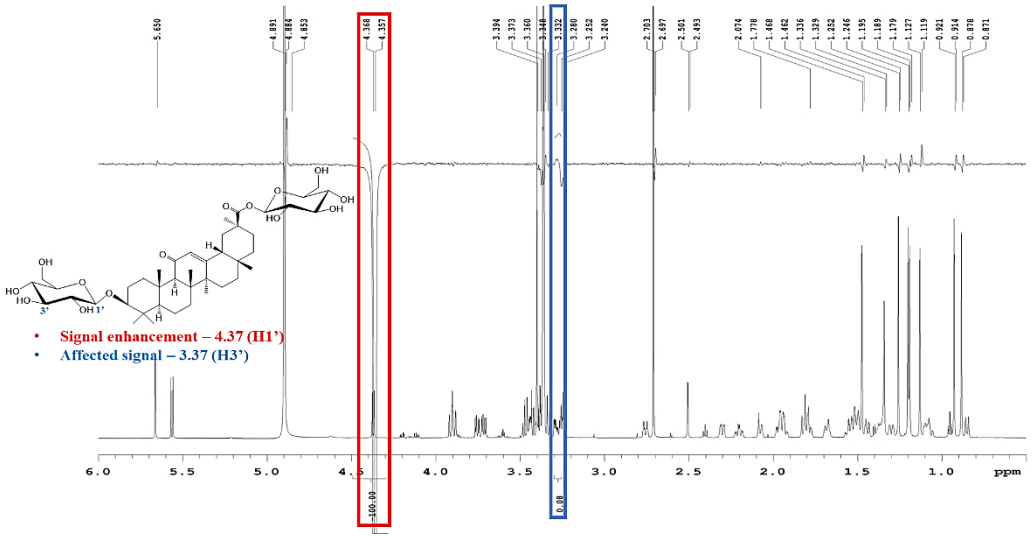
(E) HSQC


m
(C6", H6")
g
(C6', H6')
l (C5", H5")
j
(C3", H3")
f
(C5', H5')
k
(C4", H4")
c
(C2' H2')
i
(C2", H2")
e
(C4', H4')
d
(C3', H3')
h
(C1", H1")
b
(C1', H1')
a
(C12, H12)
f2 (ppm)
f1 (ppm)

(F) HMBC


(C1", H5")
c
(C3, H1')
b
(C1', H5')
d
a
(C30, H1")
f2 (ppm)
f1 (ppm)

(G) NOE


• Signal enhancement – 4.37 (H1')
• Affected signal – 3.37 (H3')
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

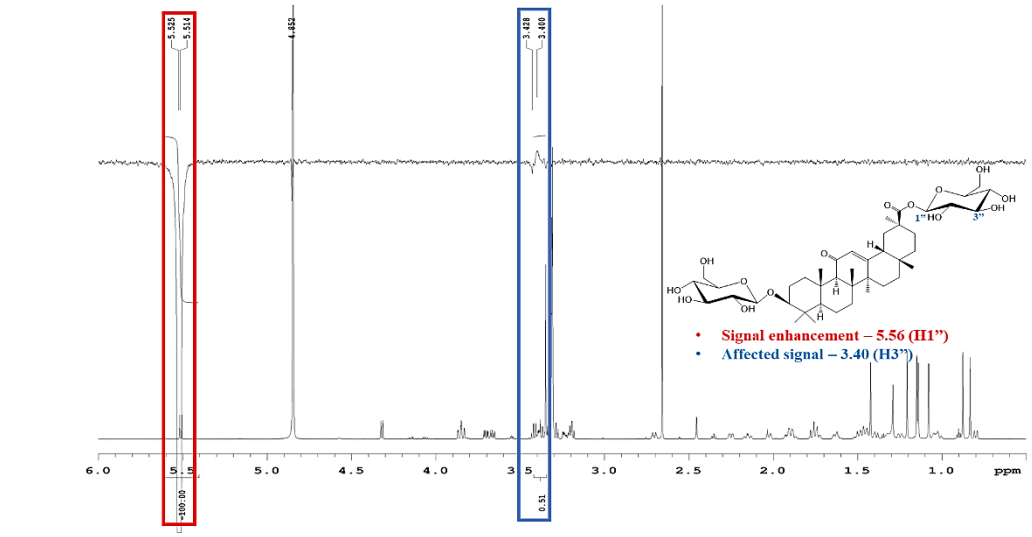
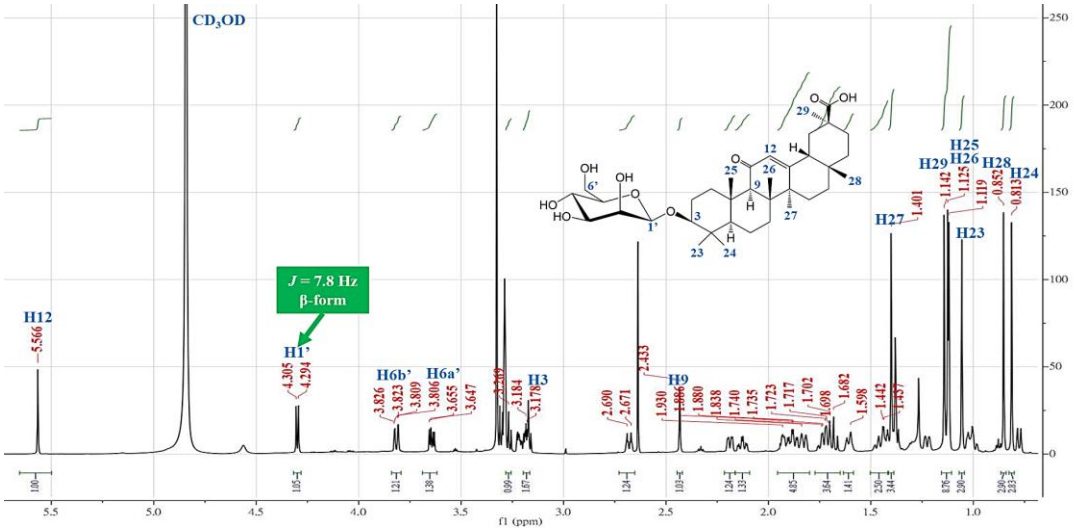
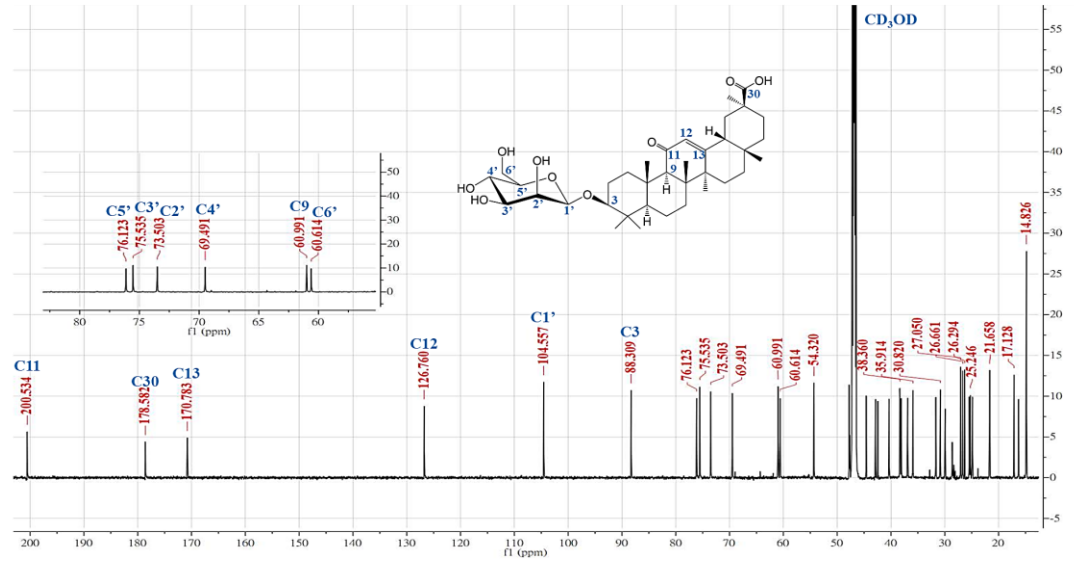

• Signal enhancement – 5.56 (H1")
• Affected signal – 3.40 (H3")
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S4 High–resolution UPLC/ESI MS and NMR of 18β-GA-3-O-β-Man (**6**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) NOE.

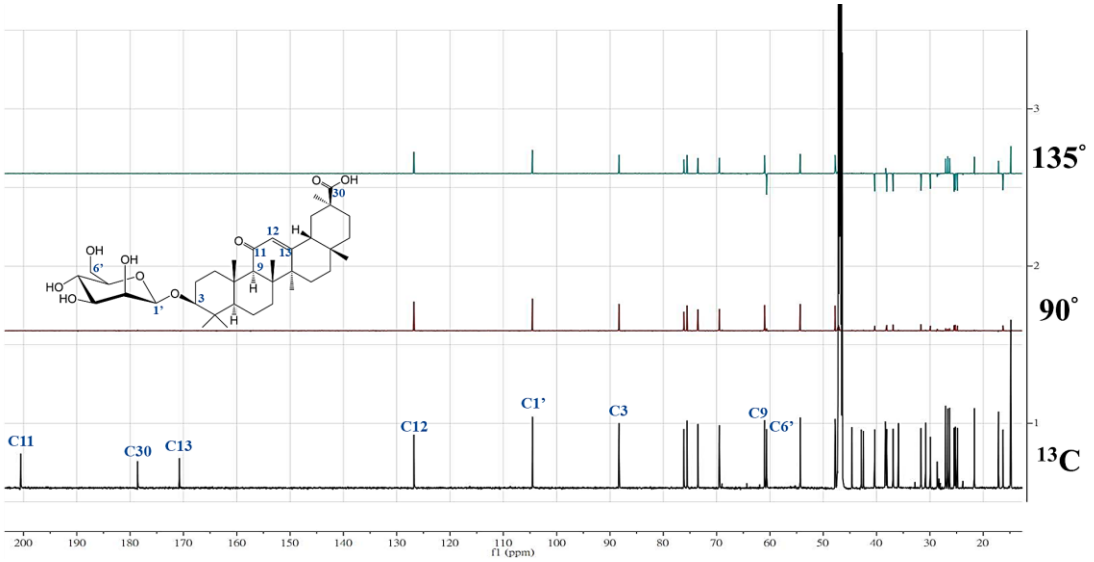
(A) ^{1}H NMR



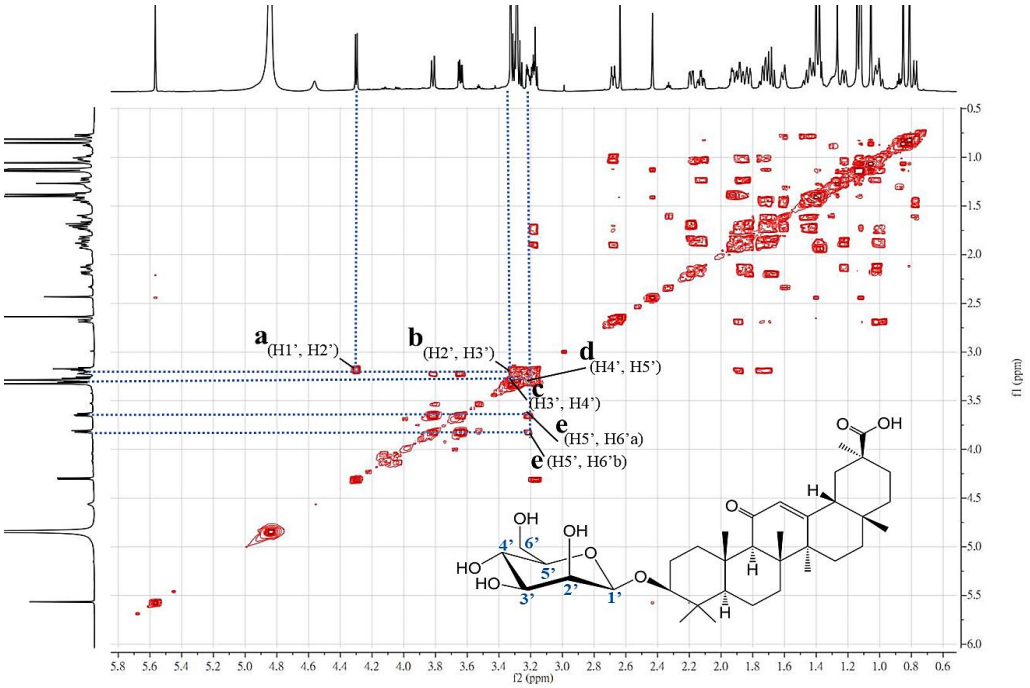
(B) ^{13}C NMR



(C) DEPT



(D) ^{1}H-^{1}H COSY



(E) NOE

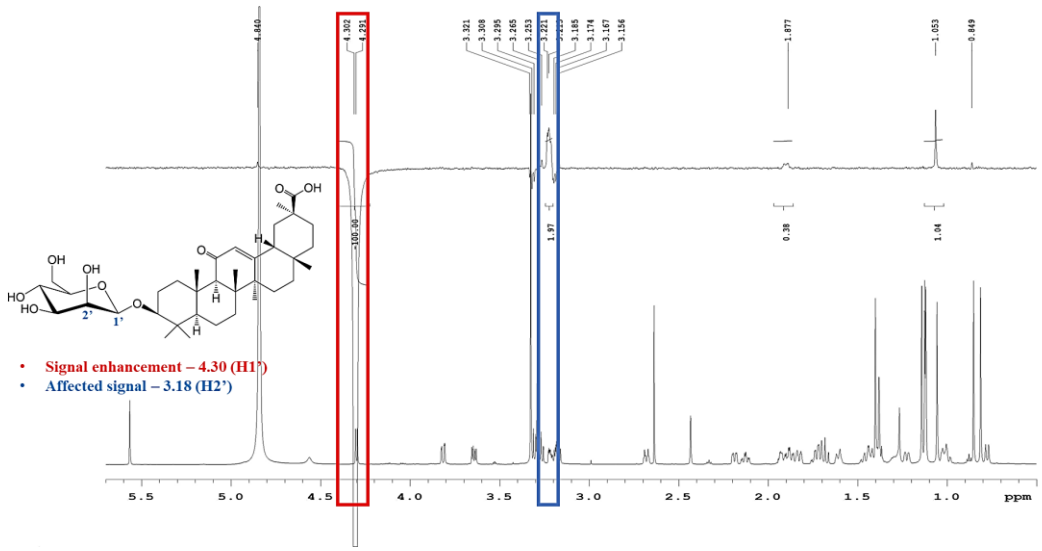
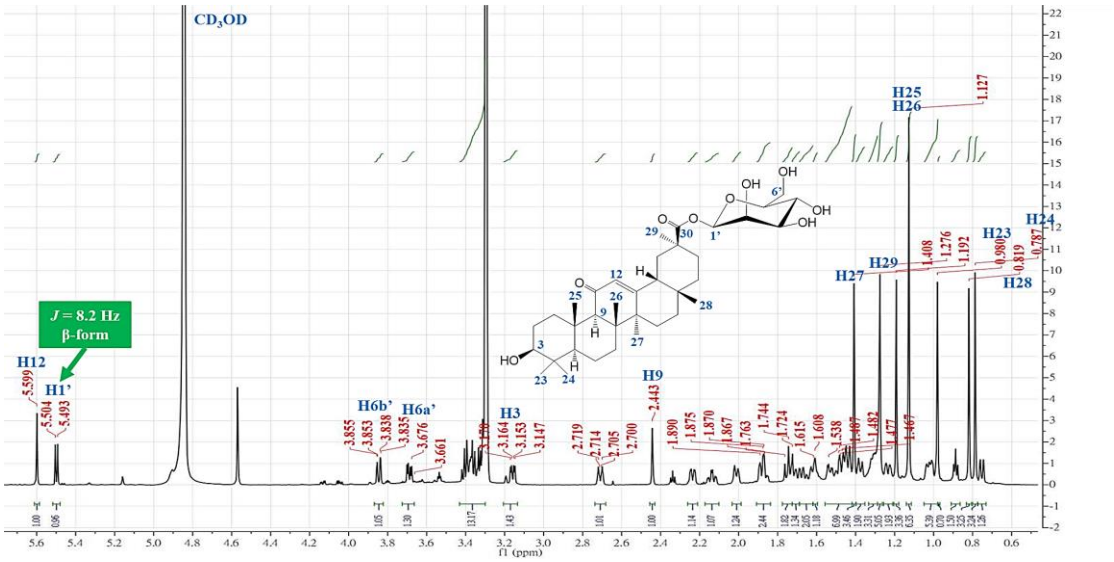
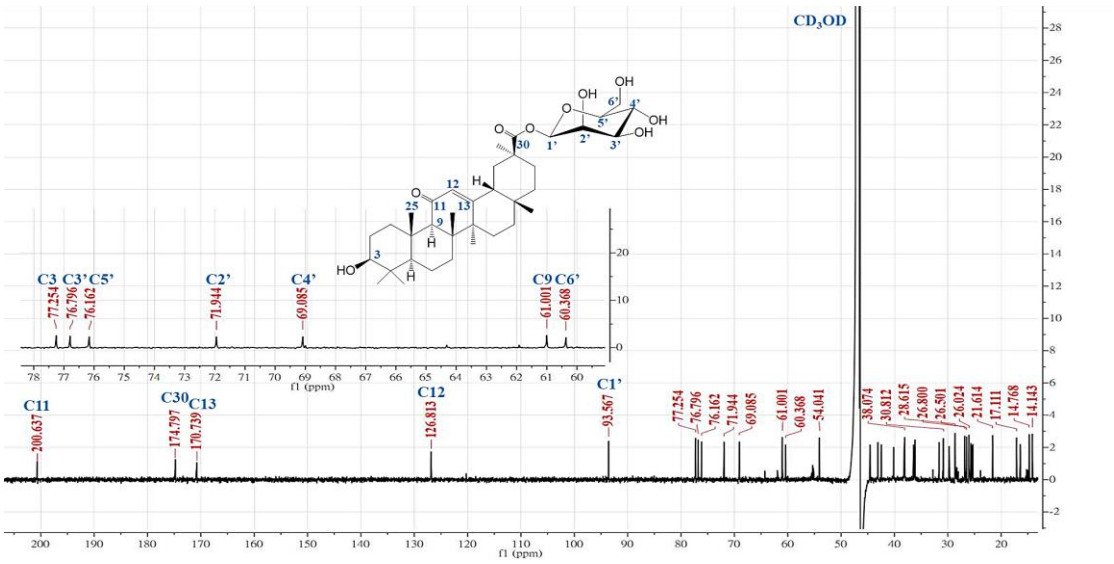

OH
OH
HO
HO
O
2'
1'
O
OH
O
H
H
H
• Signal enhancement – 4.30 (H1')
• Affected signal – 3.18 (H2')
4.840
4.302
4.291
3.321
3.308
3.295
3.265
3.253
3.221
3.185
3.174
3.167
3.156
1.877
1.053
0.849
100.00
1.97
0.38
1.04
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S5 High–resolution UPLC/ESI MS and NMR of 18β-GA-30-O-β-Man (**7**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) NOE.

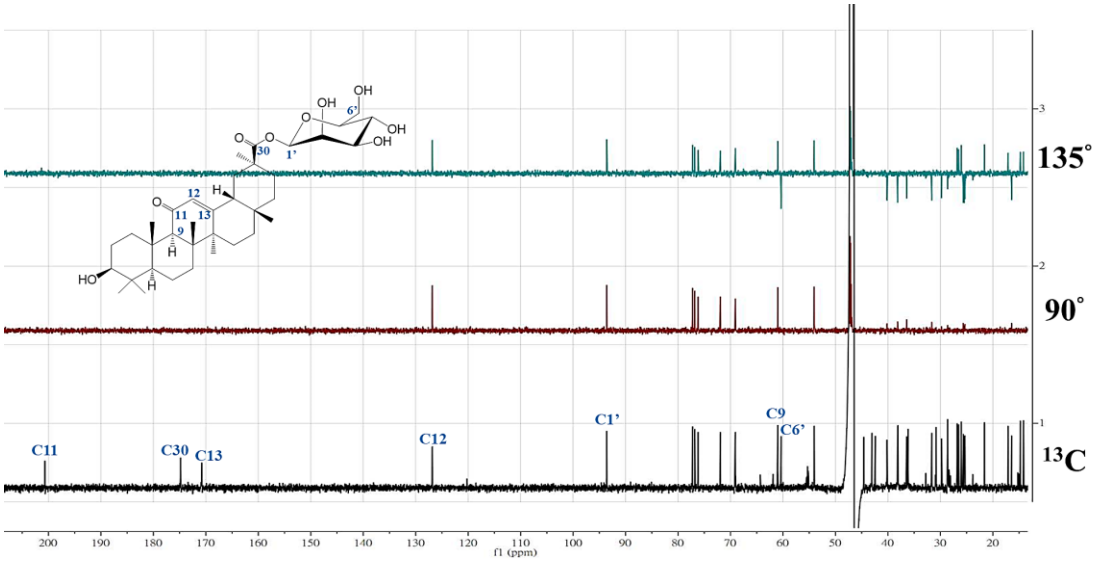
(A) ^{1}H NMR



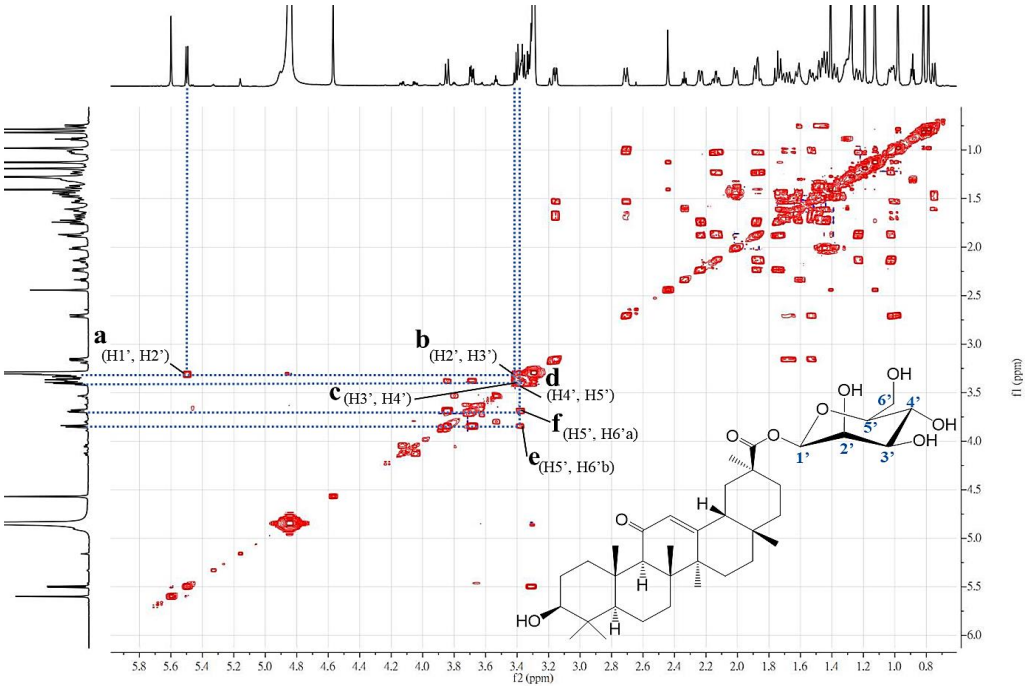
(B) ^{13}C NMR



(C) DEPT



(D) ^{1}H-^{1}H COSY



(E) NOE

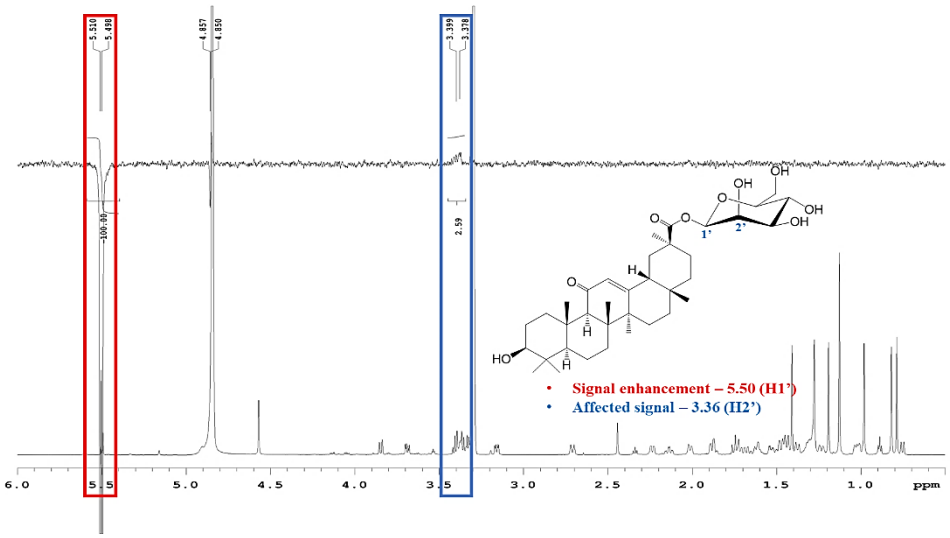
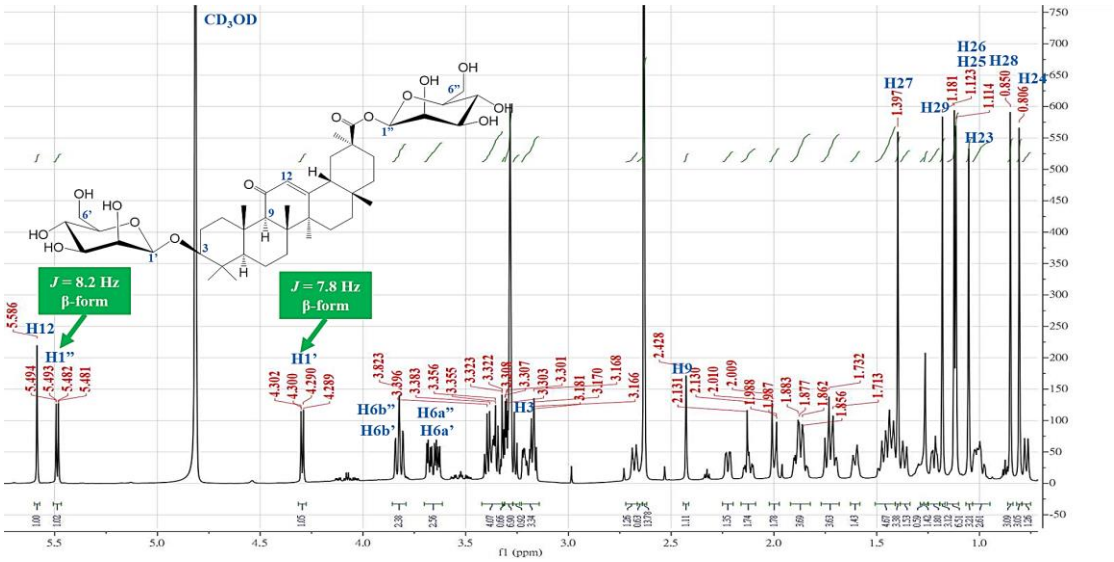
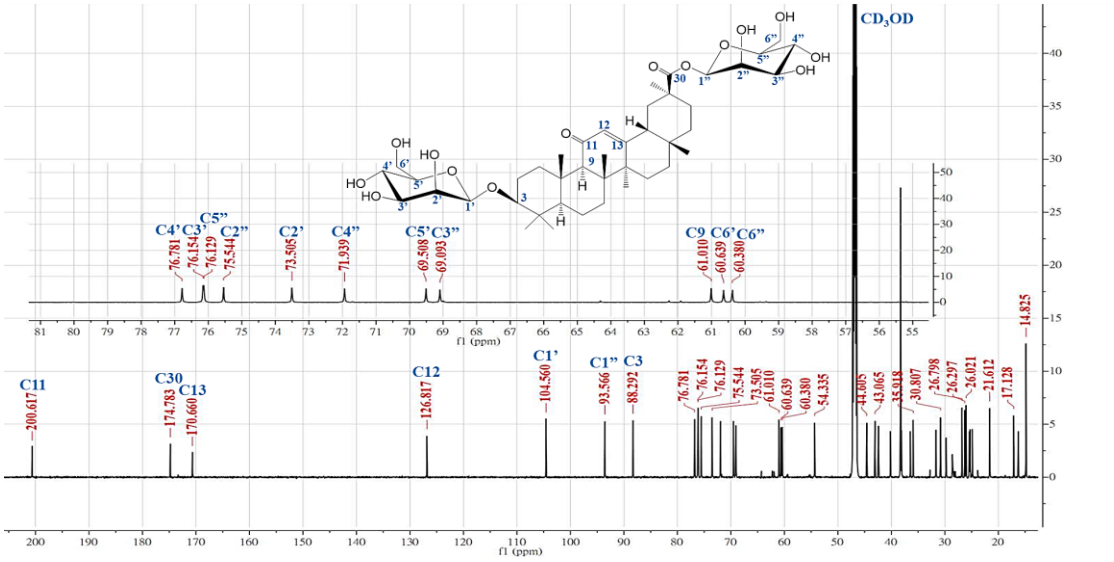

5.510
5.498
4.857
4.850
3.399
3.378
100.00
2.59
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
OH
OH
OH
OH
O
O
O
1'
2'
H
O
H
H
HO
• Signal enhancement – 5.50 (H1')
• Affected signal – 3.36 (H2')

Fig. S6 High–resolution UPLC/ESI MS and NMR of 18β-GA-3,30-O-β-bis-Man (**8**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) HSQC, (F) HMBC, and (G) NOE.

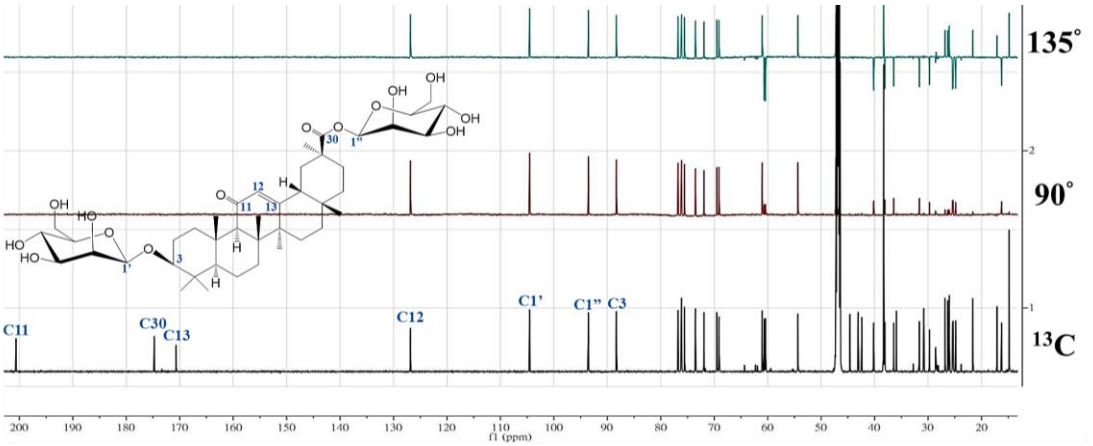
(A) ^{1}H NMR



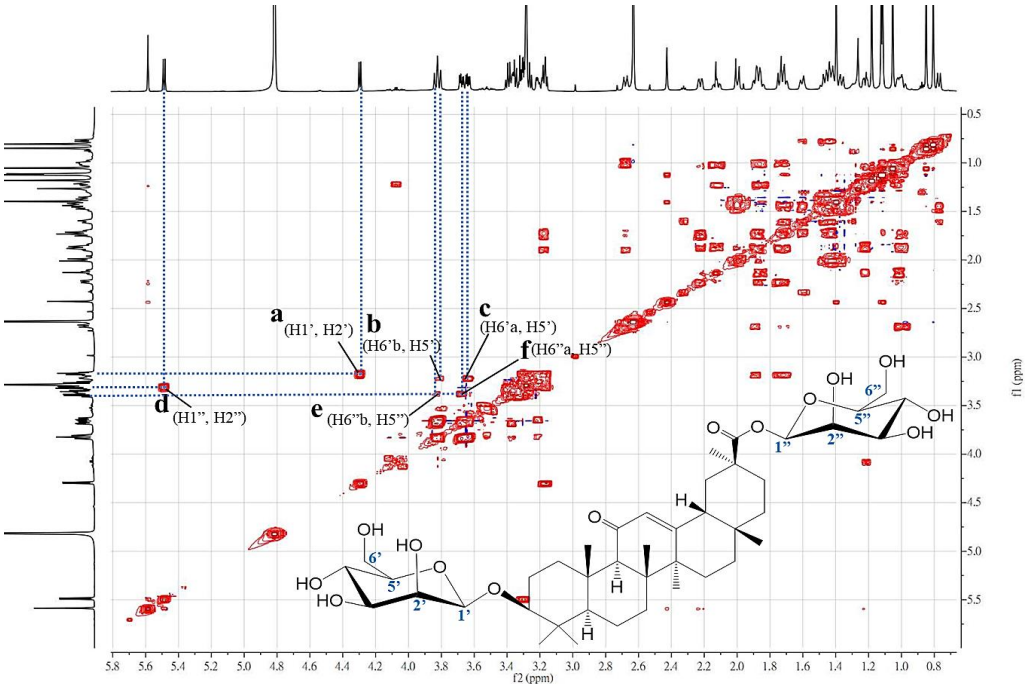
(B) ^{13}C NMR



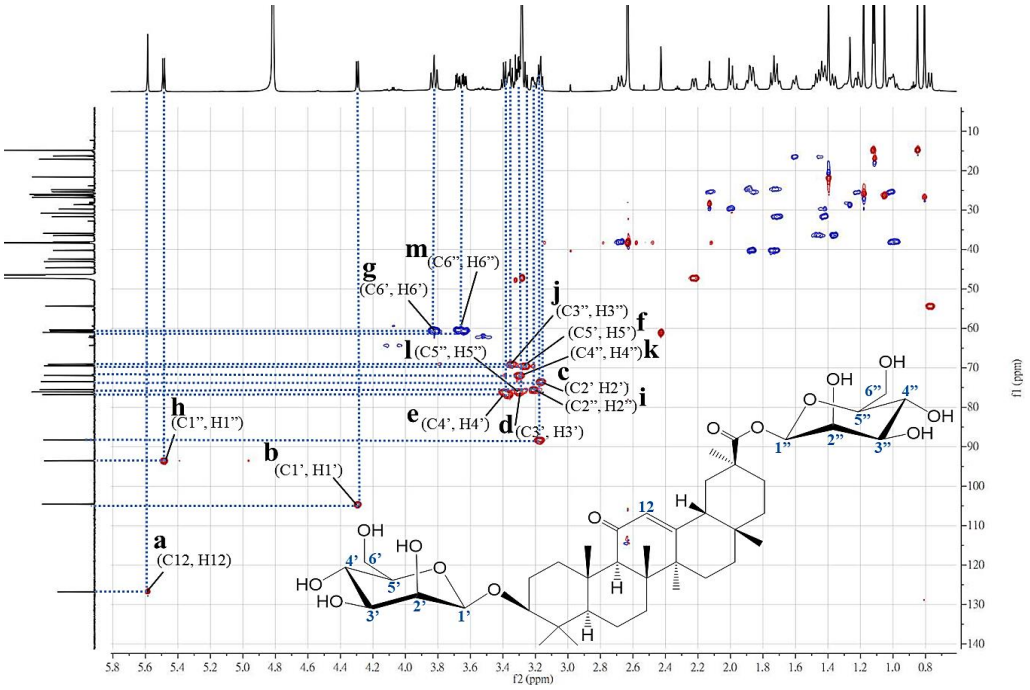
(C) DEPT



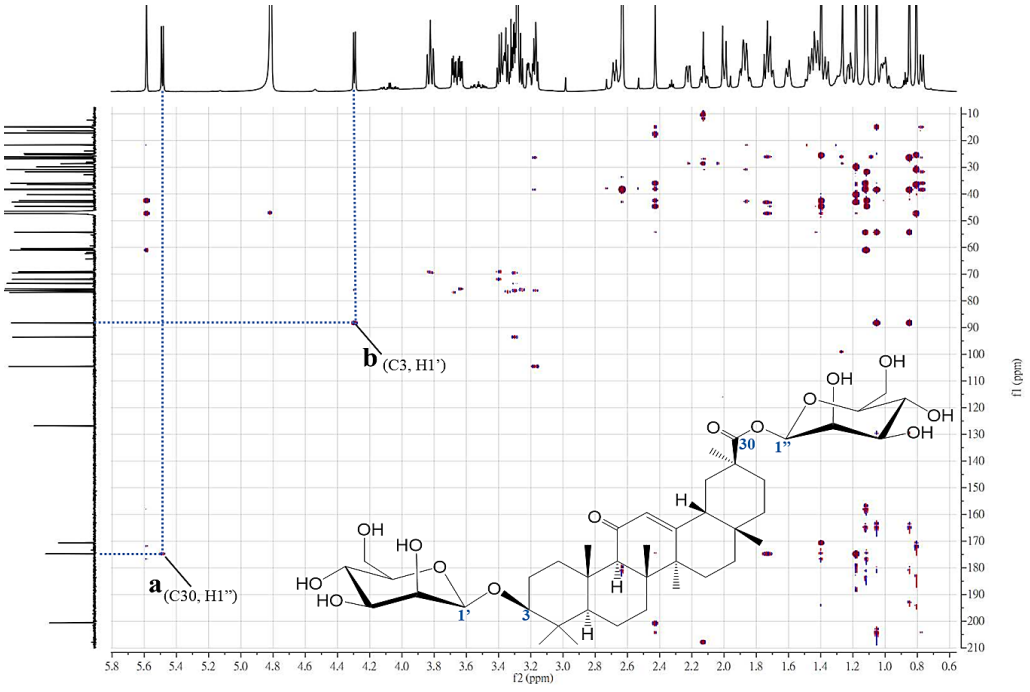
(D) ^{1}H-^{1}H COSY



(E) HSQC


m (C6”, H6”)
g (C6’, H6’)
j (C3”, H3”)
f (C5’, H5’)
l (C5”, H5”)
k (C4”, H4”)
c (C2’ H2’)
i (C2”, H2”)
e (C4’, H4’)
d (C3’, H3’)
h (C1”, H1”)
b (C1’, H1’)
a (C12, H12)
f2 (ppm)
f1 (ppm)

(F) HMBC


b (C3, H1’)
a (C30, H1”)
f2 (ppm)
f1 (ppm)

(G) NOE

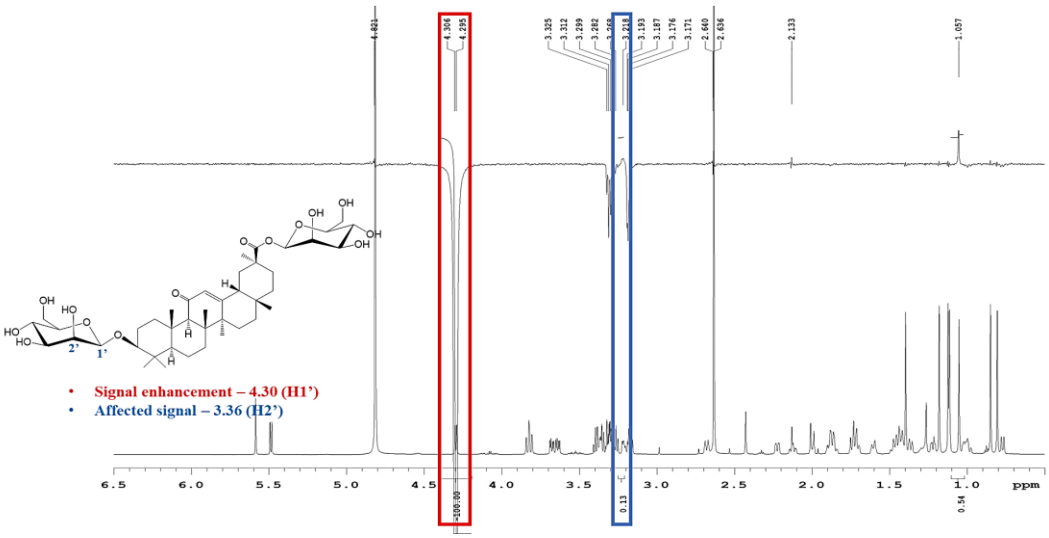
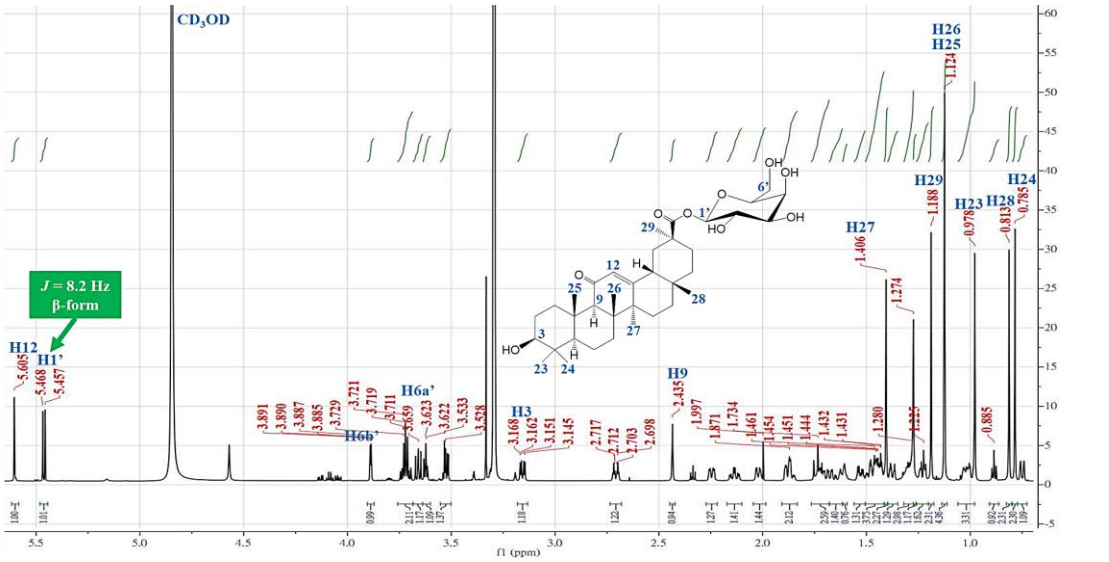
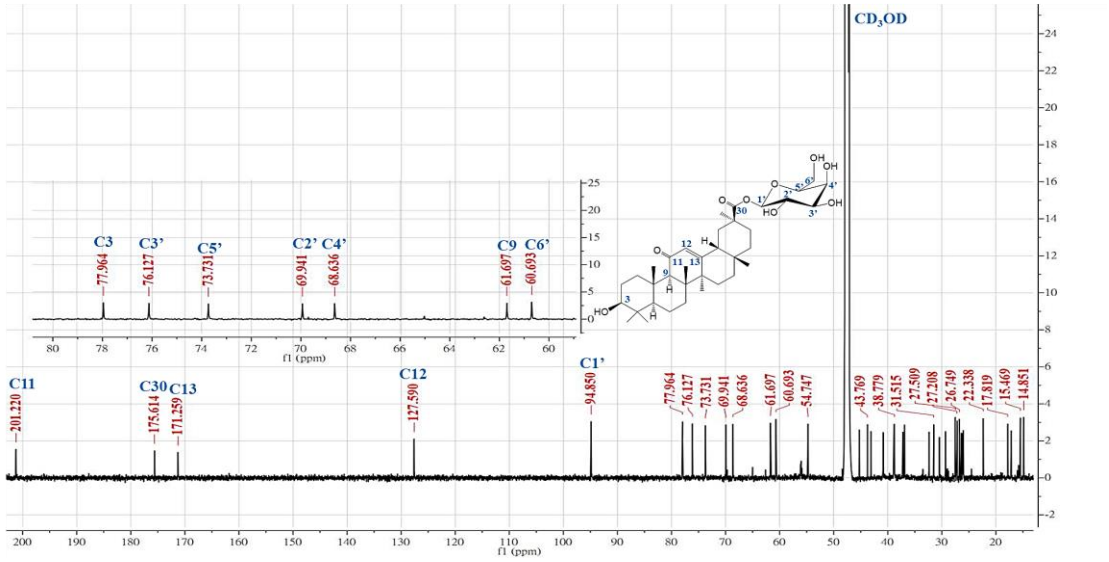

• Signal enhancement – 4.30 (H1')
• Affected signal – 3.36 (H2')
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S7 High–resolution UPLC/ESI MS and NMR of 18β-GA-30-O-β-Gal (**9**). (A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) NOE.

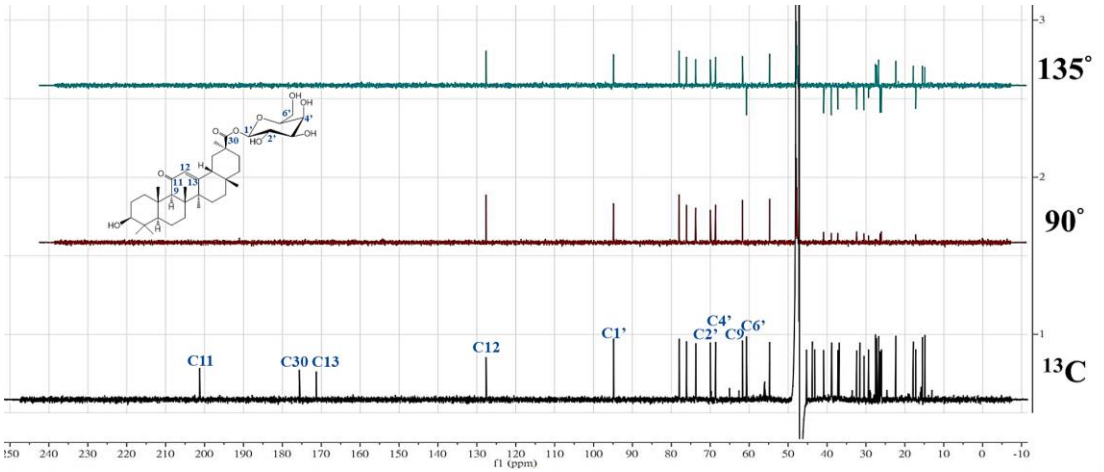
(A) ^{1}H NMR



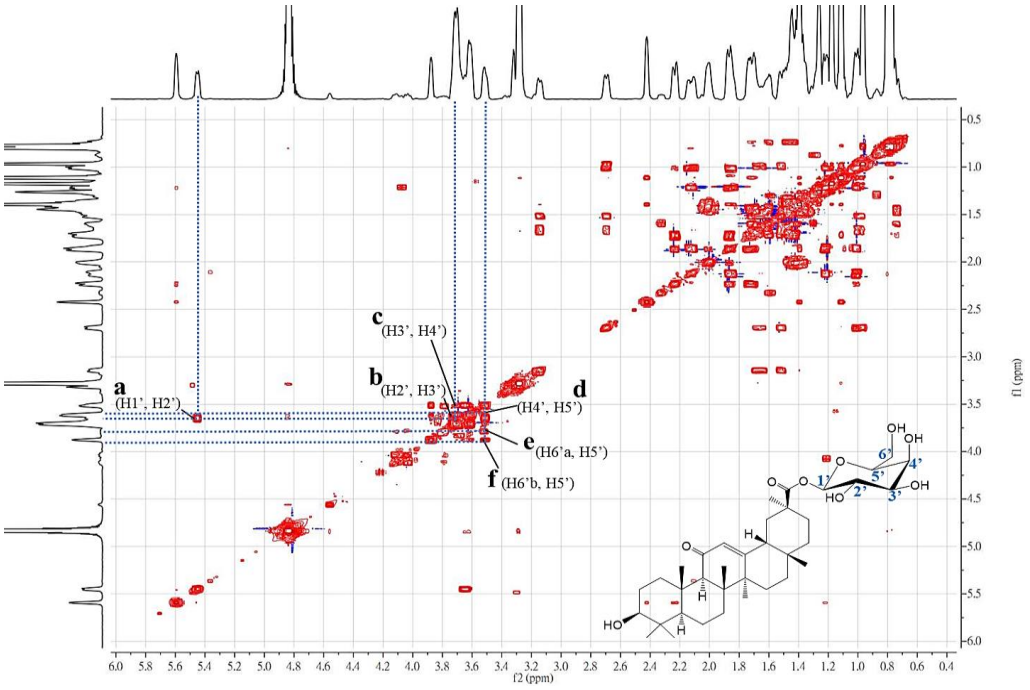
(B) ^{13}C NMR



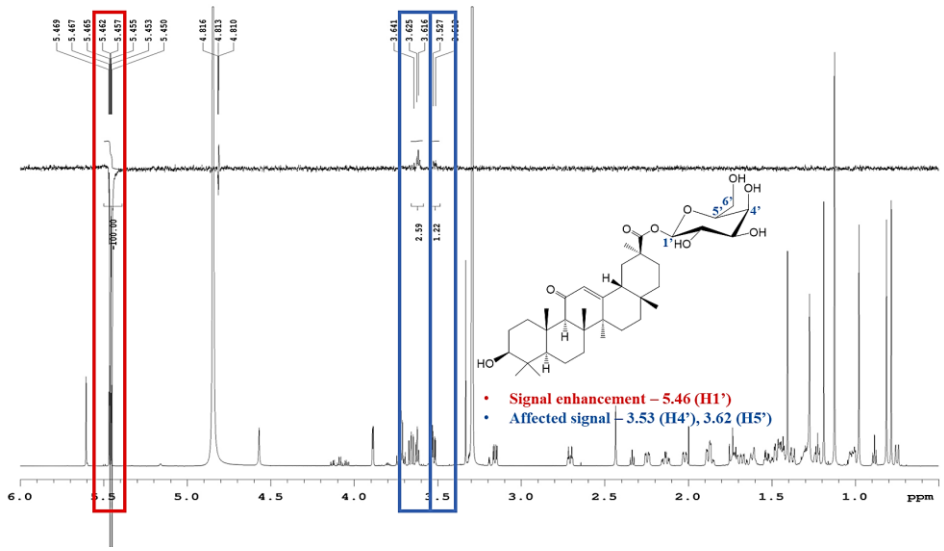
(C) DEPT

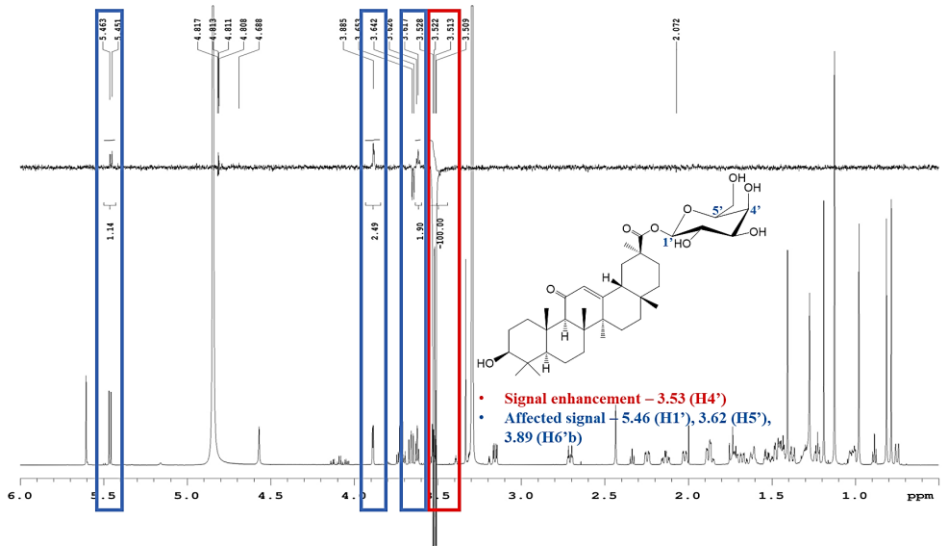


(D) ^{1}H-^{1}H COSY



(E) NOE


Signal enhancement – 5.46 (H1')
Affected signal – 3.53 (H4'), 3.62 (H5')
ppm


Signal enhancement – 3.53 (H4')
Affected signal – 5.46 (H1'), 3.62 (H5'), 3.89 (H6'b)
ppm

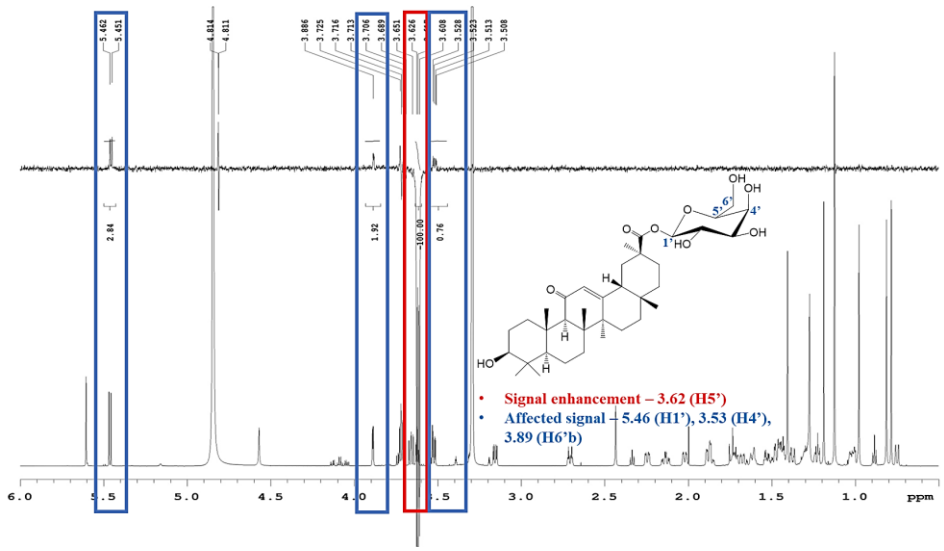
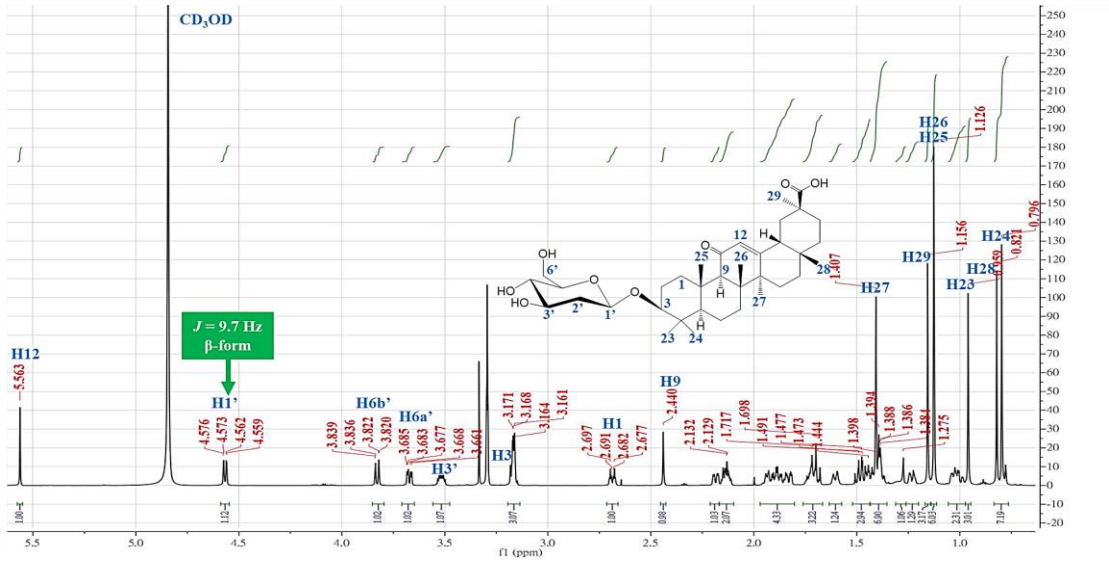

Signal enhancement – 3.62 (H5')
Affected signal – 5.46 (H1'), 3.53 (H4'), 3.89 (H6'b)
ppm

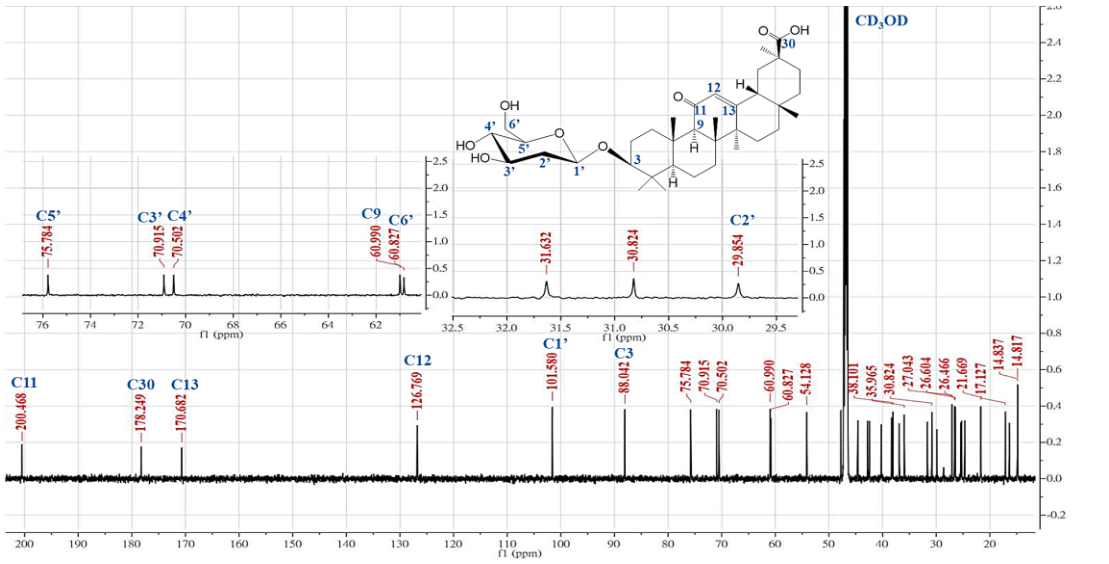
Fig. S8 High–resolution UPLC/ESI MS and NMR of 18β-GA-β-3-O-β-2DG (**10**).

(A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) NOE.

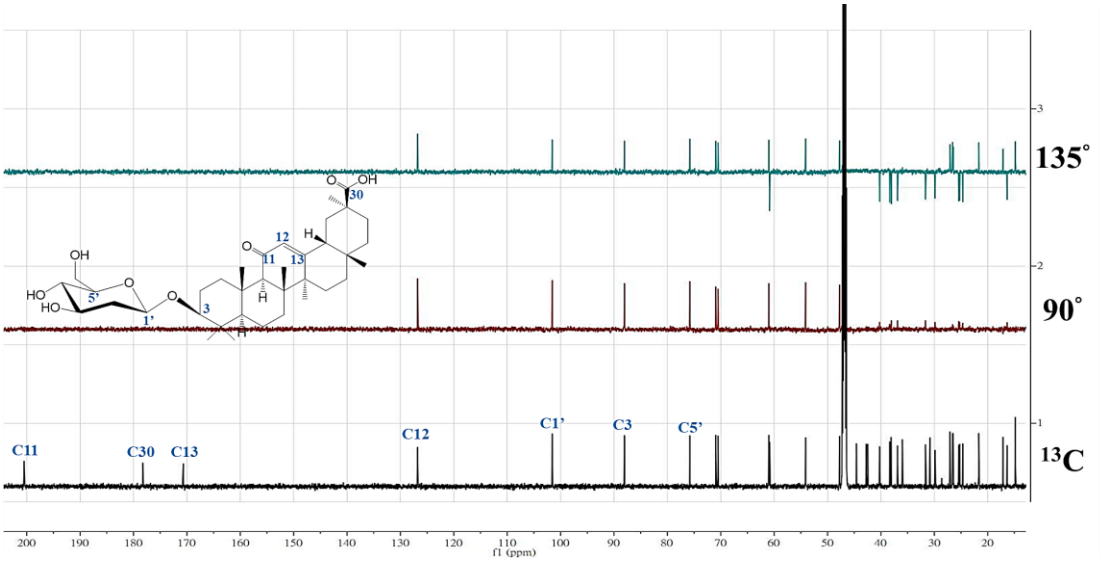
(A) ^{1}H NMR



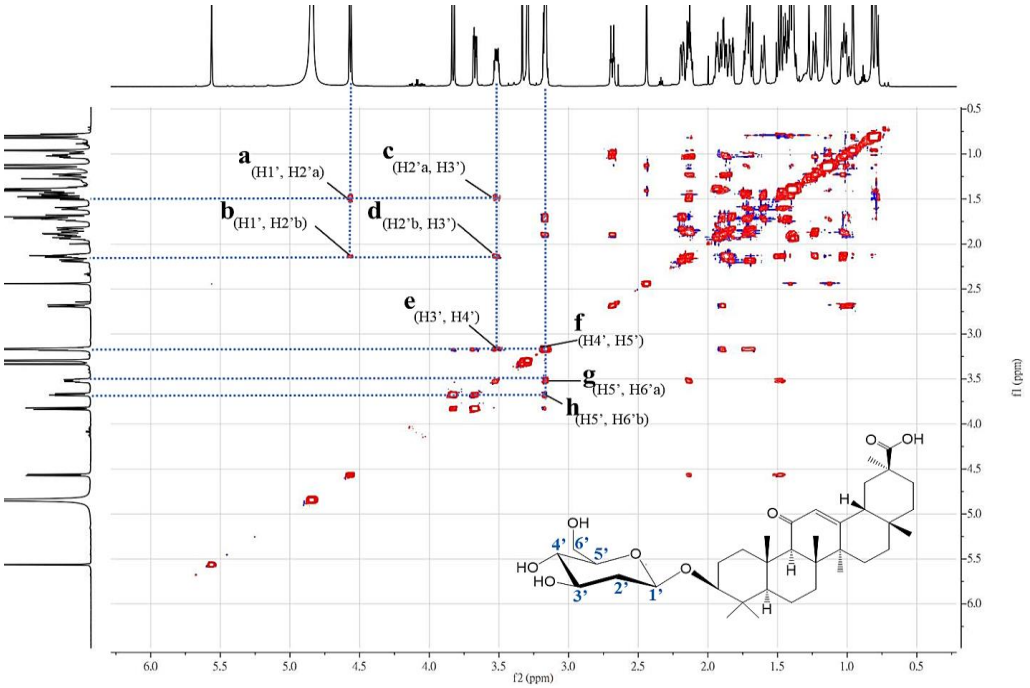
(B) ^{13}C NMR



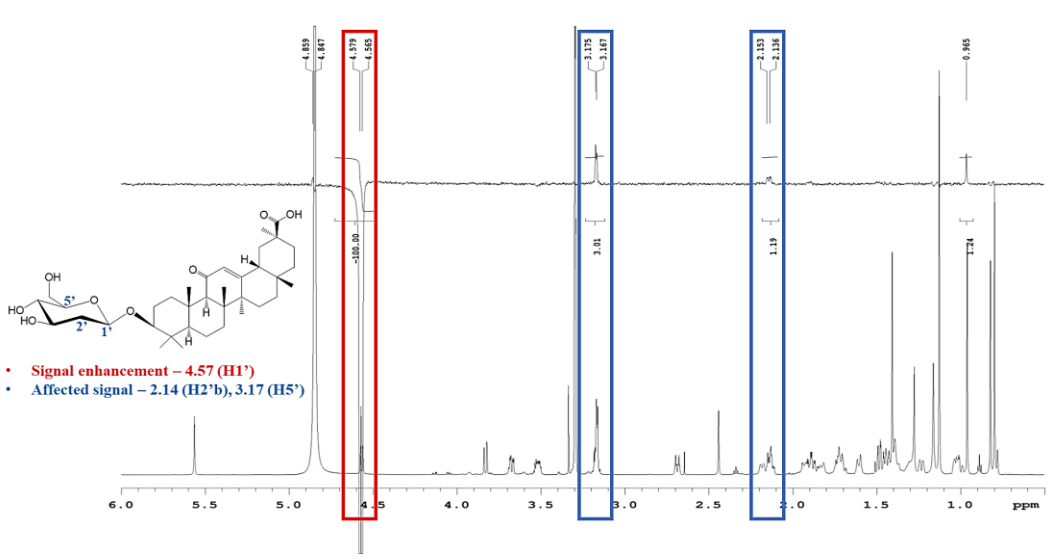
(C) DEPT



(D) ^{1}H-^{1}H COSY



(E) NOE


• Signal enhancement – 4.57 (H1')
• Affected signal – 2.14 (H2'b), 3.17 (H5')
ppm

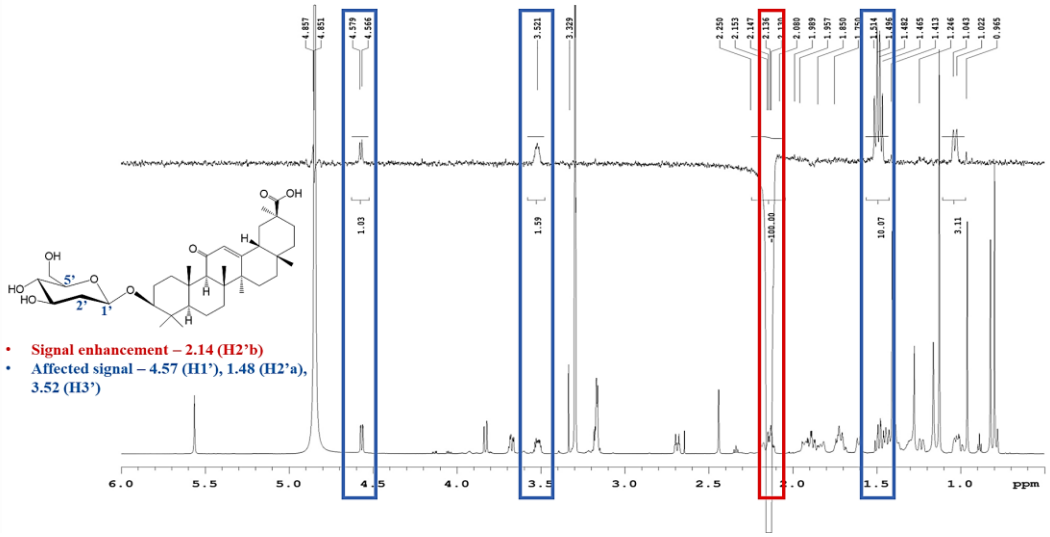
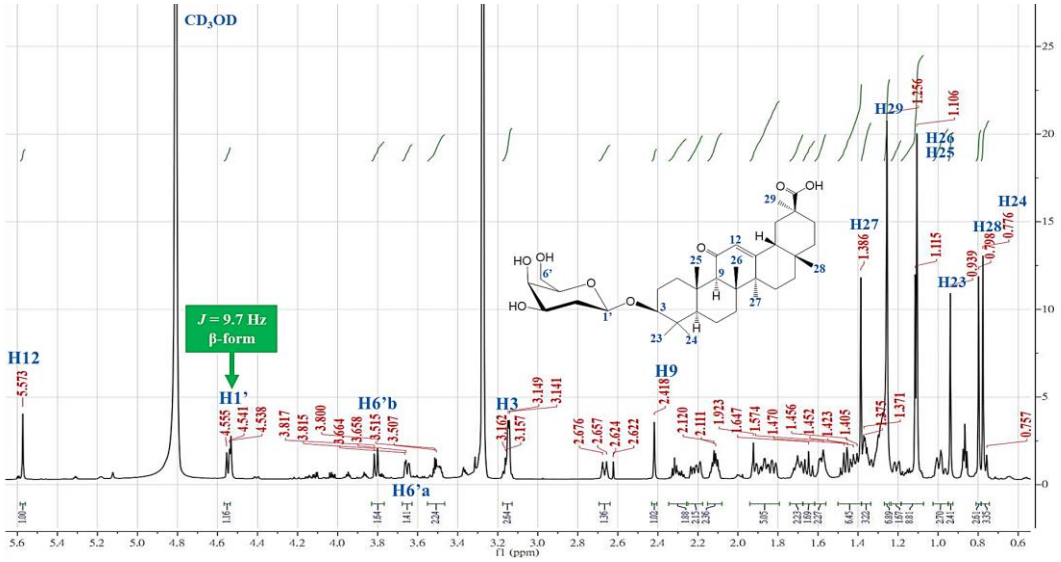

• Signal enhancement – 2.14 (H2'b)
• Affected signal – 4.57 (H1'), 1.48 (H2'a), 3.52 (H3')
ppm

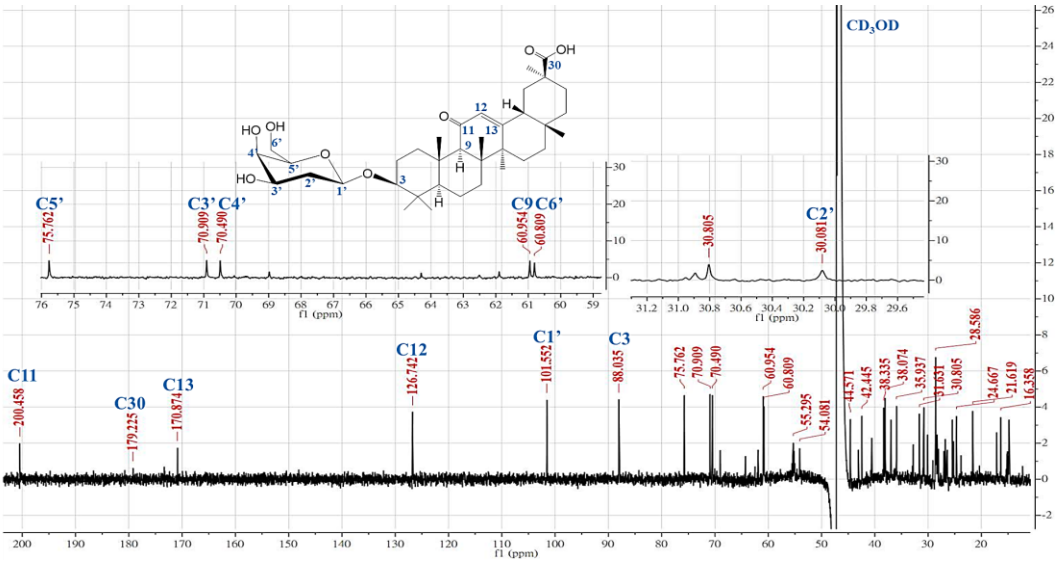
Fig. S9 High–resolution UPLC/ESI MS and NMR of 18β-GA-β-3-O-β-2Gal (**11**).

(A) ^{1}H NMR, (B) ^{13}C NMR, (C) DEPT, (D) ^{1}H-^{1}H COSY, (E) NOE.

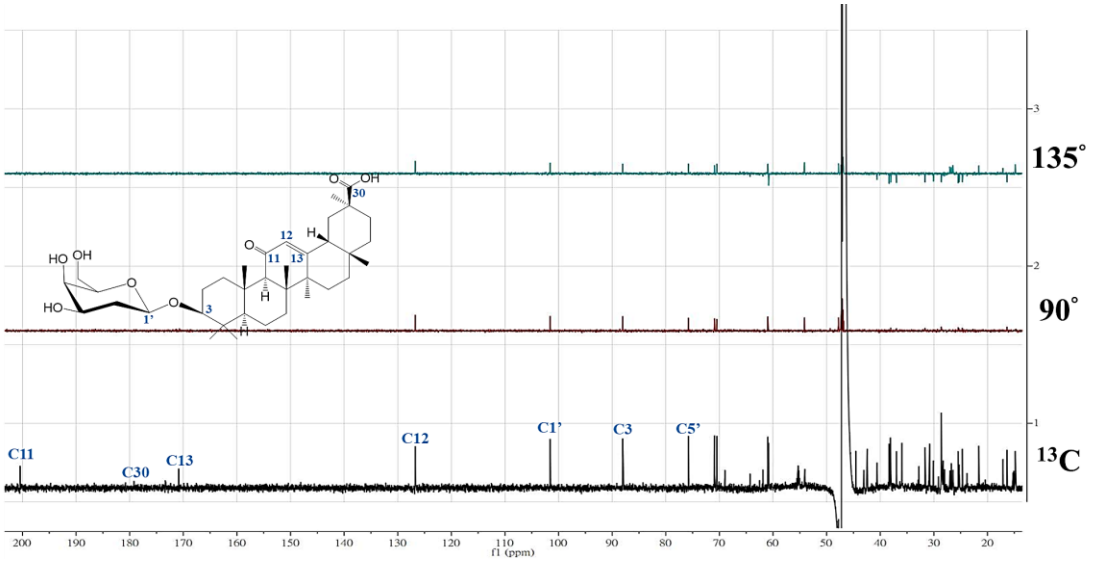
(A) ^{1}H NMR



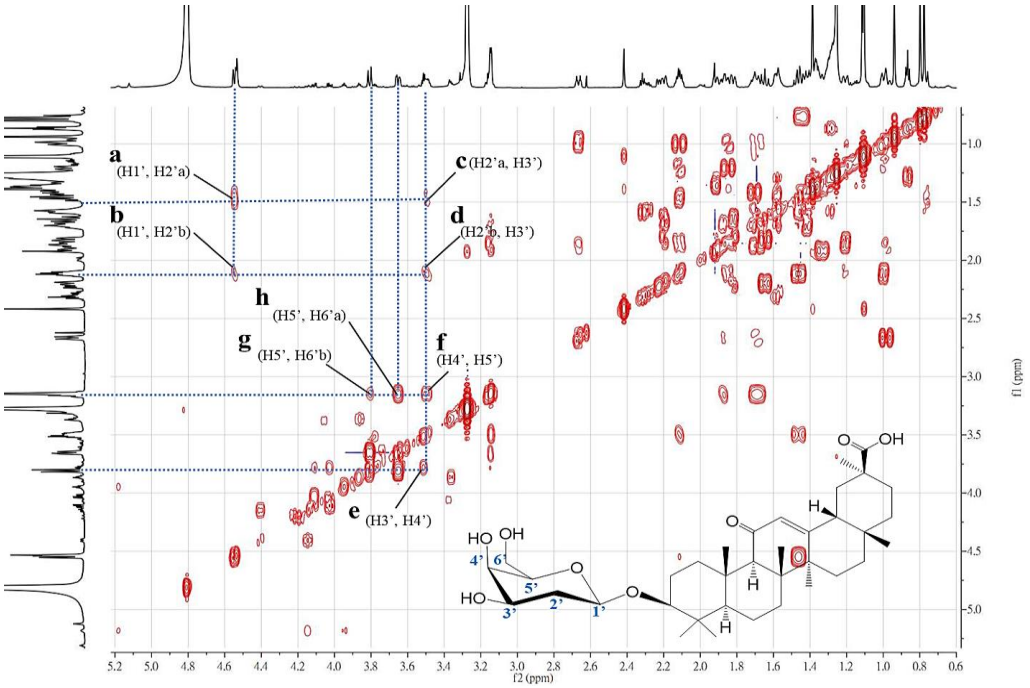
(B) ^{13}C NMR



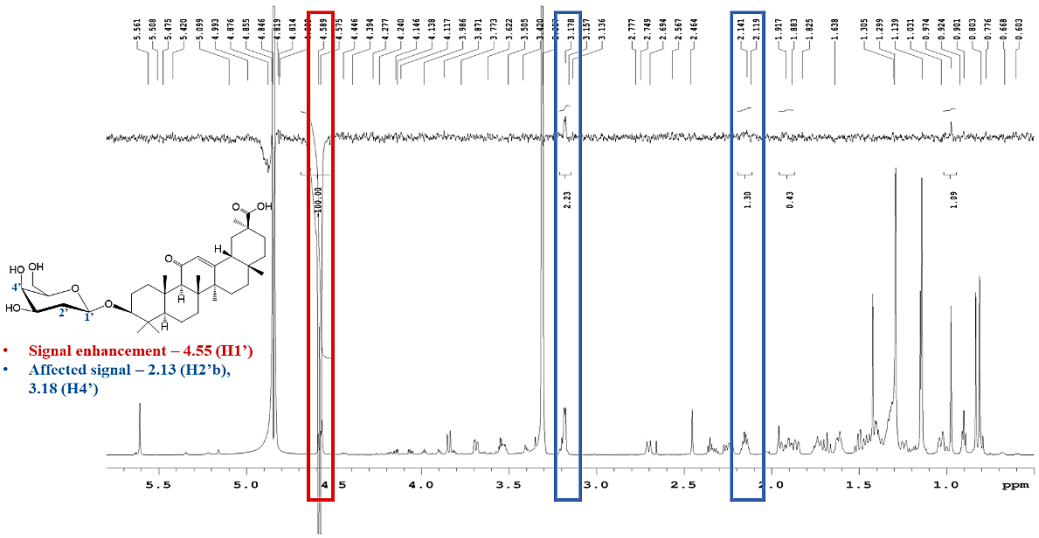
(C) DEPT

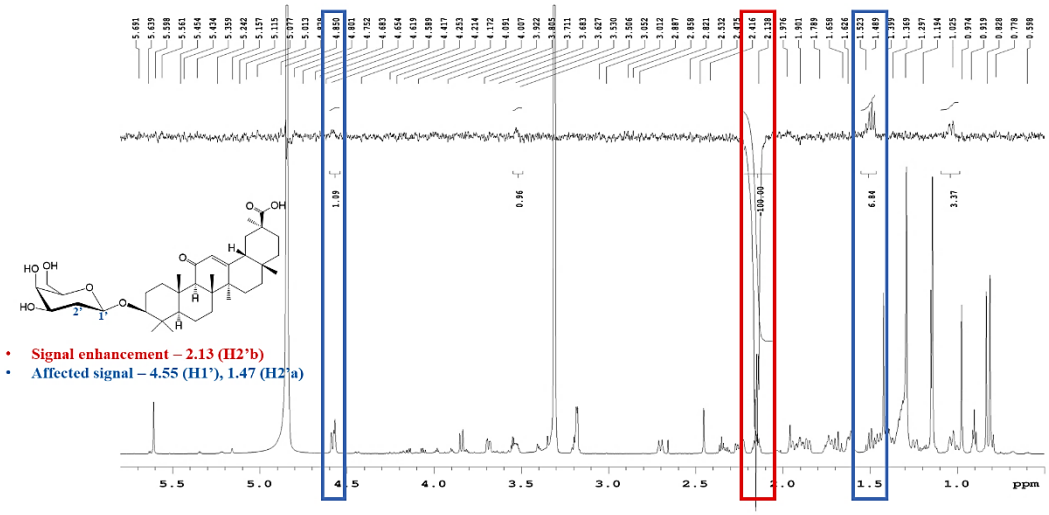


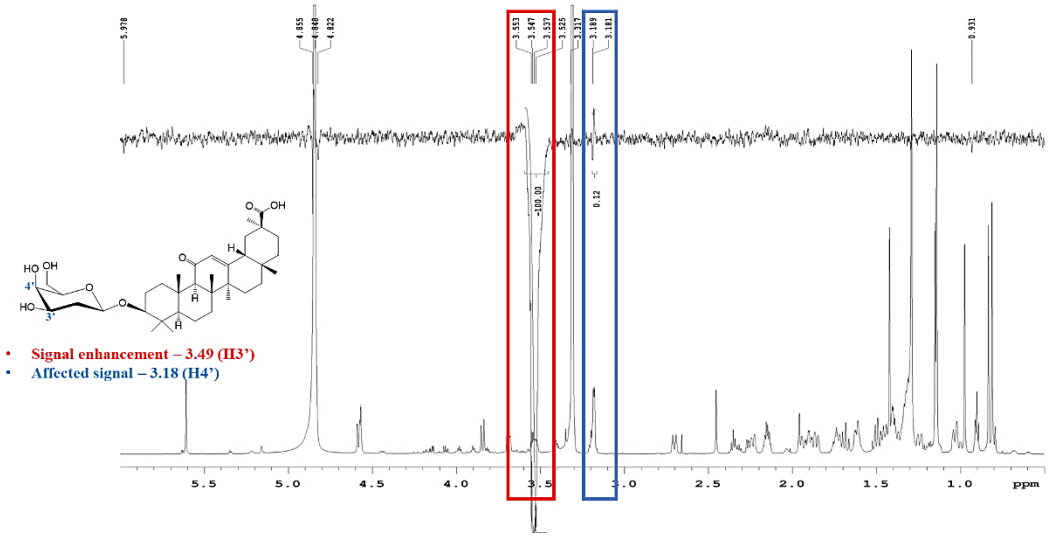
(D) ^{1}H-^{1}H COSY



(E) NOE


Signal enhancement – 4.55 (H1')
Affected signal – 2.13 (H2'b), 3.18 (H4')


Signal enhancement – 2.13 (H2'b)
Affected signal – 4.55 (H1'), 1.47 (H2'a)


Signal enhancement – 3.49 (H3')
Affected signal – 3.18 (H4')

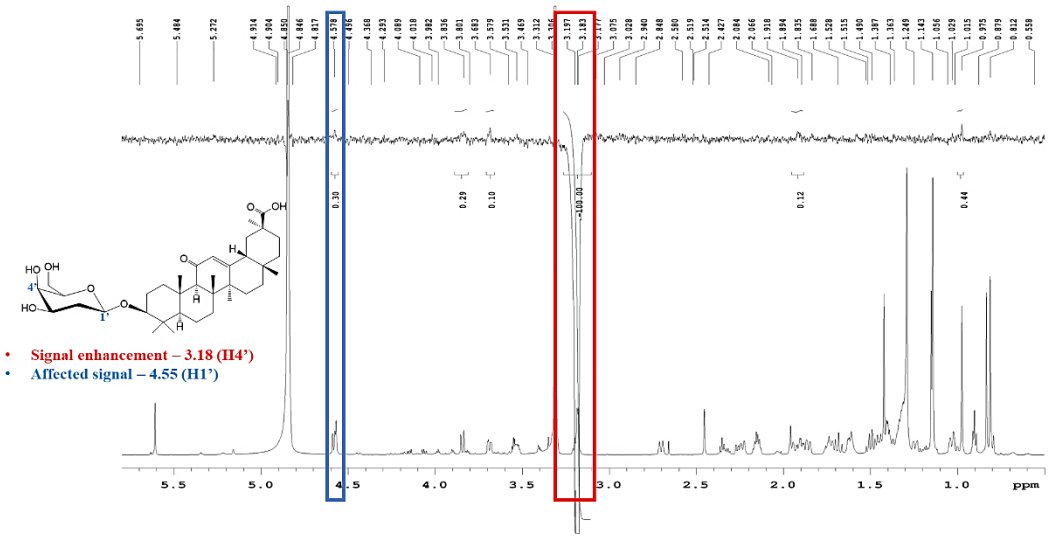

• Signal enhancement – 3.18 (H4')
• Affected signal – 4.55 (H1')
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

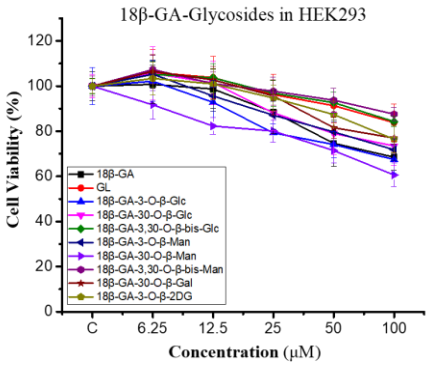
Table S1 Inhibitory effects of 18β-GA glycosides on RBD of the SARS-CoV-2 binding to ACE2.

Compound	Activity(%)	Inhibition (%)
Dalbavancin (Inhibitor control)	37.18 ± 1.20	62.82
GL (**1**)	82.32 ± 4.18	17.68
18β-GA (**2**)	94.55 ± 1.98	5.45
18β-GA-3-O-β-Glc (**3**)	94.15 ± 3.45	5.58
18β-GA-30-O-β-Glc (**4**)	76.16 ± 2.53	23.84
18β-GA-3,30-O-β-bis-Glc (**5**)	74.72 ± 3.17	25.28
18β-GA-3-O-β-Man (**6**)	78.67 ± 2.54	21.33
18β-GA-30-O-β-Man (**7**)	80.73 ± 3.18	19.27
18β-GA-3,30-O-β-bis-Man (**8**)	89.72 ± 2.68	10.28
18β-GA-30-O-β-Gal (**9**)	100.00 ± 0.00	0.00
18β-GA-3-O-β-2DG (**10**)	70.66 ± 1.00	29.34
18β-GA-3-O-β-2DGal (**11**)	96.61 ± 3.09	3.39

Table S2 IC_{50} values of GL, 18β-GA, and 18β-GA glycosides against M^{pro}.

Name	IC_{50} (μM)
GC376 (Inhibitor control)	0.82 ± 0.09
GL (**1**)	> 50
18β-GA (**2**)	23.12 ± 3.29
18β-GA-3-O-β-Glc (**3**)	8.70 ± 0.80
18β-GA-30-O-β-Glc (**4**)	4.77 ± 0.49
18β-GA-3,30-O-β-bis-Glc (**5**)	> 50
18β-GA-3-O-β-Man (**6**)	> 50
18β-GA-30-O-β-Man (**7**)	32.46 ± 7.28
18β-GA-3,30-O-β-bis-Man (**8**)	> 50
18β-GA-30-O-β-Gal (**9**)	> 50
18β-GA-3-O-β-2DG (**10**)	> 50
18β-GA-3-O-β-2DGal (**11**)	> 50

Table S3 Cytotoxicity (IC_{50} values) of GL, 18β-GA, and 18β-GA glycosides against HEK293 cells.



	HEK293 Cells IC_{50} (μM)
18β-GA	> 100
Glycyrrhizin (GL)	> 100
18β-GA-3-O-β-Glc	> 100
18β-GA-30-O-β-Glc	> 100
18β-GA-3,30-O-β-bis-Glc	> 100
18β-GA-3-O-β-Man	> 100
18β-GA-30-O-β-Man	> 100
18β-GA-3,30-O-β-bis-Man	> 100
18β-GA-30-O-β-Gal	> 100
18β-GA-3-O-β-2DG	> 100