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Convenient and chromatography-free partial syntheses of maslinic acid and augustic acid

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ABSTRACT

A convenient and chromatography-free 4-step synthesis of analytically pure maslinic acid (**1**, 41.2%) from oleanolic acid has been developed. Slight variations in the final steps gave an excellent yield of isomeric augustic acid (**7**, 71.9%).

Cancer is one of the leading causes of death in Western people, second to heart diseases. Lung and bronchial cancer is still the top killer cancer in the USA, followed by colon and rectal cancer, breast, pancreatic, and prostate cancer. According[1] to a WHO prognosis, deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030. Treatment of cancer requires a careful selection of intervention such as radiotherapy, surgery, or chemotherapy to cure the disease or considerably prolong life while improving the patient's quality. Plants provide a broad spectrum of potential drug substances[2] for chemotherapy of life. Pentacyclic triterpenes[3] of the ursane, lupane, and oleanane series are one group of promising secondary plant metabolites.

Quite recently, maslinic acid (**1**, Fig. 1) and several of its derivatives[4–6] have been identified as new and very promising compounds, and their role in the cancer setting is gradually emerging. This led to an increased need in larger amounts of **1**.

Starting with its first isolation from the leaves of Hawthorne (*Crataegus oxyacantha* L.) by Bächler[7] in 1927, many different plants have been screened as potential sources for obtaining **1**. A major breakthrough (albeit almost forgotten) was the extraction of **1** from olive cake[8] by L. Caglioti in 1960. Improvements of this technique[9–12] allowed the isolation of **1** in significant amounts. Since this pomace is readily available only in countries growing olives, the isolation of **1** from edible green or black olives has been suggested[13] as an alternative. Both procedures[13] gave yields between 0.25% (pomace) and 0.36% (olives).

Recently, we synthesized[14] several derivatives exhibiting cytotoxic effects; one of these compounds ('EM2', a diacetylated benzylamide)[13] exhibited a very high cytotoxicity for human ovarian cancer cells (IC_{50} = 0.5 μM) whereas the cytotoxicity for non-malignant human fibroblasts (IC_{50} = 156 μM) was low. During planning of extended testing of EM2 in tumor bearing animals, a large amount of **1** was needed—and its isolation from olives using typical lab-equipment ran into major problems. Known syntheses of **1**[15–17] were difficult to reproduce on a larger scale—and each of them involved extensive chromatographic work-up procedures. Hence, we decided to develop a synthesis of **1** using an inexpensive starting material[18] using only a few steps and avoiding chromatographic work-up.

Thus, commercially available and inexpensive oleanolic acid (**2**)[18] was oxidized at position C-3 by the Jones oxidation in the presence of silica gel,[19,20] and an almost quantitative yield of

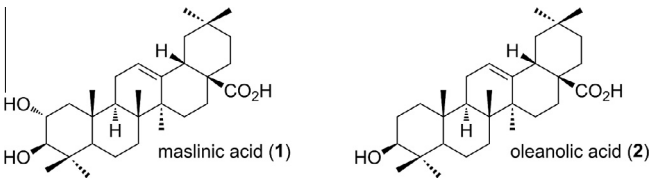

Figure 1. Structure of maslinic acid (**1**) and oleanolic acid (**2**).

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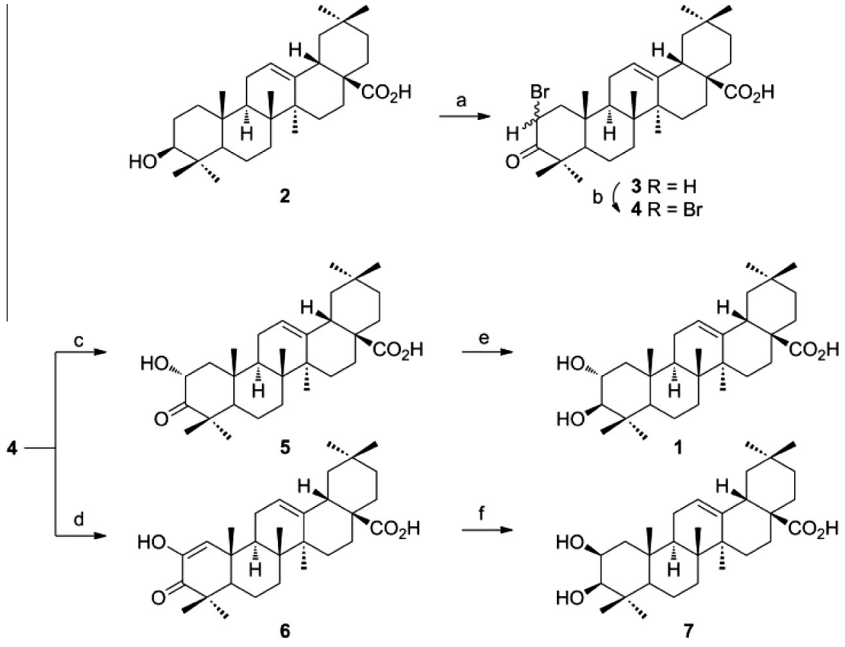

Scheme 1. Synthesis of maslinic acid (**1**) and augustic acid (**7**) from oleanolic acid (**2**): (a) CrO_3/H_2SO_4, silica gel, 0 °C; (b) pyridinium tribromide, acetic acid, 25 °C, 180 min; (c) NaOH (2 equiv), DMF, 0 °C, 30 min; (d) air, NaOH (5 equiv), DMF, 60 °C, 1 h; (e) $NaBH_4$, 0 °C, 1 h, then acetone/H_2SO_4, 30 min, 0 °C, recryst. from ethyl acetate, 41.2% (over-all); (f) $NaBH_4$, 0 °C, 1 h, recryst. from ethyl acetate, 71.9% (over-all).

slightly yellowish **3**[21] was obtained, whose reaction with pyridinium tribromide in acetic acid[22,23] furnished a mixture of bromides **4**.[24] Reaction of **4** with sodium hydroxide in DMF[24] at 0 °C for 30 min gave hydroxylated **5**[22,25] whose reduction with $NaBH_4$ at 0 °C[26] yielded the title compound **1** (together with some 3-*epi*-maslinic acid;[27] ratio maslinic acid/3-*epi*-maslinic acid = 4:1). Thus, analytically pure **1** was obtained after only one re-crystallization from ethyl acetate in a 4-step procedure with an over-all yield of 41.2% (from oleanolic acid). Reaction of **4** with air and excess of sodium hydroxide in DMF at 60 °C for an hour, however, yielded enol **6**.[28] To our surprise this reaction proceeded quite smoothly and in almost quantitative yield. Previously, similar transformations have been carried out either by microbial transformations[29] or by microwave-assisted[30] syntheses. Reduction of **6** with $NaBH_4$ at 0 °C gave an excellent yield of augustic acid (**7**)[31] (Scheme 1).

This synthetic approach can easily be scaled up and allows the preparation of **1** in large amounts. Simple re-crystallization furnishes **1** of superior purity as determined by HPLC.[32]

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18. Csuk, R.; Siewert, B. *Tetrahedron Lett.* **2011**, *52*, 6616–6618. In addition, oleanolic acid can be obtained from several suppliers in bulk quantities.
19. Thus, treatment of **2** with oxone® led to the formation of a 3,4-*seco*-olean derivative instead of an α-hydroxylation. The procedure reported by Wang et al. (Ref. 17) failed to give good yields in our hands and needed extensive chromatographic work-up.
20. The addition of silica gel allows a more convenient work-up; for a silica gel supported Jones oxidation: Ali, M. H.; Wiggin, C. J. *Synth. Commun.* **2001**, *31*, 1389–1397.
21. *Typical procedure for the synthesis of* **3**: A suspension of **2** (19.72 g, 43.18 mmol) and silica gel (150 mL) in acetone (1 L) was heated under reflux for 30 min. After cooling to 0 °C Jones reagent [prepared from CrO_3 (5.18 g, 51.82 mmol), water (16.8 mL), and concd H_2SO_4 (5 mL)] were slowly added, and the mixture was stirred for 30 min at 0 °C. Then MeOH (4 mL) was added, and stirring was continued for another 30 min. The solvents were removed under diminished pressure, and the resulting solid was extracted with ether (Soxhlet apparatus, 6 h). Evaporation of the ether furnished **3** (19.31 g, 98.4%) as a slightly yellowish solid; an analytical sample showed mp: 225–227 °C (lit.: 226–229 °C [An, R. -B. *Nat. Prod. Sci.* **2008**, *14*, 249–253]).
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24. *Typical procedure for the synthesis of* **4**: To a solution of crude **3** (19.25 g, 42.34 mmol) in acetic acid (500 mL), pyridinium tribromide (90%, 15.35 g, 43.18 mmol) was added at 25 °C within 60 min in several portions, and the mixture was stirred for 2 h. After cooling (0 °C), water (1.5 L) was added, the solid was filtered off and thoroughly washed with water, finally dried ($CaCl_2$) and **4** (22.57 g, quant.) was obtained as an off-white solid as a mixture of epimers; for analysis the epimeric bromides were separated by chromatography: *(2α)-2-bromo-3-oxoolean-12-en-28-oic acid:* colorless solid, mp 149–158; R_f = 0.15 (silica gel, hexane/ethyl acetate, 8:2); $[\alpha]_D$ +43.88° (*c*

0.35, $CHCl_3$); IR (KBr): ν = 3422v, 2949s, 2866s, 1724s, 1697s, 1461s, 1387s, 1365m, 1305w, 1270m, 1187m, 1163m, 1065m, 1012m, 763s, 727m, 646s, 603s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.28 (dd, J = 3.6, 3.6 Hz, 1H, C*H* (12)), 5.06 (dd, J = 13.4, 6.1 Hz, 1H, C*H* (2)), 2.82 (dd, J = 13.8, 4.3 Hz, 1H, C*H* (18)), 2.56 (dd, J = 13.0, 6.1 Hz, 1H, CH_a (1)), 2.14–1.88 (m, 4H, CH_b (1)+CH_a (16)+CH_a (11)+CH_b (11)), 1.88–1.54 (m, 8H, C*H* (5)+C*H* (9)+CH_a (19)+CH_a (7)+CH_b (7)+CH_a (15)+CH_a (16)+CH_b (16)), 1.54–1.40 (m, 3H, CH_a (6)+CH_b (6)+CH_a (22)), 1.39–1.01 (m, 6H, C*H* (2)+CH_b (19)+CH_b (22)+CH_a (21)+CH_b (21)+CH_b (15)), 1.21 (s, 3H, CH_3 (25)), 1.20 (s, 3H, CH_3 (27)), 1.12 (s, 3H, CH_3 (23)), 1.09 (s, 3H, CH_3 (24)), 0.93 (s, 3H, CH_3 (29)), 0.90 (s, 3H, CH_3 (30)), 0.80 (s, 3H, CH_3 (26)) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 206.9 (*C*=O, C3), 184.3 (*C*=O, C28), 144.0 (*C*=CH, C13), 122.0 (*CH*=C, C12), 56.7 (*CH*, C2), 52.4 (CH_2, C1), 52.3 (*CH*, C5), 49.5 (C_{quart}, C4), 47.1 (*CH*, C9), 46.7 (C_{quart}, C17), 45.9 (CH_2, C19), 41.9 (C_{quart}, C14), 41.1 (*CH*, C18), 40.0 (C_{quart}, C8), 39.5 (C_{quart}, C10), 33.9 (CH_2, C21), 33.2 (CH_3, C30), 32.5 (CH_2, C7), 32,3 (CH_2, C22), 30.8 (C_{quart}, C20), 27.8 (CH_2, C15), 26.5 (CH_3, C27), 26.0 (CH_3, C23), 23.7 (CH_3, C29), 23.6 (CH_2, C11), 22.8 (CH_2, C16), 22.1 (CH_3, C24), 19.4 (CH_2, C6), 17.3 (CH_3, C26), 15.4 (CH_3, C25) ppm; MS (ESI): m/z (%) = 531.3 ($[C_{30}H_{45}{}^{79}BrO_3-H]^-$, 48), 533.3 ($[C_{30}H_{45}{}^{81}BrO_3-H]^-$, 52), 1063.1 ($[2C_{30}H_{45}{}^{79}BrO_3-H]^-$, 92), 1065.1 ($[C_{30}H_{45}{}^{79}BrO_3+C_{30}H_{45}{}^{81}BrO_3-H]^-$, 100); analysis calcd for $C_{30}H_{45}BrO_3$ (533.58): C 67.53, H 8.50; found: C 67.27, H 8.71. *(2β)-2-Bromo-3-oxoolean-12-en-28-oic acid*: colorless solid; mp 146–151 °C; R_f = 0.22 (silica gel, hexane/ethyl acetate 8:2); $[\alpha]_D$ +120.03° (*c* 0.25, $CHCl_3$); IR (KBr): ν = 3425 m, 2949s, 1731s, 1697s, 1636w, 1459s, 1388s, 1365 m, 1305 m, 1268 m, 1208 m, 1162 m, 1062 m, 774s, 709 m, 646 m, 606 m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.30 (dd, J = 3.4, 3.4 Hz, 1H, C*H* (12)), 5.08 (dd, J = 11.0, 9.5 Hz, 1H, C*H* (2)), 2.84 (dd, J = 13.9, 3.9 Hz, 1H, C*H* (18)), 2.50 (dd, J = 13.6, 11.3 Hz, 1H, CH_a (1)), 2.10–1.91 (m, 3H, CH_a (11)+CH_a (16)+CH_b (1)), 1.92–1.44 (m, 10H, CH_b (16)+C*H* (5)+C*H* (9)+CH_a (22)+CH_b (22)+CH_a (15)+CH_a (19)+CH_b (11)+CH_a (7)+CH_a (6)), 1.44–1.30 (m, 3H, CH_b (6)+CH_b (7)+CH_a (21)), 1.30–1.02 (m, 3H, CH_b (21)+CH_b (19)+CH_b (15)), 1.17 (s, 3H, CH_3 (27)), 1.14 (s, 3H, CH_3 (23)), 1.13 (s, 3H, CH_3 (29)), 0.93 (s, 3H, CH_3 (24)), 0.91 (s, 3H, CH_3 (30)), 0.88 (s, 3H, CH_3 (25)), 0.76 (s, 3H, CH_3 (26)) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 209.2 (*C*=O, C3), 183.1 (*C*=O, C28), 143.6 (*C*=CH, C13), 122.4 (*CH*=C, C12), 53.5 (CH_2, C1), 52.7 (*CH*, C5), 51.2 (*CH*, C2), 47.7 (C_{quart}, C4), 46.9 (*CH*, C9), 46.8 (C_{quart}, C17), 45.9 (CH_2, C19), 42.1 (C_{quart}, C14), 41.4 (*CH*, C18), 39.6 (C_{quart}, C8), 39.3 (C_{quart}, C10), 34.0 (CH_2, C21), 33.2 (CH_3, C30), 32.5 (CH_2, C22), 31,7 (CH_2, C7), 30.8 (C_{quart}, C20), 29.6 (CH_3, C23), 27.8 (CH_2, C15), 25.9 (CH_3, C27), 23.7 (CH_3, C29), 23.6 (CH_2, C11), 23.1 (CH_2, C16), 20.4 (CH_3, C24), 20.1 (CH_2, C6), 18.3 (CH_3, C25), 16.6 (CH_3, C26) ppm; MS (ESI): m/z (%) = 531.3 ($[C_{30}H_{45}{}^{79}BrO_3-H]^-$, 100), 533.3 ($[C_{30}H_{45}{}^{81}BrO_3-H]^-$, 92), 1063.1 ($[2C_{30}H_{45}{}^{79}BrO_3-H]^-$, 54), 1065.1 ($[C_{30}H_{45}{}^{79}BrO_3+C_{30}H_{45}{}^{81}BrO_3-H]^-$, 60); analysis calcd for $C_{30}H_{45}BrO_3$ (533.58): C 67.53, H 8.50; found: C 67.32, H 8.79.

25. A similar transformation has been described very recently for a betulin derivative: Pettit, G. R.; Noelean, M.; Hempenstall, F.; Chapuis, J.-C.; Groy, T. L.; Williams, L. *J. Nat. Prod.* **2014**, *77*, 863–872. *Typical procedure for our synthesis of* **5**: To a solution of crude **4** (22.56 g, 42.28 mmol) in DMF (200 mL), an aqueous solution of NaOH (2 M, 44.4 mL, 88.78 mmol) was added at 0 °C. The mixture was stirred for 30 min. The suspension was re-dissolved by the addition of MeOH (250 mL), acidified (2 M HCl, 25 mL), and water (1.5 L) was added. The precipitate was filtered off, washed, and dried to yield *(2α)-2-hydroxy-3-oxoolean-12-en-28-oic acid* (**5**) (19.9 g, quant.) as an off-white solid; an analytical sample showed: mp 143–146 °C; R_f = 0.41 (silica gel, hexane/ethyl acetate, 6:4); $[\alpha]_D$ +64.23° (*c* 0.35, $CHCl_3$); IR (KBr): ν = 3446v, 2944s, 1700s, 1636m, 1559w, 1540w, 1507w, 1458m, 1388s, 1268m, 1162m, 1101m, 1056m, 1029s, 994m, 646m cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 5.28 (dd, J = 3.6, 3.6 Hz, 1H, C*H* (12)), 4.54 (dd, J = 12.6, 6.6 Hz, 1H, C*H* (2)), 2.83 (dd, J = 13.7, 4.3 Hz, 1H, C*H* (18)), 2.40 (dd, J = 12.6, 6.6 Hz, 1H, CH_a (1)), 2.03–1.89 (m, 2H, CH_a (11)+CH_b (11)), 1.82–1.65 (m, 2H, CH_a (22)+CH_a (15)), 1.66–1.40 (m, 9H, C*H* (9)+CH_a (19)+CH_a (7)+CH_b (7)+CH_b (22)+CH_a (16)+CH_b (16)+CH_a (6)+CH_b (6)), 1.40–1.04 (m, 6H, CH_b (1)+C*H* (5)+CH_b (19)+CH_a (21)+CH_b (21)+CH_b (15)), 1.26 (s, 3H, CH_3 (25)), 1.16 (s, 3H, CH_3 (23)), 1.10 (s, 3H, CH_3 (24)), 0.93 (s, 3H, CH_3 (29)), 0.93 (s, 3H, CH_3 (30)), 0.81 (s, 3H, CH_3 (26)) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 216.7 (*C*=O, C3), 183.9 (*C*=O, C28), 143.9 (*C*=CH, C13), 122.3 (CH=C, C12), 69.3 (*CH*, C2), 57.8 (*CH*, C5), 49.5 (CH_2, C1), 47.9 (C_{quart}, C4), 47.5 (*CH*, C9), 46.6 (C_{quart}, C17), 45.9 (CH_2, C19), 41.8 (C_{quart}, C14), 41.1 (*CH*, C18), 39.6 (C_{quart}, C8), 37.9 (C_{quart}, C10), 33.9 (CH_2, C21), 33.2 (CH_3, C30), 32.6 (CH_2, C7), 32,5 (CH_2, C22), 30.8 (C_{quart}, C20), 27.8 (CH_2, C15), 26.1 (CH_3, C27), 24.9 (CH_3, C23), 23.8 (CH_3, C29), 23.7 (CH_2, C11), 23.0 (CH_2, C16), 21.7 (CH_3, C24), 19.3 (CH_2, C6), 17.4 (CH_3, C26), 16.2 (CH_3, C25) ppm; MS (ESI): m/z (%) = 469.5 ($[M-H]^-$, 100), 939.2 ($[2M-H]^-$, 66), 961.7 ($[2M-2H+Na]^-$, 28); analysis calcd for $C_{30}H_{46}O_4$ (470.68): C 76.55, H 9.85; found: C 76.21, H 9.98..
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27. *Typical procedure for the synthesis of* **1**: To a solution of crude **5** (19.8 g, 42.28 mmol) in THF (200 mL) and MeOH (40 mL), $NaBH_4$ (0.8 g, 21.14 mmol) was added at 0 °C. The mixture was stirred at 0 °C for 60 min, HCl (aq, 2 M, 20 mL) and water (1.5 L) were added, and the precipitate was collected and dried. The crude mixture (consisting of **1** and 3-*epi*-maslinic acid) was dissolved in acetone (200 mL) at 60 °C, then cooled (0 °C), and a solution of concd H_2SO_4 (6 mL) in acetone (100 mL) (according to Tschesche, R., Henckel, E., Snatzke, G. *Liebigs Ann. Chem.* **1964**, *676*, 175–187) was added. After stirring for 30 min, water (1.5 L) was added and the solid was collected and dried. Recrystallization from ethyl acetate furnished **1** (8.41 g, 41.2%) as a colorless solid; mp: 266–270 °C (decomp.) (lit.: 269–271 °C Ref. 26). Workup of the mother liquor furnished the (2α,3α) acetonide of 3-*epi*-maslinic acid (Muthukuda, P. M. *Chem. Sri Lanka* **1985**, *2*, 13–15) whose acidic hydrolysis (aqueous H_2SO_4) furnished known 3-*epi*-maslinic acid (2.4 g, 12%, over all yield); mp 294–296 °C (lit.: 295–297 °C [Ref. 26].
28. Li, J.-F.; Zhao, Y.; Cai, M. M.; Li, X.-F.; Li, J.-X. Eur. *J. Med. Chem.* **2009**, *44*, 2796–2806. Similarly, in the betulin series from the treatment of α-bromo ketones with sodium azide and catalytic amounts of sodium iodide followed by a thermal decomposition of an α-azido ketone intermediate an enamine was obtained (cf. Ref. 25). An analytical sample of 2-hydroxy-3-oxoolean-1,12-dien-28-oic acid (**6**) showed: colorless solid; mp 137–141 °C; R_f = 0.50 (silica gel, hexane/ethyl acetate 6:4); $[\alpha]_D$ +100.97 (*c* 0.31, $CHCl_3$); UV–vis ($CHCl_3$): λ_{max} (log ε) = 272 nm (4.31); IR (KBr): ν = 3441s, 2947s, 1713s, 1697s, 1652m, 1463s, 1429m, 1396s, 1385s, 1234m, 1149m, 1123s, 1056s, 803m, 458s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.34 (s, 1H, C*H* (1)), 5.94 (s, 1H, O*H* (2)), 5.34 (dd, J = 3.6, 3.6 Hz, 1H, C*H* (12)), 2.85 (dd, J = 13.7, 4.3 Hz, 1H, C*H* (18)), 2.20–1.92 (m, 3H, CH_a (16)+CH_a (11)+CH_b (11)), 1.91–1.85 (m, 1H, C*H* (9)), 1.84–1.67 (m, 2H, CH_a (15)+CH_a (22)), 1.67–1.43 (m, 6H, CH_a (6)+CH_b (16)+CH_b (22)+CH_a (7)+CH_a (19)+C*H* (5)), 1.43–1.06 (m, 6H, CH_b (6)+CH_b (15)+CH_b (7)+CH_a (21)+CH_b (21)+CH_b (19)), 1.22 (s, 3H, CH_3 (26)), 1.22 (s, 3H, CH_3 (23)), 1.13 (s, 3H, CH_3 (27)), 1.10 (s, 3H, CH_3 (24)), 0.94 (s, 3H, CH_3 (29)), 0.91 (s, 3H, CH_3 (30)), 0.83 (s, 3H, CH_3 (25)) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ = 201.2 (*C*=O, C3), 183.5 (*C*=O, C28), 144.0 (*C*=CH, C2), 143.9 (*C*=CH, C13), 128.3 (*CH*=C, C1), 122.2 (CH=C, C12), 54.0 (*CH*, C5), 46.7 (C_{quart}, C4), 45.8 (CH_2, C19), 44.0 (C_{quart}, C17), 43.2 (*CH*, C9), 42.1 (C_{quart}, C14), 41.3 (*CH*, C18), 40.1 (C_{quart}, C8), 38.6 (C_{quart}, C10), 34.0 (CH_2, C21), 33.2 (CH_3, C30), 32.6 (CH_2, C7), 32,5 (CH_2, C22), 30.8 (C_{quart}, C20), 27.8 (CH_2, C15), 27.4 (CH_3, C23), 26.0 (CH_3, C27), 23.7 (CH_3, C29), 23.6 (CH_2, C11), 23.0 (CH_2, C16), 21.9 (CH_3, C24), 19.3 (CH_3, C26), 18.8 (CH_2, C6), 17.7 (CH_3, C25) ppm; MS (ESI): m/z (%) = 467.3 ($[M-H]^-$, 54), 935.3 ($[2M-H]^-$, 28), 958.6 ($[2M-2H+Na]^-$, 24); analysis calcd for $C_{30}H_{44}BO_4$ (468.67): C 76.88, H 9.46; found: C 76.52, H 9.71.
29. Utsukihara, T.; Okada, S.; Kato, N.; Horiuchi, C. A. *J. Mol. Catal. B: Enzym.* **2007**, *45*, 68–72.
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31. *Selected properties of* **7**: colorless solid; mp: 310–314 °C (lit.: 308–310 °C Ref. 26).
32. *For HPLC:* Prontosil C-18, MeOH/H_2O 86:6, 1% H_3PO_4.