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Pentacyclic triterpenes. Part 5: Synthesis and SAR study of corosolic acid derivatives as inhibitors of glycogen phosphorylases

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Abstract—The synthesis and biological evaluation of corosolic acid derivatives and related compounds as inhibitors of rabbit muscle glycogen phosphorylase a is described. Within this series of compounds, **8** (IC_{50} = 7.31 μM), **12d** (IC_{50} = 3.26 μM), and **12e** (IC_{50} = 5.1 μM) exhibited more potent activities than the parent compound **1** (IC_{50} = 20 μM). SAR of these compounds is also discussed.

Corosolic acid (**1**) (Fig. 1) is a naturally occurring pentacyclic triterpene acid which has attracted much attention due to its hypoglycemic activity.[1] Recently, the Japanese researchers proved for the first time that **1** exhibited a glucose-lowering effect on postchallenge plasma glucose levels in humans.[2] The mechanism of hypoglycemic action of **1** is not clear, nevertheless, according to the reported results of mechanic studies, it is likely that **1** might exert its glucose-lowing effect through multiple targets.[1a,3]

We have previously reported that **1** and related pentacyclic triterpenes are natural inhibitors of glycogen phosphorylase (GP), and they might reduce blood glucose, at least in part, through inhibiting excessive hepatic glycogenolysis.[3c] GP is the enzyme responsible for glycogen breakdown to produce glucose and related metabolites for energy supply. Recently, new insight into this enzyme has stimulated research interest in identifying safe and effective GP inhibitors as therapeutic agents for treatment of diabetes.[4] Several structural classes of GP inhibitors have been developed, and at least six binding sites have been identified in GP.[5] Although there is still a debate about whether GP could be an ideal drug target for diabetes, in our point of view, the key issue is about how to find PROPER small molecules to PROPERLY modulate GP, and to maintain chronic efficiency of these compounds with less side-effects, rather than about whether GP is a PROPER drug target.

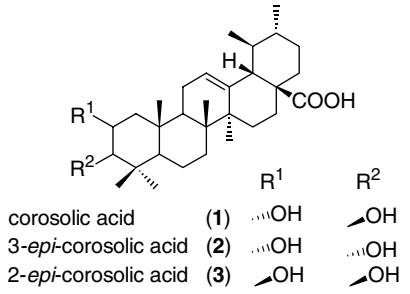

Figure 1. The structures of corosolic acid, 3-*epi*-corosolic acid and 2-*epi*-corosolic acid.

With **1** as a lead compound for GP inhibition, we carried out structural modification and SAR study. For example, in order to determine how the configuration of 2,3-dihydroxyl groups of **1** affected the enzyme inhibitory activity, we synthesized 3-*epi*-corosolic acid (**2**) and 2-*epi*-corosolic acid (**3**), which are naturally occurring isomers of **1**. Moreover, we synthesized a series of derivatives of **1** in order to determine the structure–activity relationship.

The syntheses of **1**, **2**, and **3** are summarized in Scheme 1. Herein, preparation of **1** was based on a

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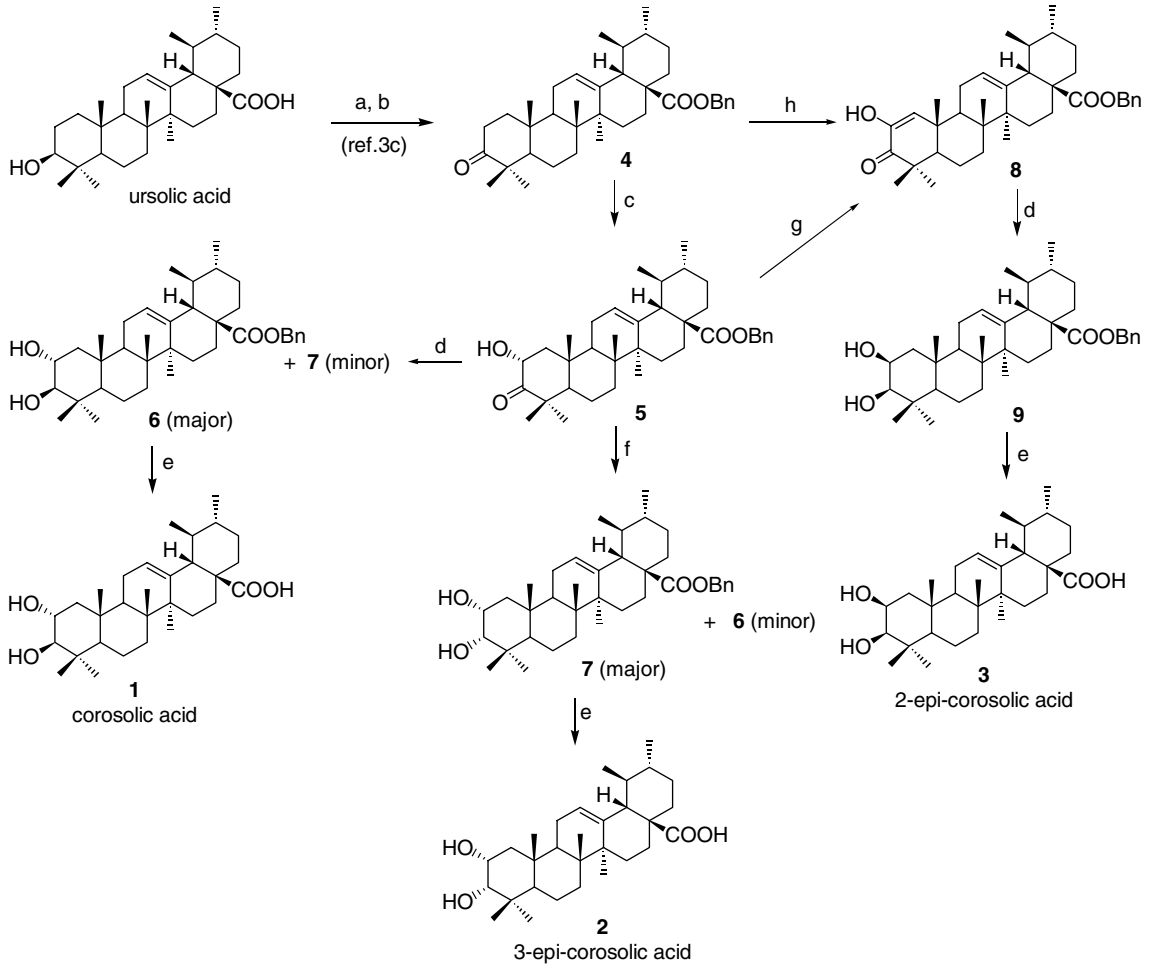
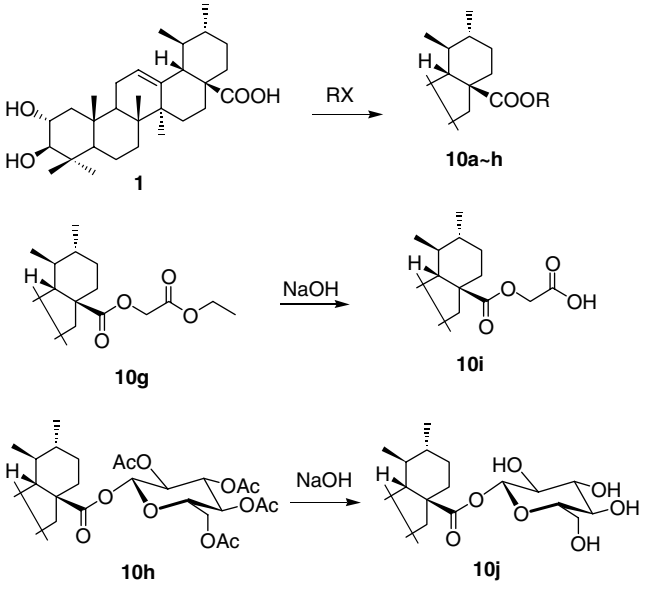


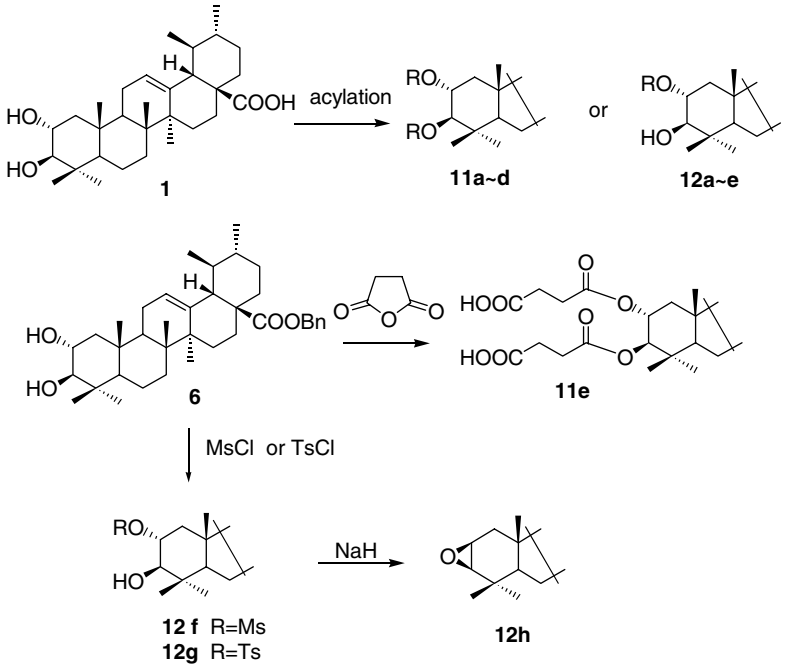
Scheme 1. Reagents and conditions: (a) BnCl, K_2CO_3/DMF, 50 °C; (b) PCC, DCM, rt, 80% for two steps; (c) *m*-CPBA, MeOH/DCM/H_2SO_4 (cat.), rt, 70%; (d) $NaBH_4$, MeOH/THF; rt, 68% for **6**; 14% for **7**; 69% for **9**. (e) H_2, Pd–C (10%), rt, 97% for **1**; 92% for **2**; 88% for **3**; (f) Al(OPr-*i*)$_3$, *i*-PrOH, reflux, 51% for **7** and 6% for **6**; (g) KOH, MeOH/DMF, rt, 62%; (h) *t*-BuOK, *t*-BuOH, air, rt, 85%.

ketone-hydroxylation strategy[6,7] which was different from our previously reported method.[3c] As shown in Scheme 1, stereoselective hydroxylation of ursonic acid benzyl ester (**4**)[3c] with *m*-CPBA in MeOH–DCM–H_2SO_4 (cat.) gave 2α-hydroxy ketone **5** in 70% yield. Reduction of **5** with $NaBH_4$ in THF–MeOH gave 2α,3β-diol **6** as the major product (68%), together with 2α,3α-diol **7** as the minor product (14%). On the other hand, Meerwein–Pondorf reduction[8] of **5** gave **7** as the major product (51%), together with **6** as the minor product (6%). Hydrogenolysis of **6** and **7** over palladium–carbon in THF furnished corosolic acid (**1**) and 3-*epi*-corosolic acid (**2**) in high yields, respectively.[9] Treatment of **5** with KOH in MeOH–DMF gave diketone **8** (62%), which existed in the form of α,β-unsaturated ketone.[10] Alternatively, treatment of ketone **4** with a large excess of *t*-BuOK under air at room temperature directly gave **8** in good yield (85%). Reduction of **8** with $NaBH_4$ gave 2β,3β-diol **9** (69%), which was further converted to 2-*epi*-corosolic acid (**3**) by hydrogenolysis over palladium–carbon.[11]

Structural modification of corosolic acid was mainly focused on C-28 and A-ring. The synthetic routes are outlined in Schemes 2 and 3. Esterification of **1** with various alkyl halides or 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide gave the corresponding esters **10a–h** in good



Scheme 2. Synthesis of derivatives **10a–j**.



Scheme 3. Synthesis of derivatives **11a–e** and **12a–h**.

yields. Hydrolysis of **10g** and **10h** afforded **10i** and **10j** in high yields, respectively. Acylation of **1** or **6** afforded diacylates **11a–e** or monoacylates **12a–e**. As we expected, the reactivity was quite different between 2-hydroxyl and 3-hydroxyl of **1** or **6**, and this kind of discrimination could be used to get 2,3-diacylates or 2-monoacylates. In this regard, diacylates **11a–e** were obtained by using a large excess of acylating agents, or by heating the reaction mixture in the presence of DMAP as a catalyst. On the other hand, monoacylates **12a–e** were obtained under relatively mild conditions such as controlled amount of acylating agents and low reaction temperature (normally room temperature). Mesylation or tosylation of **6** in pyridine at room temperature gave mesylate **12f** (26%) or tosylate **12g** (32%), respectively. Treatment of both **12f** and **12g** with NaH afforded epoxide **12h** (from **12f**: 32%; from **12g**: 73%). The detailed reagents, conditions, and yields for each reaction are shown in Tables 1 and 2.

Compounds **1**–**3**, **5**–**9**, **10a–j**, **11a–e**, and **12a–h** were biologically evaluated for their inhibitory activity against rabbit muscle glycogen phosphorylase a (RMGPa). As described previously,[12] the activity of RMGPa was measured through detecting the released amount of phosphates from glucose-1-phosphates in the direction of glycogen synthesis. The assay results are outlined in Tables 3 and 4. Initially, we investigated how the configuration of 2-hydroxy and 3-hydroxy groups affected the GP inhibitory activity. The inhibitory potency decreases sharply when both 2-hydroxy and 3-hydroxy groups are in the same side of A-ring (e.g., **1** vs **2**, **1** vs **3**, **6** vs **7**, and

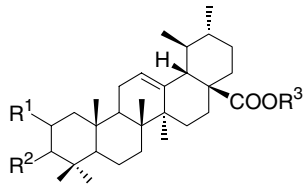
Table 1. Reagents, conditions, and yields for synthesis of **10a–j**

Products	Reagents and conditions	Yield (%)
10a	MeI, K_2CO_3/DMF, rt	69
10b	$BrCH_2CH_3$, K_2CO_3/DMF, 50 °C	90
10c	$BrCH_2CH_2CH_3$, K_2CO_3/DMF, 60 °C	92
10d	$BrCH_2CH_2CH_2CH_3$, K_2CO_3/DMF, 50 °C	89
10e	$BrCH_2CH_2{=}CH_2$, K_2CO_3/DMF, rt	65
10f	1-(Chloromethyl)-1*H*-pyrazole, K_2CO_3/DMF, rt	60
10g	$BrCH_2COOEt$, K_2CO_3/DMF, rt	90
10h	2,3,4,6-Tetra-*O*-acetyl-α-D-glucosyl bromide, K_2CO_3/DMF, rt	94
10i	NaOH (4N), H_2O/THF, rt	93
10j	NaOH (4N), H_2O/THF, rt	92

Table 2. Reagents, conditions, and yields for synthesis of **11a–e** and **12a–h**

Products	Reagents and conditions	Yield (%)
11a	Ac_2O (126 equiv), pyridine, rt	93
11b	Propionic anhydride (10 equiv), pyridine, DMAP, rt	27
11c	Butyric anhydride (19 equiv), pyridine, 80 °C	51
11d	Succinicanhydride (288 equiv), pyridine, DMAP, 80 °C	56
11e	Succinicanhydride (20 equiv), pyridine, DMAP, 80 °C	66
12a	Ac_2O (4.5 equiv), pyridine, rt	6
12b	$(CH_3CH_2CO)_2O$ (6 equiv), pyridine, rt	48
12c	Butyric anhydride (6 equiv), pyridine, rt	12
12d	Heptanoic acid anhydride (5 equiv), pyridine, rt	41
12e	Benzoic acid (8 equiv), THF/DCC/DMAP, rt	27
12f	MsCl (5 equiv), pyridine, rt	26
12g	TsCl (16 equiv), pyridine, rt	32
12h	NaH, THF, rt	32 (from **12f**) 73 (from **12g**)

Table 3. Effects of configuration of 2,3-dihydoxyl groups of corosolic acid analogues on inhibitory activity against RMGPa



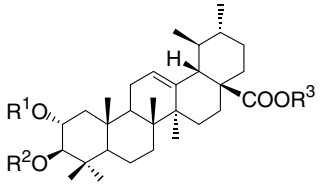
Compound	R^1	R^2	R^3	IC_{50}[a] (μM)
1	α-OH	β-OH	H	20
2	α-OH	α-OH	H	212.5
3	β-OH	β-OH	H	115.5
5	α-OH	=O	Bn	31.8
6	α-OH	β-OH	Bn	41.2
7	α-OH	α-OH	Bn	323.5
8	=O[b]	=O	Bn	7.31
9	β-OH	β-OH	Bn	107.9
caffeine				114

[a] Values are means of three experiments.
[b] Existed in the form of 1,2-enol as shown in Scheme 1.

6 vs **9**). Surprisingly, 2,3-diketone **8** (existing in the form of α,β-unsaturated ketone) is a more potent GP inhibitor (IC_{50} = 7.31 μM) than its corresponding 2,3-diol analogues (e.g., **1**, **2**, **3**, and **6**).

As shown in Table 4, the inhibitory potency decreases as the size of C-28 substituents increases (e.g., inhibitory potency: **10a** > **10b** > **10c** > **10** ≈ **10e** > **10g** > **10h**), indicating that small C-28 substituents of corosolic acid derivatives may be preferred for GP inhibition. This trend seems different from that of maslinic acid derivatives which somehow prefer big C-28 side chains for good potency.[6] Corosolic acid 28-*O*-β-D-glucopyranosyl ester (**10j**) was about 3-fold more potent than its 2,3,4,6-tetra-*O*-acetyl ester **10h**. In the series of A-ring modified triterpenes, the potency of 2,3-diacyloxyl triterpenes was not quite different from that of the corresponding 2-monoacyloxyl triterpenes (e.g., **11a** vs **12a**, **11b** vs **12b**, and **11c** vs **12c**). An increase in polarity at 2,3-diacyl substituents resulted in a significant loss of potency (e.g., **11c** vs **11d**and **11e**). The SAR of 2-monoacyl side chains was less predictive (**12a–e**). Within this series of compounds, **12d** (IC_{50} = 3.26 μM) and **12e** (IC_{50} = 5.1 μM) exhibited more potent inhibitory activity than the parent compound **1**, indicating that modification on A-ring of **1** might improve the potency of the triterpenes against GP.

Table 4. Effects of variation of substituents in corosolic acid derivatives on RMGPa



Compound	R^1	R^2	R^3	IC_{50}[a] (μM)
10a	H	H	Me	33.7
10b	H	H	Et	46.7
10c	H	H	Pr	82.5
10d	H	H	Bu	92.1
10e	H	H	Vinyl	91.2
10f	H	H	(1H-Pyrazol-1-yl)methyl	62.1
10g	H	H	CH_2COOEt	102.0
10h	H	H	β-Glc(Ac)$_4$	373.1
10i	H	H	CH_2COOH	NI[b]
10j	H	H	β-Glc	105.6
11a	Ac	Ac	H	35.0
11b	$COCH_2CH_3$	$COCH_2CH_3$	H	73.7
11c	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	H	78.7
11d	$CO(CH_2)_2COOH$	$CO(CH_2)_2COOH$	H	NI[b]
11e	$CO(CH_2)_2COOH$	$CO(CH_2)_2COOH$	Bn	722.5
12a	Ac	H	H	37.7
12b	$COCH_2CH_3$	H	H	96.7
12c	$CO(CH_2)_2CH_3$	H	H	77.9
12d	$CO(CH_2)_4CH_3$	H	H	3.26
12e	Bz	H	H	5.1
12f	Ms	H	Bn	43.4
12g	Ts	H	Bn	443.8
12h	2β,3β-epoxy		Bn	69.5

[a] Values are means of three experiments.
[b] NI, no inhibition.

Control of glycogen metabolism plays a key role in maintaining blood glucose homeostasis in both fed and fasted states.[13] Modulation of key elements in glycogen metabolism such as GP and related signaling molecules represents a promising therapeutic approach to diabetes.[14] Corosolic acid and other pentacyclic triterpenes may exert their glucose-lowering effect through multiple targets including GP. Further studies are needed to address the detailed molecular mechanisms and to biologically evaluate pentacyclic triterpenes as promising anti-diabetic agents with preventive effects against diabetic complications.

In summary, two steric isomers of corosolic acid and a series of corosolic acid derivatives have been synthesized and tested for inhibitory activity against RMGPa. Among the tested compounds, **8**, **12d**, and **12e** are much more potent than the lead compound **1**. SAR studies show that A-ring modification of corosolic acid may improve the potency for GP inhibition. Extensive structural modifications, biological evaluation, and mechanistic studies on corosolic acid and other pentacyclic triterpenes are ongoing in this laboratory in order to find potent and low-toxic anti-diabetic agents with preventive and therapeutic effects against diabetic complications.

Acknowledgments

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9. Analytical data for compound **2**: IR (KBr, cm^{-1}) 3454, 2928, 1688, 1630, 1456, 1383, 1038, 665; ^1H NMR (pyridine-d_5, 300 MHz): δ 0.88, 0.94, 1.03, 1.11, 1.25 (each 3H, s), 0.92 (3H, d, $J = 6.2$ Hz), 0.96 (3H, d, $J = 6.3$ Hz), 2.61 (1H, d, $J = 11.1$ Hz, H-18), 3.74 (1H, d, $J = 2.6$ Hz, H-3β), 4.28 (1H, m, H-2β), 5.44 (1H, t, $J = 3.3$ Hz, H-12); ^{13}C NMR (pyridine-d_5, 300 MHz): δ 16.8, 17.47, 17.50, 18.5, 21.4, 22.3, 23.7, 23.9, 24.9, 28.7, 29.5, 31.1, 33.5, 37.4, 38.6, 38.8, 39.4, 39.5, 40.2, 42.6, 43.0, 47.9, 48.1, 48.7, 53.6, 66.1, 79.4, 125.6, 139.3, 179.9; MS: 495 $[M+Na]^+$; HRMS: Calcd for $C_{30}H_{48}O_4$–H: 471.3474; Found: 471.3485.
10. Analytical data for compound **8**: IR (KBr, cm^{-1}) 3435, 2970, 2926, 2870, 1724, 1664, 1454, 1404, 1232, 1144, 1053, 746, 696, 534; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.69, 1.06, 1.12, 1.20, 1.21 (each 3H, s), 0.86 (3H, d, $J =$ 6.4 Hz), 0.95 (3H, d, $J = 6.1$ Hz), 2.31 (1H, d, $J = 11.1$ Hz, H-18), 5.01 and 5.09 (each 1H, d, $J = 12.4$ Hz, CH_2Ar), 5.29 (1H, t, $J = 3.5$ Hz, H-12), 5.93 (1H, s, HO-2), 6.35 (1H, s, H-1), 7.34 (5H, m, H-Ar); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 17.0, 17.6, 18.7, 19.7, 21.1, 21.8, 23.3, 23.5, 24.3, 27.2, 28.0, 30.7, 32.9, 36.6, 38.3, 38.9, 39.1, 40.3, 42.5, 43.1, 43.9, 48.2, 53.1, 54.0, 66.0, 125.1, 128.0, 128.2, 128.4, 136.4, 138.7, 143.8, 177.1, 201.1; MS: 581 $[M+Na]^+$; HRMS: Calcd for $C_{37}H_{50}O_4$–H–H: 557.3631; Found: 557.3651.
11. Analytical data for compound **3**: IR (KBr, cm^{-1}) 3439, 2926, 1691, 1454, 1381, 1051, 787, 766, 663; ^1H NMR (pyridine-d_5, 300 MHz): δ 0.97 (3H, d, $J = 5.7$ Hz), 1.03 (3H, d, $J = 6.4$ Hz), 1.11, 1.25, 1.27, 1.36, 1.51 (each 3H, s), 2.66 (1H, d, $J = 11.3$ Hz, H-18), 3.46 (1H, d, $J = 3.9$ Hz, H-3α), 4.41 (1H, m, H-2α), 5.50 (1H, m, H-12); ^{13}C NMR (pyridine-d_5, 300 MHz): δ 16.8, 17.5, 18.2, 18.7, 21.4, 23.9, 24.0, 25.0, 28.7, 30.3, 31.2, 33.7, 37.3, 37.5, 38.8, 39.5, 39.6, 40.2, 42.8, 45.2, 48.1, 48.5, 53.7, 56.0, 71.5, 78.4, 125.9, 139.3, 179.9; MS: 495 $[M+Na]^+$; HRMS: Calcd for $C_{30}H_{48}O_4$–H: 471.3474; Found: 471.3481.

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