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Synthesis of β-hederin and Hederacolchiside A_1: triterpenoid saponins bearing a unique cytotoxicity-inducing disaccharide moiety

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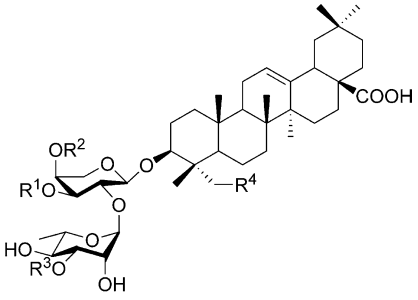
Abstract—A facile synthetic approach toward oleanolic acid glycoside bearing α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl moiety, a unique oligosaccharide that strongly induces antitumor activity of oleanane-type triterpenoid saponins, was developed. Based on this approach β-hederin (oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside) was efficiently prepared from oleanolic acid through stepwise glycosylation in linear eight steps with 52% overall yield, while Hederacolchiside A_1 (oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside) in linear 13 steps with 20% overall yield.

Keywords: β-Hederin; Hederacolchiside A_1; α-L-Rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; Cytotoxicity-inducing oligosaccharide; Triterpenoid saponin; Stepwise glycosylation

1. Introduction

β-Hederin (**1**) (Fig. 1), namely oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, is a oleanane-type triterpenoid saponin widely distributed in the nature.[1–3] The most attractive property of β-hederin is its prominent cytotoxicity to various human tumor cell lines such as HL-60 ($IC_{50} = 4.4$ μg/mL) and A549, PC3, DLD1, M4 Beu, PA 1, etc.[4,5] Notably, many oleanane-type triterpenoid saponins bearing α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl moiety at 3-OH (for instance, **2**–**7**) generally have significant antitumor activity.[4–7] For a distinguished example, Hederacolchiside A (hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside, **2**) has been used for chemotherapy of solid tumor.[8] Hederacolchiside A_1 (oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside, **3**)[2,9] has been found to have better activity than **1** and **2** to all seven tumor cell lines investigated in the literature.[5] Cytotoxicity of their aglycons, oleanolic acid (**8**) and hederagenin (**9**) (Fig. 2), is very weak, and their glycosides without α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl disaccharide moiety at C-3 commonly show much weaker activity.



	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	Glc*p*-	H	OH
3	H	Glc*p*-	H	H
4	H	H	Glc*p*-	H
5	H	H	Xyl*p*-	OH
6	H	H	Glc*p*-(1→4)-Glc*p*-	H
7	Glc*p*-	H	H	H

Figure 1. Glc*p*- = β-D-glucopyranosyl; Xyl*p*- = β-D-xylopyranosyl.

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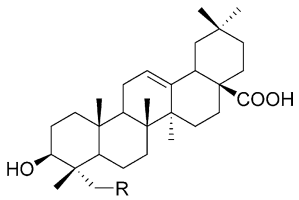


8 R = H
9 R = OH

Figure 2.

Therefore, the α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl disaccharide moiety has proved to be a unique sugar sequence which strongly induces antitumor activity of oleanane-type glycosides[4–7] and has been attracting more and more attention of researchers.

Several hypotheses have been suggested for the mechanism of their antitumor activity.[10–12] Initial structure–activity relationship (SAR) study has shown that a glucopyranosyl moiety attached to C-4 of arabinose increases the cytotoxicity against malignant melanoma M4 Beu markedly,[5] while methylation of C-28 carboxyl of the aglycon does not affect the cytotoxicity against HL-60 significantly.[4] However, research in this domain is sporadic and unsystematic mainly due to the difficulty in obtaining adequate analogical glycosides from nature for broad and thorough SAR study. Based on our experience with synthesis and bioactivity study of antitumor natural products,[13] we get excited about preparing β-hederin and a sufficiency of analogues and derivatives through chemical synthesis for SAR investigation and development of desirable antitumor agents.

2. Results and discussion

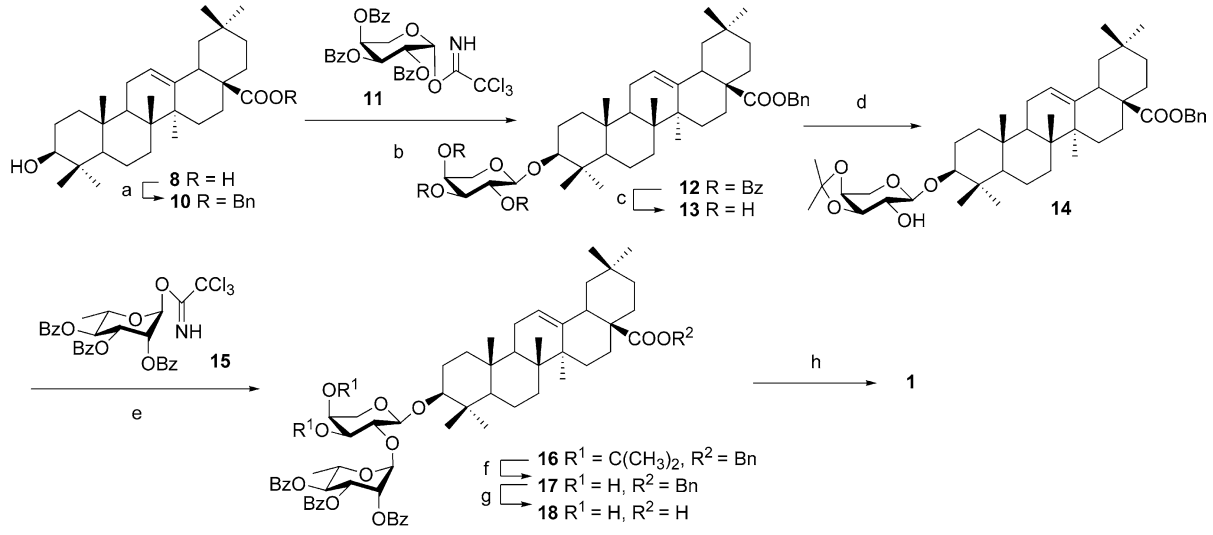
So far no synthetic approach toward oleanolic acid glycoside bearing this unique disaccharide, α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl moiety, has been reported yet. This disaccharide bears a (1→2)-linkage, thus the usual strategy of preparing the disaccharide donor followed by coupling with the aglycon is not adoptable, otherwise a mixture of α- and β-anomers would be afforded without a neighboring participating group on C-2 of arabinose residue. Alternately, stepwise glycosylation generally affords the exclusive 1,2-*trans*-glycoside linkage so long as a neighboring participating group is on C-2 of sugar donor. Moreover, this strategy provides a versatile approach for preparing a glycoside family with variety simply by altering monosaccharide donors.[14] Therefore, stepwise glycosylation was adopted in our work.

It is known that glycosylation of C-3 of triterpenoid requires a benzoyl at C-2 of sugar donor, otherwise a low yield would be obtained with the ortho ester formation and the acyl transfer being serious side reactions due to the steric hinderence of sugar acceptor.[15,16] Therefore, newly evolved protecting group AZMB(2-azidomethylbenzoyl)[17,18] is often employed in (1→2)-linkage construction,[19,20] for it is stable enough to prevent the by-product forming and can be selectively removed under the treatment of Bu_3P. However, selective introduction of a special neighboring participating protective group such as AZMB to C-2 of sugar donors is rather boring in preparation of numerous glycosides with various saccharide moiety. A linear synthetic route employing the readily available perbenzoylated sugar donors is more efficient and convenient for this work.

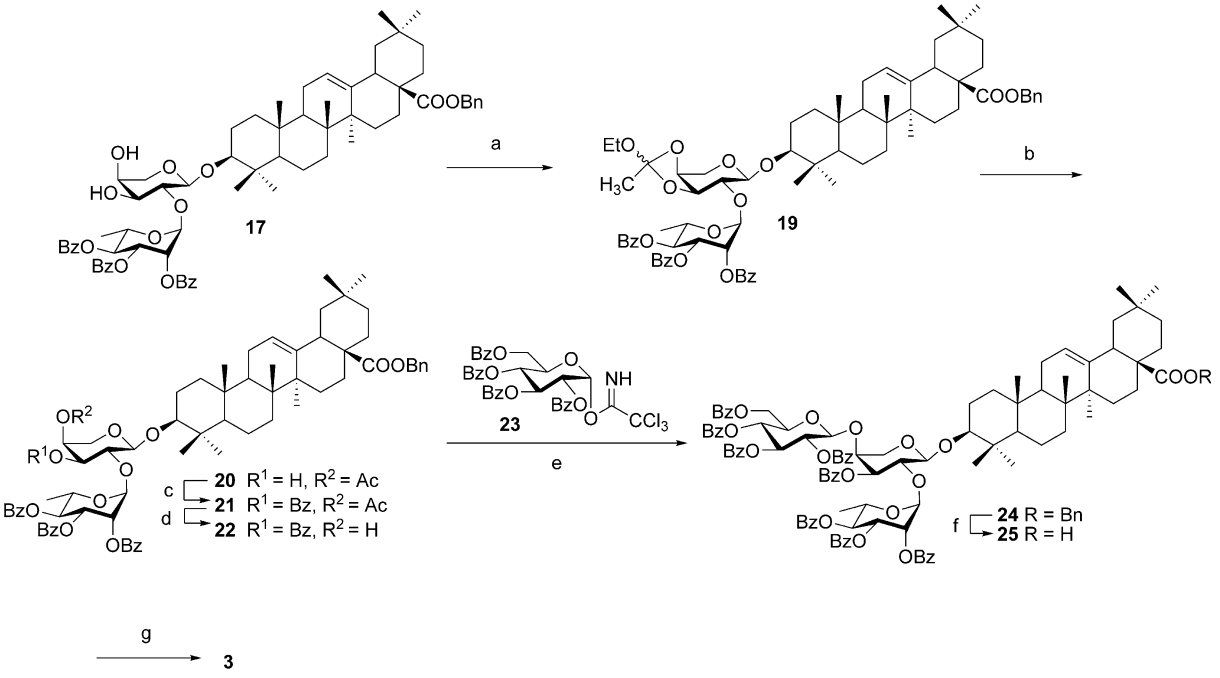
Accordingly, perbenzoylated trichloroacetimidate **11**[21] and **15**[22] (Scheme 1) were chosen as sugar donors, which can be easily prepared from L-arabinose and L-rhamnose, respectively. Ester linkage at C-28 of oleanolic acid is resistant to basic hydrolysis due to its high steric hinderence, making it difficult and inefficient to deprotect after the glycosylation.[23] Trityl,[21] *tert*-butyldiphenylsilyl,[19] and allyl[24] have been employed to protect the carboxyl of oleanolic acid in previous work. Here, we used benzyl ester (**10**) instead which can be prepared under the treatment of BnBr and Et_3N in dry THF at a high yield and easily removed through catalytic hydrogenation (double bond between C-12 and C-13 of oleanolic acid is inert to catalytic hydrogenation[25]).

As is shown in Scheme 1, esterification of oleanolic acid (**8**) followed by glycosylation with trichloroacetimidate **11** under the promotion of TMSOTf gave **12** in an excellent yield. Debenzoylation of **12** in NaOMe/MeOH without influencing benzyl ester at C-28 produced **13**. Selective shelter of hydroxyl groups at C-3 and C-4 of arabinose residue was successfully carried out using 2,2-dimethoxypropane.[26] Coupling of **14** and **15** promoted by TMSOTf led to partly breakage of isopropylidene group, so glycosylation of **14** was carried out under the promotion of $BF_3{\cdot}Et_2O$ at a low temperature in 79% yield. We had hoped that isopropylidene could be removed automatically after being warmed to rt, but the products became complicated and could not be separated very well through silica gel column chromatography. So Et_3N was added after glycosylation to neutralize the Lewis acid and an additional step of deprotection was performed to provide intermediate **17**. Finally, ready removal of benzyl group through catalytic hydrogenation and benzoyl group in NaOMe/MeOH afforded **1**, whose physical data are in well agreement with the literature reported.[1–3]

Hederacolchiside A_1 (**3**) was further synthesized from key intermediate **17** (Scheme 2). Selective protection of hydroxyl group on equatorial bond of 1,2-diol may be achieved by various methods such as BzCl[27] and Bu_2SnO/BzCl.[28] It is known that cyclic ortho ester formed from 1,2-diol and $CH_3C(OEt)_3$ cleaving under acidic condition generally results in monoacetylation of axial



Scheme 1. Reagents and conditions: (a) BnBr, Et_3N, Bu_4NI, THF, rt, 97%; (b) TMSOTf, CH_2Cl_2, MS 4 Å, rt, 94%; (c) NaOMe, CH_2Cl_2–MeOH, rt, 97%; (d) $Me_2C(OMe)_2$, TsOH, acetone, 0 °C to rt, 89%; (e) $BF_3{\cdot}Et_2O$, CH_2Cl_2, MS 4 Å, −78 °C, 79%; (f) TsOH, CH_2Cl_2–MeOH, rt, 98%; (g) Pd–C, H_2, EtOAc, reflux; (h) NaOMe, CH_2Cl_2–MeOH, rt, 86% (for two steps).



Scheme 2. Reagents and conditions: (a) $CH_3C(OEt)_3$, TsOH, toluene, rt; (b) 80% aq HOAc, rt, 89% (for two steps); (c) BzCl, pyridine, 0 °C to rt; (d) AcCl, CH_2Cl_2–MeOH, 0 °C to rt, 74% (for two steps); (e) TMSOTf, CH_2Cl_2, MS 4 Å, rt, 71%; (f) Pd–C, H_2, EtOAc, reflux; (g) NaOMe, CH_2Cl_2–MeOH, rt, 70% (for two steps).

hydroxyl group in excellent yield.[29,30] Herein, diol **17** was converted into cyclic ortho ester **19** and then cleaved in aq HOAc to furnish **20**, which would be useful in preparation of advanced derivatives glycosylated at C-3 of arabinose residue (for instance, **7**) in future work. Benzoylation of **20** and selective deacetylation at the presence of benzoyl group[31] gave **22**. Free hydroxyl of **22** was then glycosylated by 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **23**[32] under the promotion of TMSOTf. Excessive **23** (2.50 equiv) and promoter (0.40 equiv) were needed in this step to provide a satisfactory yield (71%). The final removal of benzyl and benzoyl furnished **3**, whose physical data are identical with those reported for the natural product.[2,9]

In conclusion, a facile synthetic approach toward oleanolic acid glycoside bearing α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl moiety at C-3 was developed employing perbenzoylated trichloroacetimidates

of monosaccharide as sugar donors. β-Hederin was prepared from oleanolic acid in linear eight steps at an overall yield of 52%. Hederacolchiside A_1 was further synthesized from intermediate **17** through seven steps at a yield of 33% (20% overall yield from oleanolic acid). More advanced monodesmosides and bisdesmosides can be readily synthesized from intermediates **17**, **18**, **20**, **22**, and **25**. This approach is competent for high efficient preparation of analogues and derivatives on a large scale for structure–activity relationship investigation and new chemical entity finding. Further study on preparation and bioactivity evaluation of analogues and derivatives is currently underway.

3. Experimental

3.1. General methods

Commercial reagents were used without further purification unless specialized. Solvents were dried and redistilled prior to use in the usual way. Boiling range of petroleum ether was 60–90 °C. Analytical TLC was performed with silica gel HF254. Preparation column chromatography was performed with silica gel H. Melting points were detected with BÜCHI Melting Point B-540. Optical rotations were measured at the sodium D-line at room temperature with a Perkin–Elmer 241 MC polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX 300 MHz or Avance AV 600 MHz, using Me_4Si as the internal standard if not specially mentioned. *J* Values are given in hertz. ESI-MS were obtained on an Agilent 1100 mass spectrometer. High-resolution mass spectra (FT-ICRMS) were detected on Bruker APEX II mass spectrometer.

3.2. Benzyl oleanolate (10)

A solution of oleanolic acid (1070 mg, 2.34 mmol), BnBr (0.39 mL, 3.28 mmol), Et_3N (0.45 mL, 3.28 mmol) and Bu_4NI (86 mg, 0.24 mmol) in dry THF (20 mL) was stirred at rt overnight. The solvent was evaporated in vacuum and the residue was purified through a silica gel column chromatography (8:1, petroleum ether–EtOAc) to give **10** (1240 mg, 97%) as a white amorphous solid. $[\alpha]_D$ +50.1 (*c* 2.44, $CHCl_3$); $R_f = 0.55$ (4:1, petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 5H, *Ph*CH_2), 5.29 (t, *J* 3.0, 1H, H-12), 5.07 (dd, *J* 17.8, 12.6, 2H, PhC*H*$_2$), 3.21 (dd, *J* 10.3, 4.8, 1H, H-3), 2.91 (dd, *J* 14.0, 4.2, 1H, H-18), 1.13, 0.99, 0.92, 0.90, 0.88, 0.78, 0.61 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.5, 143.7, 136.4, 128.4, 128.0, 127.9, 122.5, 79.0, 65.9, 55.2, 47.6, 46.7, 45.9, 41.7, 41.4, 39.2, 38.7, 38.4, 37.0, 33.9, 33.1, 32.7, 32.4, 30.7, 28.1, 27.6, 27.2, 25.9, 23.6, 23.4, 23.0, 18.3, 16.9, 15.6, 15.3; ESI-MS (*m*/*z*): 1115.9 (2M+Na).

3.3. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-arabinopyranoside (12)

Benzyl ester **10** (895 mg, 1.64 mmol), trichloroacetimidate **11** (1140 mg, 1.88 mmol) and powdered 4 Å molecular sieves (1600 mg) were stirred for 40 min at rt in dry CH_2Cl_2 (18 mL). A dry CH_2Cl_2 solution (1.60 mL) of TMSOTf (0.016 mL, 0.082 mmol) was added dropwise. The mixture was stirred for 20 min followed by addition of Et_3N (0.20 mL) and filtration. The filtrate was concentrated and purified by a silica gel column chromatography (8:1, petroleum ether–EtOAc) to afford **12** (1620 mg, 94%) as a white foam. $[\alpha]_D$ +108.2 (*c* 1.63, $CHCl_3$); $R_f = 0.65$ (4:1, petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09–7.26 (m, 20H, Ar–H), 5.75 (dd, *J* 8.8, 6.5, 1H, H-2′), 5.67 (m, 1H, H-4′), 5.59 (dd, *J* 8.9, 3.5, 1H, H-3′), 5.30 (t, *J* 3.0, 1H, H-12), 5.06 (dd, *J* 19.9, 12.6, 2H, PhC*H*$_2$), 4.79 (d, *J* 6.6, 1H, H-1′), 4.33 (dd, *J* 12.9, 3.8, 1H, H-5′-1), 3.87 (m, 1H, H-5′-2), 3.16 (dd, *J* 11.0, 4.9, 1H, H-3), 2.90 (dd, *J* 10.1, 3.2, 1H, H-18), 1.11, 0.92, 0.90, 0.86, 0.79, 0.66, 0.58 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4, 165.8, 165.6, 165.2, 143.6, 136.4, 133.3, 133.2, 133.1, 129.9, 129.8, 129.7, 129.4, 129.1, 128.4, 128.4, 128.3, 127.9, 127.8, 122.5, 103.1, 90.1, 70.7, 70.2, 68.6, 65.9, 62.6, 55.4, 47.5, 46.7, 45.8, 41.6, 41.3, 39.2, 38.9, 38.4, 36.7, 33.8, 33.1, 32.6, 32.3, 30.7, 27.8, 27.6, 25.8, 23.6, 23.3, 23.0, 18.1, 16.8, 16.2, 15.2; ESI-MS (*m*/*z*): 1013.5 (M+Na).

3.4. Benzyl oleanolate 3-*O*-α-L-arabinopyranoside (13)

To a solution of **12** (1100 mg, 1.11 mmol) in dry CH_2Cl_2–MeOH (1:2, 40 mL) was added a newly prepared NaOMe in MeOH solution (1.0 mol/L, 1.20 mL). The mixture was stirred at rt for 2 h and neutralized with Dowex H^+ resin to pH 7 and then filtered. The filtrate was concentrated and the resulting residue was subjected to a silica gel column chromatography (EtOAc) to gave **13** (734 mg, 97%) as a white amorphous solid. $[\alpha]_D$ +37.5 (*c* 0.77, acetone); $R_f = 0.35$ (EtOAc); ^{1}H NMR (300 MHz, Me_2SO-d_6): δ 7.33 (m, 5H, *Ph*CH_2), 5.17 (br s, 1H, H-12), 5.02 (m, 2H, PhC*H*$_2$), 4.80 (br s, 1H, O*H*), 4.53 (br s, 1H, O*H*), 4.47 (br s, 1H, O*H*), 4.09 (d, *J* 6.0, 1H, H-1′), 3.60 (m, 2H, H-5′-1, H-4′), 3.35–3.32 (m, 3H, H-2′, H-3′, H-5′-2), 2.99 (dd, *J* 10.5, 3.2, 1H, H-3), 2.80 (dd, *J* 9.8, 3.0, 1H, H-18), 1.07, 0.94, 0.87, 0.85, 0.82, 0.73, 0.51 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, Me_2SO-d_6): δ 176.4, 143.4, 136.4, 128.5, 128.0, 127.8, 122.1, 106.0, 87.7, 72.8, 71.1, 67.8, 65.4, 65.3, 55.0, 47.0, 46.2, 45.5, 41.3, 41.1, 38.1, 36.3, 33.2, 32.8, 32.4, 32.1, 30.5, 27.7, 27.1, 25.8, 25.6, 23.4, 23.0, 22.7, 17.8, 16.7, 16.5, 15.2; ESI-MS (*m*/*z*): 701.4 (M+Na).

3.5. Benzyl oleanolate 3-*O*-3,4-*O*-isopropylidene-α-L-arabinopyranoside (14)

To a solution of compound **13** (679 mg, 1.00 mmol) in dry acetone (12 mL) stirred at 0 °C was added $Me_2C(OMe)_2$ (0.31 mL, 2.50 mmol) and TsOH (17.2 mg). The mixture was then allowed to warm up to rt and stirred for 4 h before Et_3N (0.20 mL) was added. The solution was concentrated and purified through a silica gel column chromatography (6:1, petroleum ether–EtOAc) to afford **14** (634 mg, 89%) as a white foam. $[\alpha]_D$ +45.0 (*c* 1.60, $CHCl_3$); $R_f = 0.45$ (4:1, petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H, *Ph*CH_2), 5.28 (t, *J* 3.0, 1H, H-12), 5.07 (dd, *J* 18.7, 12.6, 2H, PhC*H*$_2$), 4.22–4.17 (m, 3H, H-1′, H-4′, H-5′-1), 4.06 (dd, *J* 7.8, 6.1, 1H, H-3′), 3.75 (dd, *J* 13.9, 3.5, 1H, H-5′-2), 3.63 (dd, *J* 7.8, 7.8, 1H, H-2′), 3.12 (dd, *J* 11.5, 4.6, 1H, H-3), 2.91 (dd, *J* 13.8, 3.3, 1H, H-18), 2.30 (br s, 1H, O*H*), 1.54, 1.36 (s each, 3H each, O–(C*H*$_3$)$_2$C–O), 1.11, 0.98, 0.92, 0.89, 0.88, 0.82, 0.60 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4, 143.6, 136.3, 128.3, 127.9, 127.8, 122.4, 110.0, 104.3, 88.9, 78.1, 74.2, 73.2, 65.9, 63.0, 55.4, 47.5, 46.6, 45.8, 41.6, 41.3, 39.2, 39.0, 38.4, 36.6, 33.8, 33.1, 32.6, 32.3, 30.6, 28.2, 28.0, 27.5, 26.0, 25.8, 23.6, 23.3, 22.9, 18.1, 16.8, 16.6, 15.2; ESI-MS (*m*/*z*): 741.4 (M+Na); HRMS: calcd for $C_{38}H_{59}O_7$ (M−Bn): 627.4261; found: *m*/*z* 627.4255.

3.6. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-α-L-arabinopyranoside (16)

A suspension of **14** (560 mg, 0.779 mmol), **15** (630 mg, 1.01 mmol) and powdered 4 Å molecular sieves (850 mg) in dry CH_2Cl_2 (8 mL) was stirred for 40 min and then cooled to −78 °C. $BF_3{\cdot}Et_2O$ (0.070 mL, 0.545 mmol) was added and the mixture was stirred at −78 °C for 2 h before the reaction was quenched by Et_3N (0.20 mL). The suspension was then filtered and the filtrate was concentrated and subjected to a silica gel chromatography (8:1, petroleum ether–EtOAc) to furnish **16** (730 mg, 79%) as a white foam. $[\alpha]_D$ +96.7 (*c* 2.58, $CHCl_3$); $R_f = 0.60$ (4:1, petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12–7.21 (m, 20H, Ar–H), 5.87 (dd, *J* 10.2, 3.3, 1H, H-3″), 5.76 (br s, 1H, H-1″), 5.65 (m, 2H, H-2″, H-4″), 5.30 (t, *J* 3.0, 1H, H-12), 5.07 (dd, *J* 22.4, 12.6, 2H, PhC*H*$_2$), 4.53 (m, 1H, H-5″), 4.47 (d, *J* 3.0, 1H, H-1′), 4.25 (m, 2H, H-3′, H-4′), 4.17 (m, 1H, H-5′-1), 3.90 (dd, *J* 3.0, 3.0, 1H, H-2′), 3.79 (m, 1H, H-5′-2), 3.17 (dd, *J* 11.3, 4.1, 1H, H-3), 2.92 (m, 1H, H-18), 1.55, 1.35 (s each, 3H each, O–(C*H*$_3$)$_2$C–O), 1.34 (d, *J* 6.1, 3H, H-6″), 1.14, 0.95, 0.93, 0.92, 0.90, 0.89, 0.64 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4, 165.7, 165.5, 165.4, 143.7, 136.4, 133.3, 133.2, 132.9, 130.0, 129.7, 129.6, 129.5, 129.4, 129.3, 128.5, 128.4, 128.3, 128.2, 127.9, 122.5, 110.4, 103.3, 95.2, 89.1, 79.2, 75.3, 73.4, 72.0, 70.6, 69.9, 66.5, 65.9, 62.7, 55.8, 47.6, 46.7, 45.8, 41.7, 41.4, 39.3, 39.2, 38.7, 36.7, 33.8, 33.1, 32.7, 32.4, 30.7, 28.2, 27.8, 27.6, 26.1, 25.9, 23.6, 23.4, 23.0, 18.1, 17.5, 16.9, 16.7, 15.3; ESI-MS (*m*/*z*): 1200.0 (M+Na); HRMS: calcd for $C_{65}H_{81}O_{14}$ (M−Bn): 1085.5626; found: *m*/*z* 1085.5619.

3.7. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (17)

TsOH (78 mg) was added to a solution of **16** (705 mg, 0.600 mmol) in CH_2Cl_2–MeOH (1:2, 45 mL) and the solution was stirred at rt. When TLC (2:1, petroleum ether–EtOAc) showed that deprotection had completed, Et_3N (0.40 mL) was added and the mixture was concentrated and purified through a silica gel column chromatography (2:1, petroleum ether–EtOAc) to give **17** (666 mg, 98%) as a white amorphous solid. $[\alpha]_D$ +77.3 (*c* 2.27, $CHCl_3$); $R_f = 0.36$ (2:1, petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–7.23 (m, 20H, Ar–H), 5.84 (dd, *J* 10.2, 3.1, 1H, H-3″), 5.65 (m, 2H, H-2″, H-4″), 5.36 (s, 1H, H-1″), 5.29 (br s, 1H, H-12), 5.07 (dd, *J* 18.7, 12.6, 2H, PhC*H*$_2$), 4.81 (d, *J* 1.3, 1H, H-1′), 4.34 (m, 1H, H-5″), 4.11–3.98 (m, 3H, H-2′, H-4′, O*H*), 3.82 (m, 1H, H-5′-1), 3.67 (m, 1H, H-5′-2), 3.45 (d, *J* 7.9, 1H, H-3′), 3.18 (dd, *J* 11.0, 3.2, 1H, H-3), 2.91 (m, 1H, H-18), 2.52 (br s, 1H, O*H*), 1.34 (d, *J* 6.1, 3H, H-6″), 1.12, 1.05, 0.92, 0.89, 0.88, 0.84, 0.61 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4, 165.7, 165.5, 143.6, 136.4, 133.5, 133.3, 133.1, 129.9, 129.7, 129.6, 129.2, 129.2, 129.1, 128.5, 128.4, 128.2, 127.9, 127.8, 122.4, 102.0, 98.2, 90.3, 76.2, 71.5, 70.7, 70.7, 69.7, 67.3, 65.9, 65.5, 61.1, 55.4, 47.6, 46.7, 45.8, 41.6, 41.3, 39.2, 39.1, 38.5, 36.7, 33.8, 33.1, 32.6, 32.3, 30.6, 28.1, 27.6, 25.8, 25.7, 23.6, 23.4, 23.0, 18.2, 17.5, 16.8, 16.4, 15.3; ESI-MS (*m*/*z*): 1159.6 (M+Na).

3.8. Oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (1)

A suspension of **17** (63 mg, 0.055 mmol) and 10% Pd–C (30 mg) in EtOAc (16 mL) was refluxed and bubbled up with H_2 (20 mL/min) for 3 h. The mixture was then filtered and the filtrate was concentrated to dryness to afford crude **18** as a colorless oil. Crude **18** was dissolved in dry CH_2Cl_2–MeOH (1:2, 18 mL), to which a newly prepared NaOMe in MeOH solution (1.0 mol/L, 0.80 mL) was added. The mixture was stirred at rt for 2 h and then neutralized with Dowex H^+ resin to pH 7 and filtered. The filtrate was concentrated and purified with a silica gel column chromatography (15:1, EtOAc–MeOH) to gave **1** (35 mg, 86%) as a white powder. Mp 237–240 °C, lit.[1] 222–225 °C, lit.[2] 230–240 °C, lit.[3] 240–241 °C; $[\alpha]_D$ +4.5 (*c* 0.60, MeOH), lit.[1] +9 (*c*

1.83, MeOH), lit.[2] +6 (*c* 1.0, MeOH), lit.[3] +4.29 (*c* 0.35, MeOH); $R_f = 0.23$ (7:1, $CHCl_3$–MeOH); ^1H NMR (300 MHz, pyridine-d_5, solvent peak as internal standard (7.57 ppm)): δ 6.15 (s, 1H, H-1″), 5.46 (br s, 1H, H-12), 4.88 (d, *J* 5.0, 1H, H-1′), 4.74 (m, 1H, H-2″), 4.64–4.56 (m, 3H, H-2′, H-3″, H-5″), 4.33–4.27 (m, 4H, H-3′, H-4′, H-5′-1, H-4″), 3.82 (m, 1H, H-5′-2), 3.26 (m, 2H, H-3, H-18), 1.61 (d, *J* 5.7, 3H, H-6″), 1.29, 1.17, 1.06, 1.00, 0.98, 0.95, 0.82 (s each, 3H each); ^{13}C NMR (150 MHz, pyridine-d_5, solvent peak as internal standard (135.6 ppm)): δ 180.3 (C-28), 144.9 (C-13), 122.6 (C-12), 105.0 (C-1′), 101.8 (C-1″), 88.8 (C-3), 76.0 (C-2′), 74.1 (C-4″), 74.0 (C-3′), 72.6 (C-3″), 72.5 (C-2″), 69.9 (C-5″), 68.8 (C-4′), 64.9 (C-5′), 56.0 (C-5), 48.1 (C-9), 46.7 (C-17), 46.5 (C-19), 42.2 (C-14), 42.0 (C-18), 39.8 (C-4), 39.6 (C-8), 38.9 (C-1), 37.1 (C-10), 34.3 (C-21), 33.4, 33.3, 33.2 (C-7, C-22, C-29), 31.0 (C-20), 28.4 (C-15), 28.1 (C-23), 26.6 (C-2), 26.2 (C-27), 23.8, 23.7, 23.7 (C-11, C-16, C-30), 18.6, 18.6 (C-6, C-6″), 17.4 (C-26), 17.1 (C-24), 15.6 (C-25); ESI-MS (*m*/*z*): 757.5 (M+Na); HRMS: calcd for $C_{41}H_{65}O_{11}$ (M−H): 733.4527; found: *m*/*z* 733.4529.

3.9. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-α-L-arabinopyranoside (20)

A solution of **17** (285 mg, 0.250 mmol), $CH_3C(OEt)_3$ (0.23 mL, 1.25 mmol) and TsOH (16 mg) in dry toluene (8 mL) was stirred at rt for 1 h before the reaction was quenched with Et_3N (0.2 mL). The mixture was diluted with toluene (20 mL) and washed with water (20 mL × 3). The solvent was removed in vacuum to furnish crude **19** as a colorless oil, which was then dissolved in 80% aq HOAc (8 mL) and stirred at rt for another 1 h. Coevaporation with toluene yielded a straw yellow solid, which was then subjected to a silica gel column chromatography (5:1, petroleum ether–EtOAc) to give **20** (262 mg, 89%) as a white foam. $[\alpha]_D$ +85.8 (*c* 2.28, $CHCl_3$); $R_f = 0.27$ (3:1, petroleum ether–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 8.10–7.22 (m, 20H, Ar–H), 5.86 (m, 1H, H-3″), 5.75–5.65 (m, 2H, H-2″, H-4″), 5.47 (s, 1H, H-1″), 5.29 (t, *J* 2.5, 1H, H-12), 5.14 (m, 1H, H-4′), 5.10 (dd, *J* 20.1, 12.6, 2H, PhCH_2), 4.72 (d, *J* 4.5, 1H, H-1′), 4.43 (m, 1H, H-5″), 4.12 (m, 1H, H-3′), 3.98 (m, 1H, H-5′-1), 3.95 (m, 1H, H-2′), 3.69 (dd, *J* 12.1, 3.0, 1H, H-5′-2), 3.35 (br s, 1H, O*H*), 3.19 (dd, *J* 11.3, 4.4, 1H, H-3), 2.91 (dd, *J* 12.3, 2.8, 1H, H-18), 2.15 (s, 3H, CH_3CO), 1.35 (d, *J* 6.3, 3H, H-6″), 1.12, 1.10, 0.92, 0.89, 0.89, 0.88, 0.61 (s each, 3H each, 7 × Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 177.4, 170.8, 165.8, 165.5, 165.5, 143.7, 136.4, 133.4, 133.3, 133.1, 129.9, 129.8, 129.7, 128.5, 128.4, 128.2, 127.9, 127.9, 122.4, 103.0, 98.2, 90.3, 76.2, 71.6, 70.7, 70.5, 69.8, 69.6, 67.2, 65.9, 59.9, 55.6, 47.6, 46.7, 45.8, 41.6, 41.3, 39.3, 39.1, 38.6, 36.7, 33.8, 33.1, 32.6, 32.3, 30.7, 28.1, 27.6, 25.8, 23.6, 23.4, 23.0, 21.1, 18.2, 17.5, 16.8, 16.5, 15.3; ESI-MS (*m*/*z*): 1201.7 (M+Na); HRMS: calcd for $C_{64}H_{79}O_{15}$ (M−Bn): 1087.5419; found: *m*/*z* 1087.5419.

3.10. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-benzoyl-α-L-arabinopyranoside (22)

A solution of **20** (240 mg, 0.203 mmol) in dry pyridine (5 mL) was stirred and cooled to 0 °C. After slow addition of BzCl (0.070 mL, 0.610 mmol), the mixture was warmed up to rt and stirred overnight. Water (0.40 mL) was added slowly to quench the reaction and the solvent was evaporated in vacuum. The resulting residue was dissolved in CH_2Cl_2 (20 mL) and washed with water (15 mL × 3) and dried over $MgSO_4$. The mixture was filtered and the filtrate was concentrated to afford crude **21** as a white amorphous solid, which was then dissolved in dry CH_2Cl_2–MeOH (1:2, 15 mL). To the solution was added AcCl (0.30 mL) at 0 °C, and the solution was warmed to rt and stirred until TLC (3:1, petroleum ether–EtOAc) showed the starting material had disappeared. Et_3N (1.20 mL) was added to neutralize the acid. The solution was then concentrated and purified with a silica gel column chromatography (5:1, petroleum ether–EtOAc) to furnish **22** (187 mg, 74%) as a white foam. $[\alpha]_D$ +88.7 (*c* 2.32, $CHCl_3$); $R_f = 0.40$ (3:1, petroleum ether–EtOAc); ^1H NMR (300 MHz, $CDCl_3$): δ 8.08–7.19 (m, 25H, Ar–H), 5.80 (dd, *J* 10.2, 3.3, 1H, H-3″), 5.64 (m, 2H, H-2″, H-4″), 5.36 (s, 1H, H-1″), 5.34–5.29 (m, 2H, H-3′, H-12), 5.07 (dd, *J* 20.0, 12.6, 2H, PhCH_2), 4.76 (d, *J* 4.1, 1H, H-1′), 4.41 (m, 1H, H-5″), 4.33–4.27 (m, 2H, H-2′, H-4′), 4.08 (m, 1H, H-5′-1), 3.72 (dd, *J* 10.5, 2.7, 1H, H-5′-2), 3.17 (dd, *J* 11.5, 3.8, 1H, H-3), 2.91 (dd, *J* 10.3, 3.6, 1H, H-18), 1.33 (d, *J* 6.1, 3H, H-6″), 1.12, 1.03, 0.92, 0.90, 0.87, 0.70, 0.60 (s each, 3H each, 7 × Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 177.4, 165.9, 165.7, 165.2, 165.2, 143.7, 136.4, 133.5, 133.3, 133.3, 133.2, 133.0, 130.1, 129.9, 129.8, 129.7, 129.6, 129.5, 129.5, 129.2, 129.1, 128.5, 128.4, 128.4, 128.2, 128.1, 128.0, 127.9, 122.5, 102.7, 98.1, 89.3, 73.8, 73.8, 71.7, 70.6, 69.5, 67.3, 65.9, 65.5, 62.6, 55.6, 47.6, 46.7, 45.8, 41.6, 41.3, 39.3, 39.2, 38.7, 36.7, 33.8, 33.1, 32.6, 32.3, 30.7, 29.7, 28.0, 27.6, 26.0, 25.8, 23.6, 23.4, 23.0, 18.1, 17.4, 16.8, 16.4, 15.4; ESI-MS (*m*/*z*): 1263.7 (M+Na); HRMS: calcd for $C_{69}H_{81}O_{15}$ (M−Bn): 1149.5576; found: *m*/*z* 1149.5569.

3.11. Benzyl oleanolate 3-*O*-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-3-*O*-benzoyl-α-L-arabinopyranoside (24)

A suspension of **22** (160 mg, 0.129 mmol), **23** (240 mg, 0.322 mmol) and powdered 4 Å molecular sieves

(400 mg) in dry CH_2Cl_2 (6 mL) were stirred for 40 min at rt. A dry CH_2Cl_2 solution (1.00 mL) of TMSOTf (0.010 mL, 0.052 mmol) was added dropwise. The mixture was stirred for 1 h before Et_3N (0.16 mL) was added to quench the reaction. The mixture was then diluted with CH_2Cl_2 (25 mL) and filtered. The filtrate was concentrated and purified by a silica gel column chromatography (5:1, petroleum ether–EtOAc) to afford **24** (166 mg, 71%) as a white foam. $[\alpha]_D$ +47.0 (*c* 1.38, $CHCl_3$); $R_f = 0.42$ (4:1, petroleum ether–EtOAc); ^1H NMR (600 MHz, $CDCl_3$): δ 8.08–7.10 (m, 45H, Ar–H), 5.88 (dd, *J* 9.6, 9.6, 1H, H-3‴), 5.78 (m, 1H, H-3″), 5.73 (dd, *J* 9.6, 9.6, H-4‴), 5.64 (m, 2H, H-2″, H-4″), 5.55 (m, 1H, H-2‴), 5.27 (m, 1H, H-12), 5.21–5.05 (m, 4H, PhCH_2, H-1″, H-1‴), 4.71 (m, 2H, H-1′, H-3′), 4.53 (dd, *J* 9.0, 1.4, 1H, H-2′), 4.43 (m, 1H, H-6‴-1), 4.33 (m, 2H, H-4′, H-5″), 4.25–4.20 (m, 3H, H-5′-1, H-5‴, H-6‴-2), 3.77 (m, 1H, H-5′-2), 3.06 (m, 1H, H-3), 2.83 (dd, *J* 13.3, 3.1, 1H, H-18), 1.33 (d, *J* 6.3, 3H, H-6″), 1.10, 1.04, 0.93, 0.90, 0.87, 0.81, 0.57 (s each, 3H each, 7 × Me); ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.4, 166.0, 165.8, 165.7, 165.6, 165.4, 165.2, 165.1, 165.0, 143.6, 136.4, 133.3, 133.0, 132.8, 129.9, 129.8, 129.2, 129.1, 128.9, 128.8, 128.4, 128.2, 128.1, 127.9, 122.5, 101.8, 100.6, 98.2, 89.2, 73.7, 73.7, 72.8, 72.1, 71.6, 71.6, 70.5, 70.3, 69.7, 69.6, 67.3, 65.9, 62.8, 60.4, 55.5, 47.6, 46.7, 45.8, 41.6, 41.3, 39.3, 39.1, 38.6, 36.7, 33.8, 33.1, 32.6, 32.4, 30.7, 28.0, 27.8, 27.6, 25.8, 23.6, 23.4, 23.0, 18.1, 17.5, 16.8, 16.2, 15.3; ESI-MS (*m*/*z*): 1842.7 (M+Na); HRMS: calcd for $C_{103}H_{107}O_{24}$ (M−Bn): 1727.7152; found: *m*/*z* 1727.7149.

3.12. Oleanolic acid 3-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside (3)

A suspension of **24** (122 mg, 0.067 mmol) and 10% Pd–C (45 mg) in EtOAc (18 mL) was refluxed and bubbled up with H_2 (20 mL/min) for 4 h. Pd–C was removed through filtration and the filtrate was concentrated to dryness. The resulting residue was dissolved in dry CH_2Cl_2–MeOH (1:2, 24 mL), to which a newly prepared NaOMe in MeOH solution (1.0 mol/L, 1.00 mL) was added. The solution was stirred at rt for 2 h and then neutralized with Dowex H^+ resin to pH 7 and filtered. The filtrate was concentrated and subjected to a silica gel column chromatography (20:10:1, $CHCl_3$–MeOH–H_2O) to give a pale yellow powder, which was recrystallized in MeOH to afford **3** (42 mg, 70%) as a white powder. Mp 251–253 °C, lit.[2] 250–260 °C; $[\alpha]_D$ −2.1 (*c* 0.16, MeOH), lit.[2] 0 (*c* 2.97, MeOH), lit.[9] −6.11 (*c* 0.135, EtOH); $R_f = 0.35$ (20:10:1, $CHCl_3$–MeOH–H_2O); ^1H NMR (300 MHz, pyridine-d_5, solvent peak as internal standard (7.56 ppm)): δ 6.20 (s, 1H, H-1″), 5.46 (m, 1H, H-12), 5.14 (d, *J* 7.8, 1H, H-1‴), 4.77–4.73 (m, 2H, H-1′, H-2″), 4.67–4.60 (m, 3H, H-2′, H-3″, H-5″), 4.53–4.48 (m, 2H, H-6‴), 4.41–4.34 (m, 2H, H-4″, H-5′-1), 4.30–4.16 (m, 4H, H-3′, H-4′, H-3‴, H-4‴), 4.03 (dd, *J* 7.9, 7.9, 1H, H-2‴), 3.91 (m, 1H, H-5‴), 3.80 (d, *J* 11.3, 1H, H-5′-2), 3.27–3.22 (m, 2H, H-3, H-18), 1.64 (d, *J* 6.2, 3H, H-6″), 1.28, 1.17, 1.10, 1.00, 0.97, 0.94, 0.82 (s each, 3H each, 7 × Me); ^{13}C NMR (150 MHz, pyridine-d_5, solvent peak as internal standard (135.6 ppm)): δ 180.2 (C-28), 144.9 (C-13), 122.6 (C-12), 106.4 (C-1‴), 105.0 (C-1′), 101.8 (C-1″), 88.7 (C-3), 79.7 (C-4′), 78.8 (C-5‴), 78.6 (C-3‴), 76.4 (C-2′), 75.5 (C-2‴), 74.1, 74.1 (C-3′, C-4″), 72.5, 72.4 (C-2″, C-3″), 71.3 (C-4‴), 69.9 (C-5″), 64.6 (C-5′), 62.6 (C-6‴), 56.0 (C-5), 48.1 (C-9), 46.7 (C-17), 46.5 (C-19), 42.2 (C-14), 42.0 (C-18), 39.8 (C-4), 39.5 (C-8), 38.9 (C-1), 37.1 (C-10), 34.3 (C-21), 33.3, 33.2, 33.2 (C-7, C-22, C-29), 31.0 (C-20), 28.4 (C-15), 28.1 (C-23), 26.6 (C-2), 26.2 (C-27), 23.8, 23.7, 23.7 (C-11, C-16, C-30), 18.7 (C-6″), 18.5 (C-6), 17.4 (C-26), 17.1 (C-24), 15.6 (C-25); ESI-MS (*m*/*z*): 919.5 (M+Na); HRMS: calcd for $C_{47}H_{75}O_{16}$ (M−H): 895.5055; found: *m*/*z* 895.5050.

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