Synthesis of Enantiomerically Pure α-Hydroxylated Nervonic Acid – A Chiral Pool Approach to α-Hydroxylated Unsaturated Fatty Acids

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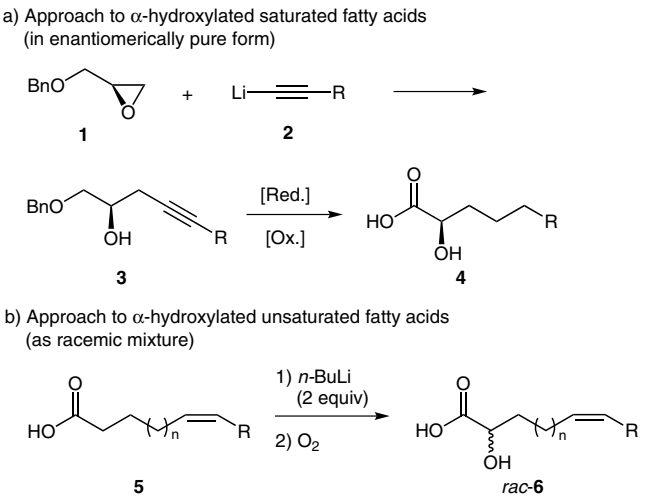
Abstract: A scalable and robust synthesis to α-hydroxylated nervonic acid is presented. The chiral-pool approach starts with malic acid and employs only highly reliable reactions such as Wittig reactions, protecting group conversions, and reductions. Depending on the configuration of malic acid being used as starting material both enantiomers can be obtained.

Key words: lipids, fatty acids, malic acid, nervonic acid

Biological membranes act as boundaries between the interior and the exterior of living cells. In most eukaryotes they consist of a lipid bilayer. Although the bilayer possesses a fluid character the constituents commonly do not mix uniformly. Inner and outer leaflet of the lipid bilayer differ strongly, and in addition different types of lipids segregate in the plane of the membrane driven by lipid–lipid interactions.[1] As a result domains (lipid rafts) are formed. These rafts are postulated to be of crucial importance in a plethora of cellular functions such as signaling, endocytic traffic, and adhesion of viruses, bacteria, and other cells.[2–4] Depending on the type and the percentage of different lipids (sphingomyelin, cholesterol, glycosphingolipids) in the mixture the fate and the dynamics of the rafts heavily change. In order to investigate how rafts are formed and evolve on a molecular level prerequisite is access to pure compounds. It is well-known that hydroxylated fatty acids in glycosphingolipids change their function significantly.[5–7] One idea is that a hydroxyl group next to the carbonyl group enhances exposure of the carbohydrate part on the cell surface.[8] Due to the enzymatic machinery which is responsible for hydroxylations only one of two possible stereoisomers is formed during this process.

In order to investigate biophysical questions in detail one relies on pure compounds being not isolated, but synthesized by chemical means. In the case of hydroxylated saturated fatty acids of type **4** one usually takes advantage of a ring opening of an enantiomerically pure epoxide **1** by a lithiated acetylide **2** (Scheme 1, a).[9] Later, the triple bond of **3** gets reduced to an ethano unit. However, this method is not suitable for unsaturated hydroxylated fatty acids since reduction would also eliminate the double bond. Another approach to reach α-hydroxylation is based on oxidation of the carboxylate enolate of the corresponding fatty acid **5** (Scheme 1, b).[10–12] Such a highly electron-rich species is easily attacked by molecular oxygen which is bubbled through the solution. The drawback of this method is that a racemic mixture of **6** is obtained. Therefore, we became interested to design a simple and scalable route to access enantiomerically pure α-hydroxylated *cis*-unsaturated fatty acids starting from a compound of the chiral pool.[13] Our idea was to use malic acid, an α-hydroxylated diacid, which is commercially available in both enantiomeric forms.



Scheme 1 Approaches to α-hydroxylated fatty acids

The synthesis of α-hydroxylated nervonic acid started with the transformation of D-malic acid to the corresponding aldehyde **8** by a literature-known sequence consisting of protection, reduction, and oxidation (Scheme 2).[14] Afterwards, the alkyl chain was elongated via Wittig reaction using phosphonium salt **9**.

This phosphonium salt containing the required eleven carbons as well as a terminal hydroxyl group for further transformations was prepared starting from 11-bromoundecanol (**7**) by benzyl protection followed by subsequent formation of the Wittig salt using triphenylphosphine as described in the literature.[15]

With both starting materials in hand, the first olefination was performed using NaHMDS in THF to obtain the desired product **10** in 62% yield. Hydrogenation of the double bond by $Pd(OH)_2$ on charcoal under hydrogen atmosphere removes the benzyl protecting group in the same step and furnishes the corresponding primary alco-

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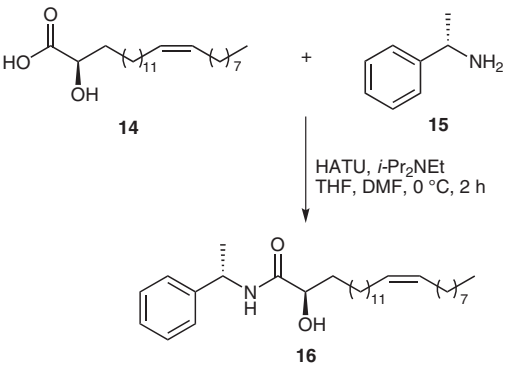
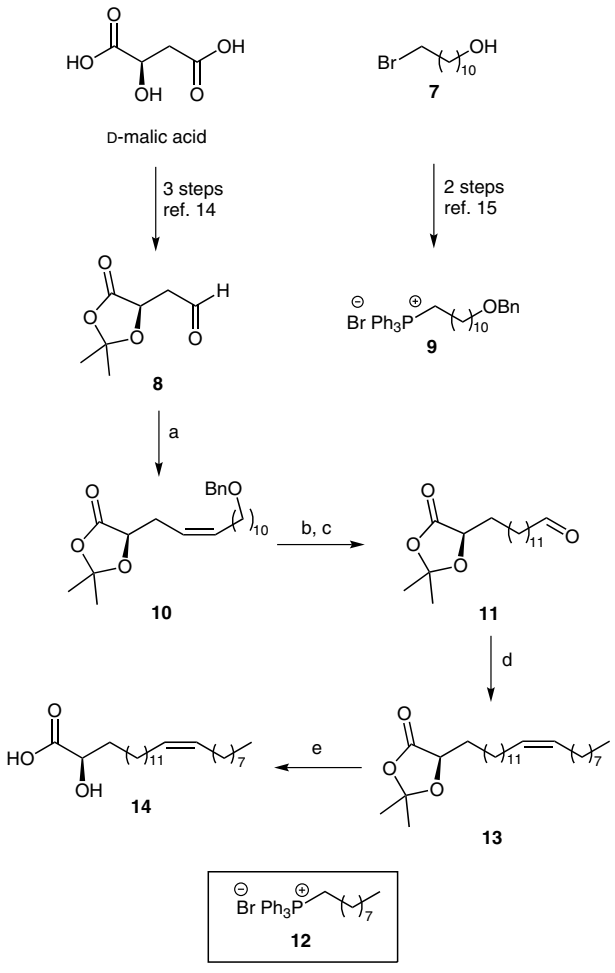
hol, which was readily oxidized with pyridinium chlorochromate (PCC) to afford aldehyde **11** within 30 minutes. The latter was used without further purification in the final *Z*-selective Wittig reaction to accomplish the fatty acid chain of α-hydroxy nervonic acid. The coupling between phosphonium salt **12** with NaHMDS as base (leading to an unstabilized phosphorus ylide) and aldehyde **11** yielded the protected *Z*-configured fatty acid **13** in 23% overall yield from **10**, whereas only traces of the *E*-configured product could be observed which were easily removed by silica gel column chromatography. Final deprotection of **13** was achieved using a mixture of 1 M aq HCl in THF to obtain the free α-hydroxy nervonic acid **14** in 96% yield. Scheme 2 depicts the synthetic route to the *R*-configured congener. In addition we prepared also the *S*-configured enantiomer starting from L-malic acid in a completely analogous way with similar yields.

In order to prove that our transformations did not lead to (partial) racemization of the stereocenter we performed an HPLC experiment. However, we came across separation problems utilizing a chiral stationary phase. Therefore, the enantiomeric purity of α-hydroxylated nervonic acid (**14**) was determined by a coupling experiment using the enantiomerically pure chiral amine **15** (Scheme 3). HATU-mediated coupling in the presence of Hünig base[16] produced only one observable diastereomer of **16** as proven by ^{13}C NMR spectroscopy of the crude reaction mixture, wherefore an enantiomeric ratio of >95:5 was obtained. The use of the crude mixture is necessary to ensure that a potentially occurring diastereomer is not removed by column chromatography.



Scheme 3 HATU-mediated formation of **16** to determine the enantiomeric purity of α-hydroxylated nervonic acid (**14**)

In summary, we developed a fast and efficient synthetic method to prepare α-hydroxylated nervonic acid (**14**) in five steps from readily available aldehyde **8**. Prerequisite was a chiral-pool approach using malic acid as starting material combined with the choice of suitable protecting groups. Depending on the configuration of malic acid both enantiomers of α-hydroxylated nervonic acid can be obtained. The presented approach should be applicable to access a variety of enantiomerically pure unsaturated fatty acids with an α-hydroxyl group, even such with more than one unsaturation. Studies incorporating nervonic acid into complex glycolipids to study their phase behavior in membranes are under way and will be reported in due course.



Scheme 2 Synthesis of α-hydroxylated nervonic acid from malic acid. *Reagents and conditions*: a) **9**, NaHMDS, THF, 0 °C to r.t., 1 h; then **9**, –78 °C to r.t., 16 h, 62%; b) $Pd(OH)_2/C$, H_2, MeOH, r.t., 16 h; c) PCC, CH_2Cl_2, 0 °C to r.t., 1 h; d) **12**, NaHMDS, THF, 0 °C to r.t., 1 h; then **11**, –78 °C to r.t., 16 h, 23% (over 3 steps); e) THF–HCl, 60 °C, 96%; THF = tetrahydrofuran, NaHMDS = sodium bis(trimethylsilyl)amide.

(*R*,*Z*)-5-[13-(Benzyloxy)tridec-2-en-1-yl]-2,2-dimethyl-1,3-dioxolan-4-one (10)

To a suspension of phosphonium bromide **9** (724 mg, 1.20 mmol, 1.00 equiv) in THF (4 mL) was added NaHMDS (1 M in THF, 1.20 mL, 1.20 mmol, 1.00 equiv) at 0 °C and further stirred for 1 h at r.t. Afterwards, the reaction mixture was cooled to –78 °C, and aldehyde **8** (190 mg, 1.20 mmol, 1.20 equiv) dissolved in THF (2 mL) was added dropwise. The reaction mixture was allowed to warm to r.t. overnight. Then H_2O was added, the phases were separated, and the aqueous layer was extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (pentane–EtOAc = 40:1 to 20:1) to obtain the title compound (301 mg, 749 μmol, 62%) as a colorless oil.

^1H NMR (200 MHz, $CDCl_3$): δ = 1.25–1.40 (m, 14 H), 1.54 (d, *J* = 0.6 Hz, 3 H), 1.57–1.66 (m, 5 H), 2.05 (q, *J* = 7.0 Hz, 2 H), 2.42–2.72 (m, 2 H), 3.46 (t, *J* = 6.7 Hz, 2 H), 4.44 (dd, *J* = 6.6, 4.6 Hz, 1 H), 4.50 (s, 2 H), 5.32–5.47 (m, 1 H), 5.52–5.68 (m, 1 H),

7.27–7.36 (m, 5 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 26.0, 26.2, 27.1, 27.4, 29.3, 29.4, 29.4, 29.5, 29.5, 29.5, 29.6, 29.8, 70.5, 72.8, 74.1, 110.6, 122.1, 127.4, 127.6, 128.3, 134.5, 138.7, 172.8 ppm. IR (ATR): ν = 2993, 2925, 2853, 1794, 1455, 1271, 1113 cm^{-1}. ESI-HRMS: *m/z* calcd for $C_{25}H_{38}O_4$ [M + Na]$^+$: 425.26623; found: 425.26662.

(*R*,*Z*)-5-(Docos-13-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-one (13)

To a solution of **10** (427 mg, 1.06 mmol, 1.00 equiv) in MeOH (10 mL) was added $Pd(OH)_2$/C (20 wt%, 100 mg, 100 μmol, 10 mol%), and the atmosphere was changed to H_2 and stirred for 16 h at r.t. The suspension was filtered through a short pad of Celite and eluted with additional MeOH. The solvents were removed in vacuo. The residue was dissolved in anhydrous CH_2Cl_2 (10 mL), cooled to 0 °C and PCC (434 mg, 2.02 mmol, 1.90 equiv) was added. After 10 min at 0 °C the reaction was stirred for additional 20 min at r.t., filtered through a short pad of Celite and eluted with additional CH_2Cl_2. Afterwards, activated carbon was added to the filtrate and stirred for further 30 min before the mixture was filtered through a short pad of silica to obtain the corresponding crude aldehyde **11** (172 mg) after concentration, which was used without further purification.

To a suspension of phosphonium bromide **12** (285 mg, 600 μmol, 0.57 equiv) in THF (6 mL) was added NaHMDS (1 M in THF, 0.60 mL, 600 μmol, 0.57 equiv) at 0 °C, and the resulting mixture was stirred for an additional hour at r.t. Then, the solution was cooled to –78 °C, and the crude aldehyde **11** dissolved in THF (3 mL) was added dropwise. The resulting mixture was stirred for 10 min at –78 °C and then 30 min at r.t. Afterwards, silica (300 mg) was added, the solvent was removed in vacuo, and the residue was purified by column chromatography on silica (pentane–EtOAc = 150:1) to obtain the title compound (105 mg, 23% over three steps) as a colorless oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 7.0 Hz, 3 H), 1.24–1.36 (m, 30 H), 1.38–1.58 (m, 2 H), 1.54 (d, *J* = 0.5 Hz, 3 H), 1.60 (d, *J* = 0.5 Hz, 3 H), 1.68–1.75 (m, 1 H), 1.84–1.90 (m, 1 H), 2.01 (q, *J* = 6.7 Hz, 4 H), 4.39 (dd, *J* = 7.2, 4.3 Hz, 1 H), 5.35 (t, *J* = 5.1 Hz, 2 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.9, 25.8, 27.2, 27.2, 27.2, 29.2, 29.3, 29.3, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.6, 29.8, 29.8, 31.6, 31.9, 74.1, 110.4, 129.9, 129.9, 173.4 ppm. IR (ATR): ν = 2998, 2922, 2853, 1797, 1462, 1383, 1265, 1125 cm^{-1}. ESI-HRMS: *m/z* calcd for $C_{27}H_{50}O_3$ [M + Na]$^+$: 445.36522; found: 445.36553.

(–)-(*R*)-α-Hydroxynervonic Acid (14)

Protected acid **13** was dissolved in a mixture of THF (4 mL) and HCl (1 M, 4 mL) and stirred for 48 h at 60 °C. After cooling to r.t. NaCl was added until saturation of the aqueous phase was observed. The phases were separated, and the aqueous phase was extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4. After removal of the solvent in vacuo the title compound (85.5 mg, 223 μmol, 96%) was obtained as a white solid (mp 67 °C).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 7.2 Hz, 3 H), 1.22–1.36 (m, 30 H), 1.39–1.52 (m, 2 H), 1.67–1.74 (m, 1 H), 1.83–1.90 (m, 1 H), 2.01 (q, *J* = 5.7 Hz, 4 H), 4.78 (q, *J* = 3.3 Hz, 1 H), 5.34–5.36 (m, 2 H) ppm. We could neither detect the proton of the carboxylic acid nor the one of the hydroxyl group. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1, 22.7, 24.8, 27.2, 27.2, 29.3, 29.3, 29.3, 29.3, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 29.7, 29.8, 29.8, 31.9, 34.2, 70.2, 129.9, 129.9, 178.9 ppm. IR (ATR): ν = 3543, 3163, 2917, 2848, 1709, 1664, 1464, 1207, 1092 cm^{-1}. ESI-HRMS: *m/z* calcd for $C_{24}H_{46}O_3$ [M + Na]$^+$: 405.33392; found: 405.33405. $[\alpha]_D^{21.5}$ –2.5 (*c* 1.76, $CHCl_3$).

(*R*,*Z*)-2-Hydroxy-*N*-[(*S*)-1-phenylethyl]tetracos-15-enamide (16)

To a solution of fatty acid **14** (25.0 mg, 65.0 μmol, 1.00 equiv), amine **15** (8.40 μL, 65.0 μmol, 1.00 equiv), and *i*-Pr_2NEt (13.6 μL, 78.0 μmol, 1.20 equiv) in THF (0.5 mL) was added HATU (32.0 mg, 85 μmol, 1.30 equiv) dissolved in DMF (0.5 mL) at 0 °C, and the mixture was stirred at this temperature for 2 h. Then brine and aq HCl (4:1) were added, and the aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over Na_2SO_4, and the solvent was removed in vacuo to obtain the crude coupling product **16**. To prove the enantiomeric purity, ^{1}H and ^{13}C NMR spectra were recorded using the crude product mixture.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.3 Hz, 3 H), 1.21–1.40 (m, 32 H), 1.50 (d, *J* = 1.5 Hz, 3 H), 1.53–1.67 (m, 1 H), 1.74–1.88 (m, 1 H), 2.06 (q, *J* = 5.8 Hz, 4 H), 4.11 (dd, *J* = 7.8, 3.7 Hz, 1 H), 5.12 (dd, *J* = 7.8, 7.0 Hz, 1 H), 5.35 (t, *J* = 5.1 Hz, 2 H), 6.80 (d, *J* = 6.8 Hz, 1 H), 7.27–7.36 (m, 5 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1, 21.9, 24.9, 27.2, 29.3, 29.3, 29.3, 29.3, 29.4, 29.4, 29.5, 29.5, 29.5, 29.6, 29,6, 29.6, 29.7, 29.8, 29.8, 31.9, 34.9, 39.5, 48.4, 72.1, 126.0, 127.3, 128.6, 129.9, 143.0, 172.9 ppm. ESI-HRMS: *m/z* calcd for $C_{34}H_{55}O_2N$ [M + Na]$^+$: 508.41250; found: 508.41238.

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References

(1) Simons, K.; Ikonen, E. *Nature (London)* **1997**, *387*, 569.
(2) Cinek, T.; Horejsi, V. *J. Immunol.* **1992**, *149*, 2262.
(3) Hakomori, S.; Igarashi, Y. *J. Biochem.* **1995**, *118*, 1091.
(4) Krauss, K.; Altevogt, P. *J. Biol. Chem.* **1999**, *274*, 36921.
(5) Hakomori, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 225.
(6) Ladisch, S.; Sweeley, C. C.; Becker, H.; Gage, D. *J. Biol. Chem.* **1989**, *264*, 12097.
(7) Kolter, T.; Sandhoff, K. *Angew. Chem. Int. Ed.* **1999**, *38*, 1532.
(8) Windschiegl, B.; Steinem, C. *Langmuir* **2006**, *22*, 7454.
(9) Iwayama, Y.; Ando, H.; Ishida, H.; Kiso, M. *Chem. Eur. J.* **2009**, *15*, 4637.
(10) Konen, D. A.; Silbert, L. S.; Pfeffer, P. E. *J. Org. Chem.* **1975**, *40*, 3253.
(11) Andelic, I.; Myhren, F.; Skattebøl, L.; Öberg, B.; Datema, R.; Chattopadhyaya, J. *Acta Chem. Scand.* **1985**, *39*, 231.
(12) Patterson, J. E.; Ollmann, I. R.; Cravatt, B. F.; Boger, D. L.; Wong, C.-H.; Lerner, R. A. *J. Am. Chem. Soc.* **1996**, *118*, 5938.
(13) A Bn-protected nervonic acid was obtained from glyceraldehyde; however, to remove the Bn group Birch conditions were necessary to retain the double bond: Cateni, F.; Zacchigna, M.; Zillic, J.; Luca, G. D. *Helv. Chim. Acta* **2007**, *90*, 282.
(14) Dutton, F.; Lee, B. H.; Johnson, S. S.; Coscarelli, E. M.; Lee, P. H. *J. Med. Chem.* **2003**, *46*, 2057.
(15) Muller, T.; Coowar, D.; Hanbali, M.; Heuschling, P.; Luu, B. *Tetrahedron* **2006**, *62*, 12025.
(16) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.