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ORIGINAL PAPER

Polyhydroxy Fatty Acids Derived from Sophorolipids

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Abstract Starting from 17-hydroxyoleic acid, which is readily available from acid alcoholysis of sophorolipids, several new polyhydroxy fatty acids have been synthesized. These compounds contain from 2 to 4 hydroxy groups, in some instances combined with other functional groups. The added hydroxy groups can be incorporated in the C_{18} chain in a variety of geometries, for example spaced widely throughout the chain at C1, C8, and C17. This regiochemical control will be of use in structure/function studies involving materials constructed from these hydroxy fatty acids. A further benefit is that the hydroxy groups can be present in protected or free states. The principal reactions used to introduce extra hydroxy groups are selenium oxide-mediated allylic hydroxylation, osmium-catalyzed dihydroxylation, and borohydride reduction of a carboxylic ester. These new compounds are expected to be of use in a number of areas, but particularly as building blocks for polymers or components of lubricant formulations.

Keywords Biorenewables · Fatty alcohols · Hydroxy fatty acids · Sophorolipids

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Introduction

Hydroxylated fatty acids (HFA) are the objects of a great deal of study due to their potential for application in a number of technologies. On the bulk scale, these include building blocks for polymers, especially polyurethanes and polyesters, lubricants, and grease thickeners [1–3 and references therein]. More specialized uses are as components in slow-release polymers for drug delivery [4], building blocks in biosurfactants [5], humectants [6], solubilizers [7], and monolayers compatible with biomacromolecules [8]. The principal HFA that has attracted attention over the years has been ricinoleic acid, although lesquerolic acid has recently emerged as an alternative [9]. Unfortunately, both of these compounds have limitations. Ricinoleic acid is associated with toxic byproducts from the castor bean source, but a different kind of limitation is structural: both ricinoleic and lesquerolic acids possess their hydroxy functional group roughly in the middle of the alkanoic chain. This geometry is not necessarily a drawback, but it does limit the range of options in structure/function studies that can be applied during an effort to fine-tune the properties of materials constructed from these HFA.

Fortunately, there are other HFA that are available from biorenewable sources. Fermentation of fats and oils by yeasts such as *Candida bombicola* can provide glycolipids in good yields. For example, sophorolipids are disaccharides with a lipid chain that is hydroxylated at the ω or $\omega - 1$ positions. Isolation of this HFA is easy with acid treatment [10]. Molecule **1** is an isomer of ricinoleic acid that has the important advantage of possessing three sites that can be used for chemical derivatization (carboxy, olefin, and hydroxy) that are widely spaced throughout the chain. This geometry should impart alternative properties

to materials constructed from this chain. A polyester constructed from **1**, for example, would not contain the six-carbon side chain that a polymer from ricinoleic acid would have; glass-transition temperatures, among other properties, of two such polymers should be quite different. Our goal was to show how the abundant bioderived starting material **1** can be altered into a number of polyhydroxylated fatty acids and alcohols—diols, triols, and tetrols—with the OH groups distributed in a range of geometries, and in some cases with other functional groups present that can be reacted further. The hydroxy groups themselves can be incorporated either free or in masked (''protected'') style, allowing for their use in a selective fashion. This latter point represents an important difference with HFA that are prepared through microbial routes; while a great deal of elegant work has appeared, and regio- and stereoselectivity are likely to be good, the tolerance of biosynthetic pathways to bulky protecting groups might be low, so chemical routes such as those presented here will remain a necessary complement to microbial methods [11]. We expect that these polyfunctional compounds will find use in a number of the technological applications listed above, especially as polymer building blocks.

Experimental Procedures

General

Compound **1** was prepared, as previously reported, from sophorolipids [10], which were in turn prepared using oleic acid and glucose as carbon sources [12]. Chemicals and reagents were obtained commercially from Sigma-Aldrich (St. Louis, MO) and Lancaster Synthesis (Alfa Aesar, Ward Hill, MA). All solvents and reagents were used as received. Silica gel used for column chromatography was obtained from Fisher Scientific (Fairlawn, NJ). NMR spectra were recorded at room temperature in $CDCl_3$ on either a Varian Associates (Walnut Creek, CA) Gemini 200 MHz or Inova 400 MHz instrument; HMQC and COSY experiments were used to aid in assignments. LCMS data were recorded on a Waters/Micromass (Milford, MA) ZMD instrument with APCI, using an elution gradient of 40:60 water/acetonitrile to 100% acetonitrile over 30 min (or minor variations on those conditions) on a 2.1 × 150 mm Waters Symmetry C18 3.5 μ column. GCMS data were collected with a Hewlett-Packard (Agilent, Wilmington, DE) HP 5890 instrument using an HP DB-5 column (30 m × 0.25 mm × 0.25 μm) and an HP 5972 mass detector (EI). The heating profile was: initial temperature 80 °C, hold 1 min; heat at 10 °C/min to 230 °C; hold 10 min. See Table 1 for spectroscopic data.

Diols 2a and 2b

A solution of SeO_2 (275 mg, 2.5 mmol) and *t*BuOOH (3 mL of a 5–6 M solution in decane, 15–18 mmol) was prepared in 10 mL CH_2Cl_2 at room temperature and stirred for 20 min. To this solution was then added **1** (1.44 g, 4.6 mmol) in another 10 mL CH_2Cl_2. Glacial acetic acid was added (10 μL). The reaction was allowed to proceed for 48 h, then solvent was removed on the rotary evaporator, 100 mL EtOAc was added, and extraction was performed three times with $Na_2S_2O_3$ solution (approx. 1 M, 100 mL each). Solvent was again removed on the rotary evaporator, and the residue was chromatographed on silica gel with 1:1 hexane/ethyl acetate. Diols **2a** (R_f = 0.4) and **2b** (R_f = 0.3) were obtained in a combined yield of 66% (495 and 504 mg respectively); 309 mg starting material was also recovered.

Diol 3a and Ketone 3b

Molecule **1** (1.05 g, 3.3 mmol) was dissolved in 10 mL MeOH and 3 mL water, $LiOH{\cdot}H_2O$ (282 mg, 6.6 mmol) was added, and the mixture was heated at 45 °C for 18 h. MeOH was removed on the rotary evaporator, 50 mL saturated citric acid solution was added, and the resulting suspension was extracted with 1:1 EtOAc/Et_2O (2 × 50 mL). Approximately half (0.5 g, 1.7 mmol) of the free acid thus obtained after removal of organic solvent was dissolved in 10 mL tetrahydrofuran (THF), and *N*, *N*-diisopropylethylamine (DIEA) (366 μL, 2.1 mmol) followed by benzotriazol-1-yloxytris(pyrrolidino) phosphonium hexafluorophosphate (PyBOP) (983 mg, 1.9 mmol) were added. The mixture was stirred for 30 min under N_2, then $NaBH_4$ (65 mg, 1.7 mmol) was added. After another 30 min, another portion of $NaBH_4$ (30 mg, 0.9 mmol) was added, and stirring was continued for another 2 h. Solvent was removed on the rotary evaporator, and the residue was taken up in 100 mL EtOAc and extracted with 2% HCl, then H_2O. Solvent was again removed and the crude was chromatographed on silica gel with 1:1 hexane/ethyl acetate (R_f = 0.5) to afford **3a**, 203 mg [42% (75% allowing for recovered starting material, 218 mg, 0.7 mmol)]. To form **3b**, **3a** (190 mg, 0.7 mmol) was dissolved in 10 mL CH_2Cl_2, and *tert*-butyldiphenylsilyl chloride (*t*BDPSCl, 182 mg, 0.7 mmol), 4-dimethylaminopyridine (DMAP, 10 mg, 0.1 mmol), and NEt_3 (102 μL, 0.7 mmol) were added. The reaction was stirred under N_2 for 36 h at room temperature, then concentrated, applied to a silica gel column, and eluted with 3:1 hexane/ethyl acetate (R_f = 0.6) to afford the mono-protected diol (305 mg, 87%). This compound (280 mg, 0.5 mmol) was then dissolved in 10 mL CH_2Cl_2, and 3-Å molecular sieves and pyridinium dichromate (PDC, 300 mg, 0.8 mmol) were added. The

Table 1 Spectra of products

Compound	^{13}C NMR[a,b]	^{1}H NMR[b]	Mass spec[c]
2a	23.6(C18), 25.0(C3), (25.4, 25.8, C6, C15), 29.2 (3), 29.4, 29.5, 32.2, 34.2(C2), 37.3(C7), 39.4(C16), 51.6(Me), 68.3(C17), 73.3(C8), 132.3, 133.1(C9,10), 174.5(C1)	1.20 (d, 3H, 6.2 Hz, H18), 1.33 (br s, 16H), 1.52–1.73 (m, 4H), 2.02 (m, 2H, H11), 2.32 (t, 2H, 7.4 Hz, H2), 3.68 (s, 3H, Me), 3.80 (m, 1H, H17), 4.04 (m, 1H, H8), 5.43 (dd, 1H, 15.8, 6.6 Hz, H9), 5.60 (m, 1H, H10)	351 (+ Na^+), 311 (loss of H_2O), 293 (loss of 2 H_2O)
2b	23.7(C18), 25.1(C3), (25.7, 25.9, C6 and C15), 29.1, 29.3 (3), 29.7, 32.3, 34.3(C2), 37.4(C12), 39.5(C16), 51.7(Me), 68.3(C17), 73.4(C11), (132.4, 133.3, C9, C10), 174.6(C1)	1.17 (d, 3H, 6.9 Hz, H18), 1.29 (br s, 16H), 1.40 – 1.63 (m, 4H), 2.00 (q, 2H, 6.7 Hz, H8), 2.29 (t, 2H, 7.5 Hz, H2), 3.65 (s, 3H, Me), 3.77 (m, 1H, H17), 4.01 (q, 1H, 6.6 Hz, H11), 5.42 (dd, 1H, 15.4, 7.1 Hz, H10), 5.60 (m, 1H, H9)	
3a	23.7(C18), 26.0 (2, C8, C11), 27.4(C3), 29.4, 29.5, 29.6, 29.7, 29.8, 29.9 (3), 33.0(C2), 39.6(C16), 63.3(C1), 68.4(C17), 130.1 (2, C9,10)	1.17 (d, 3H, 6.4 Hz, H18), 1.29 (br s, 14H), 1.42 (m, 4H), 1.55 (m, 4H), 2.00 (m, 4H, H8, H11), 3.62 (t, 2H, 6.6 Hz, H1), 3.77 (m, 1H, H17), 5.34 (m, 2H, H9, H10)	285, 267 (loss of H_2O), 249 (loss of 2 H_2O)
3b	19.3(tBu-C), 23.9(C18), 25.9, 27.0 (3, tBu), (27.2, 27.3, C8,11), 29.2 (2), 29.4, 29.5, 29.6, 29.7, 29.8, 32.7(C2), 43.9(C16), 64.1(C1), 127.7(Ar), 129.6(Ar), (129.8, 130.2, C9, C10), 134.3(Ar), 135.7(Ar), 209.4(C17)	1.08 (s, 9H, tBu), 1.30 (br s, 16H), 1.59 (m, 4H), 2.04 (m, 4H, H8, H11), 2.15 (s, 3H, H18), 2.44 (t, 2H, 7.5 Hz, H16), 3.68 (t, 2H, 6.5 Hz, H1), 5.38 (m, 2H, H9, H10), 7.43 (m, 6H, Ar), 7.70 (m, 4H, Ar)	521, 443 (loss of C_6H_6), 365 (loss of 2 C_6H_6)
4	23.6(C1, C18), 25.9, 27.3*, 29.2 (2), 29.3*, 29.6, 29.8*, 32.6(C8, C11), 39.4(C3, C16), 68.3(C2, C17), 130.0*, 130.4(C9, C10)	1.20 (d, 6H, 6.0 Hz, H1, H18), 1.31 (br s, 12H), 1.43 (m, 4H), 1.60 (m, 4H), 2.00 (m, 4 H, H8, H11), 3.81 (m, 2H, H2, H17), 5.40 (m, 2H, H9, H10)	285, 267 (loss of H_2O), 249 (loss of 2 H_2O)
5	23.6(C18), 25.0(C3), 25.8, 26.0, 29.1, 29.2, 29.5, 29.6, 29.7 (2), 31.3 (2, C8, C11), 34.2(C2), 39.4(C16), 51.6(Me), 68.2(C17), 74.7 (2, C9, C10), 174.5(C1)	1.21 (d, 3H, 6.2 Hz, H18), 1.34 (br s, 16H), 1.45 (m, 4H), 1.55–1.8 (m, 4H), 2.33 (t, 2H, 7.6 Hz, H2), 3.62 (m, 2H, H9, H10), 3.69 (s, 3H, Me), 3.82 (m, 1H, H17)	347, 329 (loss of H_2O), 311 (loss of 2 H_2O), 293 (loss of 3 H_2O)
5b	20.1(C18), 25.1(C3), 25.4, 26.2 (2), 29.2, 29.4, 29.5, 29.6, 29.7, 31.4 (2, C8, C11), 34.3(C2), 36.0(C16), 51.7(Me), 69.5(C17), 74.9 (2, C9, C10), 75.8(Bn), (128.5 (2), 128.6, 128.9 (2), 135.6, 6 Ar), 174.6(C1)	1.26 (d, 3H, 6.2 Hz, H18), 1.30 (br s, 16H), 1.40 (m, 4H), 1.49 (m, 2H), 1.60 (m, 2H), 2.29 (t, 2H, 7.5 Hz, H2), 3.56 (m, 2H, H9, H10), 3.65 (s, 3H, Me), 4.75 (hex, 1H, 6.4 Hz, H17), 5.13 (s, 2H, Bn), 7.35 (m, 5H, Ar)	437, 419 (loss of H_2O), 311 (loss of 2 H_2O and C_7H_6)
6	19.4(tBu-C), 23.3, 24.9, 25.3, 25.8(C3), 27.2 (3, tBu), 29.2, 29.4, 29.6, 32.2, 32.9(C2), 38.2(C7), 39.6(C16), 63.2(C1), 69.7(C17), 74.8(C8), 127.5(Ar), (129.5, 131.3, C9, C10), 132.9(Ar-C), 134.7(Ar), 136.0(Ar)	1.09 (br s, 21H, tBu, H18), 1.11–1.36 (m, 18H), 1.36–1.63 (m, 4H), 1.84 (m, 2H, H11), 3.64 (t, 2H, 6.6 Hz, H1), 3.85 (m, 1H, H17), 4.08 (m, 1H, H8), 5.16–5.46 (m, 2H, H9, H10), 7.31–7.46 (m, 12 H, Ar), 7.65–7.74 (m, 8H, Ar)	800 (+ Na^+), 760 (loss of H_2O), 559 (+ Na^+, loss of *t*BDPS)
9a	17.8(C18), 25.0(C3), 25.6, 26.1, 26.3 (2, acet), 28.8, 29.2, 29.3, 29.6, 29.7 (2), 34.2(C2), 37.2(C14), 51.6(Me), 73.1(C15), 78.1 (2, C9, C10), 107.4(acet), 126.9(C17), 134.4(C16), 174.4(C1)	1.34 (br s, 14H), 1.38–1.67 (m, 6H), 1.42 (s, 6H, acet), 1.71 (d, 3H, 6.2 Hz, H18), 2.32 (t, 2H, 7.2 Hz, H2), 3.68 (s, 3H, Me), 3.96–4.11 (m, 3H, H9, H10, H15), 5.48 (ddt, 1H, 15.4, 6.9, 1.5 Hz, H16), 5.67 (dq, 1H, 15.4, 6.2 Hz, H17)	385, 367 (loss of H_2O), 327 (loss of H_2O and acetonide), 309 (loss of 2 H_2O, acetonide), 291
9b	25.0(C3), 25.3, 26.1, 26.3 (2, acet), 28.8, 29.2, 29.3, 29.6, 29.7 (3), 34.2(C2), 37.0(C15), 51.6(Me), 73.3(C16), 78.1 (2)(C9, C10), 107.4(acet), 114.7(C18), 141.4(C17), 174.4(C1)	1.35 (br s, 16H), 1.44 (s, 6H, acet), 1.47–1.74 (m, 6H), 2.32 (t, 2H, 7.3 Hz, H2), 3.69 (s, 3H, Me), 4.03 (m, 2H, H9, H10), 4.13 (m, 1H, H16), 5.12 (dt, 1H, 10.2, 1.4 Hz, H18 *cis* to H17), 5.24 (dt, 1H, 17.2, 1.4 Hz, H18 *trans* to H17), 5.89 (ddd, 1H, 17.2, 10.3, 6.2 Hz, H17)	
9c	25.0(C3), 26.1, 26.3 (2, acet), 28.8, 29.1, 29.2, 29.3, 29.6, 29.7 (3), 32.2(C15), 34.2(C2), 51.6(Me), 64.0(C18), 78.1 (2, C9, C10), 107.4(acet), 129.1(C17), 133.5(C16), 174.4(C1)	1.36 (br s, 16H), 1.44 (s, 6H, acet), 1.48–1.68 (m, 4H), 2.07 (m, 2H, H15), 2.32 (t, 2H, 7.4 Hz, H2), 3.69 (s, 3H, Me), 4.05 (m, 2H, H9, H10), 4.11 (d, 2H, 6.4 Hz, H18), 5.68 (m, 2H, H16, H17)	

Table 1 continued

Compound	^{13}C NMR[a,b]	^{1}H NMR[b]	Mass spec[c]
10a	20.2(C18), 21.3(Ac), 21.6(Ac), 25.1, 25.5 (2), 26.1, 29.2, 29.4(2), 29.6, 30.9, 33.1(C2), 34.3(C8), 36.0(C16), 51.7(Me), 69.6, 71.2(C9, C17), 74.5, 75.0(C10, C11), (171.1, 172.5, Ac), 174.6(C1)	1.18 (d, 3H, 6.2 Hz, H18), 1.30 (br s, 14 H), 1.41–1.52 (m, 4H), 1.52–1.66 (m, 4H), 2.01 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.29 (t, 2H, 7.5 Hz, H2), 3.27 (m, 1H, H10), 3.45 (m, 1H, H9), 3.65 (s, 3H, Me), 4.74 (m, 1H, H11), 4.87 (hex, 1H, 6.2 Hz, H17)	429 (loss of H_2O), 387 (loss of CH_3CO_2H), 369 (loss of CH_3CO_2H and 2 H_2O), 309 (loss of 2 CH_3CO_2H and 2 H_2O)
10b	20.2(C18), 21.4(Ac), 21.6(Ac), 25.1, 25.5(2), 25.6, 29.2(2), 29.5(2), 31.1, 33.8(C2), 34.3(C8), 36.0(C16), 51.7(Me), (71.2, 72.0, C9, C17), (75.0, 75.2, C10, C11), (171.1, 171.4, Ac), 174.6(C1)	1.19 (d, 3H, 6.5 Hz, H18), 1.28 (br s, 14H), 1.42 (m, 4H), 1.60 (m, 4H), 2.03 (s, 3H, Ac), 2.08 (s, 3H, Ac), 2.29 (t, 2H, 7.4 Hz, H2), 3.38 (t, 1H, 5.2 Hz, H10), 3.53 (m, 1H, H9), 3.66 (s, 3H, Me), 4.87 (hex, 1H, 6.2 Hz, H17), 4.99 (m, 1H, H11)	
12	19.4(tBu-C), 23.3(C18), 25.3, 25.8, 26.2, 26.3 (2, acet), 27.1 (3, tBu), 28.8, 29.5, 29.6 (2), 29.8 (m), 32.9(C2), 39.5(C16), 63.2(C1), 69.7(C17), 78.2 (2, C9, C10), 107.4(acet), 127.5(Ar), 129.5(Ar-C), 134.7(Ar), 136.0(Ar)	1.08 (br s, 12H, tBu, H18), 1.38 (br s, 20 H), 1.46 (s, 6H, acet), 1.60 (m, 6H), 3.66 (t, 2H, 6.6 Hz, H1), 3.84 (hex, 1H, 5.6 Hz, H17), 4.03 (m, 2H, H9, H10), 7.38 (m, 6H, Ar), 7.70 (m, 4H, Ar)	598, 580 (loss of H_2O), 503 (loss of C_6H_5 + H_2O), 461 (loss of C_6H_5, H_2O, and acetonide), 383 (loss of 2 C_6H_5, H_2O, and acetonide)
13a	19.6(C18), 25.0(C3), 25.6, 26.1, 26.2 (2, acet), 28.7, 29.1, 29.2, 29.5, 29.7 (3), 33.3(C2), 34.1(C15), 51.5(Me), 70.9(C17), 76.1(C16), 78.1 (2, C9, C10), 107.4(acet), 174.5(C1)	1.15 (d, 3H, 6.2 Hz, H18), 1.28 (br s, 16H), 1.39 (s, 6H, acet), 1.41–1.69 (m, 6H), 2.28 (t, 2H, 7.4 Hz, H2), 3.30 (m, 1H, H16 or H17), 3.54 (m, 1H, H16 or H17), 3.64 (s, 3H, Me), 3.98 (m, 2H, H9, H10)	403, 385 (loss of H_2O), 367 (loss of 2 H_2O), 345 (loss of H_2O and acetonide), 327 (loss of 2 H_2O and acetonide)
13b	25.1(C3), 25.7, 26.2 (2, acet), 26.4, 28.9, 29.3, 29.4, 29.7 (2), 29.8 (3), 33.4(C2), 34.3(C16), 51.7(Me), 67.0(C18), 72.5(C17), 78.2 (2, C9, C10), 107.5(acet), 174.6(C1)	1.29 (br s, 18H), 1.39 (s, 6H, acet), 1.45 (m, 4H), 1.58 (m, 2H), 2.28 (t, 2H, 7.4 Hz, H2), 3.41 (dd, 1H, 11.0, 7.5 Hz, H18), 3.60–3.72 (m, 2H, H17, H18), 3.65 (s, 3H, Me), 3.99 (m, 2H, H9, H10)	
13c	16.9(C18), 25.1(C3), 25.7, 26.3, 26.4 (2, acet), 28.9, 29.3, 29.4, 29.7, 29.8 (2), 31.9, 33.5(C2), 34.3(C15), 51.7(Me), 71.1(C17), 75.1(C16), 78.2 (2, C9, C10), 107.5(acet), 174.6(C1).	1.20 (d, 3H, 6.5 Hz, H18), 1.30 (br s, 16H), 1.40–1.71 (m, 6H), 1.42 (s, 6H, acet), 2.31 (t, 2H, 7.3 Hz, H2), 3.55–3.72 (m, 2H, H16, H17), 3.67 (s, 3H, Me), 4.02 (m, 2H, H9, H10)	

[a] In carbon spectra, numbers in parentheses indicate the number of peaks that are closely overlapped at the indicated chemical shift; ''m'' indicates a larger number of peaks that cannot be distinguished. Overlapped peaks in the aromatic units of *t*BDPS are not enumerated. Asterisks indicate minor peaks from the *cis* isomer

[b] Me = methyl ester; acet = acetonide; Ac = acetyl group; Ar = aromatic; Bn = CH_2 of benzyl. ''H*n*'' indicates protons attached to Carbon *n*

[c] Unless otherwise indicated, the first peak listed is MH^+. Blank entries indicate that the peaks are the same as observed for the preceding isomer, with only minor differences in relative intensities

slurry was stirred under N_2 at room temperature, with a $CaSO_4$ drying tube affixed to the flask. After 18 h, TLC showed that no starting material remained, so the slurry was filtered through celite, concentrated on the rotary evaporator, and chromatographed on silica gel with 3:1 hexane/ethyl acetate (R_f = 0.8) to yield ketone **3b** (256 mg, 92%).

Diol 4

Compound **1** (314 mg, 1.0 mmol) was dissolved in 10 mL CH_2Cl_2 that had been dried over activated 3-Å molecular sieves, and the solution was stirred under N_2 in a round bottom flask fitted with a drying tube filled with $CaSO_4$ and sieves. The catalyst (benzylidene-bis(tricyclohexylphosphine)dichlororuthenium, ''Grubbs' 1st-generation catalyst'', 60 mg) was added, dissolving immediately to give a purple solution. The solution was refluxed for 90 min, over the course of which the color changed from purple to brownish. After the mixture cooled to RT, the solvent was removed under a gentle N_2 stream, then the residue was taken up in 3:1 hexane/ethyl acetate and chromatographed on a silica gel column. Diol **4** was obtained in 89% theoretical yield (63 mg, 0.2 mmol, R_f = 0.3), allowing for the production of other metathesis products at equilibrium, since 1 mol of nonsymmetrical olefin A:B can only give 0.25 mol A:A (such as compound **4**), 0.5 mol A:B, and 0.25 mol B:B.

Triol 5

To a suspension of **1** (1.7 g, 5.4 mmol) in 50 mL 1:1 water/*t*BuOH at 0 °C was added methanesulfonamide (530 mg, 5.6 mmol) and AD-mix-α reagent (commercially available from Aldrich, a mixture of chiral ligand and osmium oxidant, 6.5 g). The mixture was stirred overnight and allowed to warm to room temperature, then solid $Na_2S_2O_3$ (approx 2.5 g) was added until the color changed from yellow to tan. The reaction was extracted with EtOAc (2 × 75 mL) and $CHCl_3$ (2 × 75 mL), the organic layers combined and concentrated to dryness on the rotary evaporator, and the residue was chromatographed on silica gel in ethyl acetate to yield **5** (1.79 g, 96%, R_f = 0.5).

Triol 6

Diol **2a** (200 mg, 0.6 mmol) was dissolved in 5 mL pyridine, and *t*BDPSCl (419 mg, 1.5 mmol), DMAP (43 mg, 0.4 mmol), and imidazole (48 mg, 0.7 mmol) were added. The reaction was heated to 50–55 °C for 48 h. TLC showed no remaining starting material. Solvent was removed on the rotary evaporator, and the residue was applied to a short silica gel column and eluted with 3:1 hexane/ethyl acetate (R_f = 0.9) to give the doubly-protected ester (450 mg, 93%). A portion of this material (220 mg, 0.3 mmol) was hydrolyzed with LiOH·H_2O as for **3a**. The resulting free acid (140 mg, 0.2 mmol) was dissolved in 10 mL THF, then DIEA (37 μL, 0.2 mmol) and PyBOP (103 mg, 0.2 mmol) were added. After 30 min, $NaBH_4$ (11 mg, 0.3 mmol) was added, followed by the same amount after another 30 min. Workup was the same as for **3a**, with chromatography in 2:1 hexane/ethyl acetate yielding 91 mg, 65% (R_f = 0.5) from the free acid, 40% from **2a**.

Triol 9

Triol **5** (1.35 g, 3.9 mmol) was suspended in 7 mL 2,2-dimethoxypropane and 15 mL CH_2Cl_2. On the addition of pyridinium *p*-toluenesulfonate (PPTS) (230 mg, 0.9 mmol) all the suspended material dissolved in less than a minute. The reaction was stirred under N_2 at RT for 20 h, the solvent was removed on the rotary evaporator, and the residue chromatographed on silica gel with 3:1 hexane/ethyl acetate to yield 1.41 g, 94% (R_f = 0.3). This 17-hydroxy 9,10-acetonide (0.91 g, 2.4 mmol) and triphenylphosphine (1.24 g, 4.7 mmol) were dissolved in 50 mL 1,2-dichloroethane, then a solution of 1,2-dibromotetrachloroethane (1.54 g, 4.7 mmol) and triethylamine (1.31 mL, 9.2 mmol) in 3 mL 1,2-dichloroethane was added. The reaction was stirred at room temperature under N_2 for 30 min, then filtered through a glass frit. The precipitate was washed twice with 2 mL of 1,2-dichloroethane. The filtrate was evaporated, then applied to a silica gel column and eluted with 4:1 hexane/ethyl acetate to give molecule **7**, 0.95 g, 91% (R_f = 0.8).

Bromide **7** (675 mg, 1.5 mmol) was dissolved in 3 mL toluene and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (330 μL, 2.3 mmol) was added. A $CaSO_4$ drying tube was attached to the flask, and the mixture was heated under N_2 at 60–65 °C for 24 h, then another 100 μL DBU was added and heating was continued for a further 48 h. Solvent was removed on the rotary evaporator, and the residue was chromatographed on silica with 4:1 hexane/diethyl ether to afford **8** (363 mg, 66%, R_f = 0.6) and unreacted **7** (93 mg, 0.21 mmol). For the final step, a procedure analogous to that used for preparation of **2** was followed, with molecule **8** (220 mg, 0.6 mmol), SeO_2 (34 mg, 0.3 mmol), and *t*BuOOH (0.24 mL of a 5–6 M solution in decane) in 10 mL CH_2Cl_2. Careful chromatography on silica gel with either 2:1 hexane/ethyl acetate or 5% MeOH in $CHCl_3$ (R_f's = 0.4, 0.35, 0.3 in both solvent systems) afforded three products **9b**, **a**, and **c** (24, 78, and 19 mg, respectively, see ''Results and Discussion'' for structures) for a total yield of 53% as well as unreacted **8** (76 mg, 0.21 mmol).

Tetrol 10

Diol **2b** (310 mg, 1.0 mmol) was dissolved in 5 mL pyridine, and DMAP (45 mg, 0.4 mmol) and acetic anhydride (Ac_2O, 1.5 mL) were added. The reaction was stirred under N_2 at room temperature. After a few minutes, a yellow color developed. The reaction continued for 3 h, at which point TLC showed no starting material, so the solvent was removed on the rotary evaporator, and the crude mixture was dried under a nitrogen stream for 2 h, then placed on the vacuum line overnight. It was then dissolved in 10 mL 1:1 H_2O/*t*BuOH, and methanesulfonamide (100 mg) and AD-mix-α (1.4 g) were added. The reaction was stirred for 24 h and worked up as for compound **5** above. Silica gel chromatography in 1:1 hexane/ethyl acetate afforded two closely eluting spots (R_f's = 0.3 and 0.2) **10a** (197 mg) and **10b** (174 mg) for a total yield of 88%.

Tetrol 12

Compound **1** (1.2 g, 3.8 mmol) was converted to its *t*BDPS ether as for **3b** and **6**. After workup, silica gel chromatography with 3:1 hexane/ethyl acetate afforded 1.72 g *t*BDPS-protected **1** (3.2 mmol, 84 %, R_f = 0.9). This compound was hydrolyzed with LiOH·H_2O as for **3a**, and after workup was chromatographed in 9:1 $CHCl_3$/MeOH to afford 1.1 g (2.0 mmol) free acid (R_f = 0.4). A portion of this material (823 mg, 1.5 mmol) was dissolved in 20 mL THF, and DIEA (320 μL, 1.8 mmol), *N*,*N*-diisopropylcarbodiimide (DIC, 263 μL, 1.7 mmol), and 1-hydrox-

ybenzotriazole hydrate (HOBt, 257 mg, 1.7 mmol) were added. After 10 min, $NaBH_4$ (120 mg, 3.2 mmol) was added. After another 30 min, the same amount of $NaBH_4$ was added again. The mixture was stirred for a further 2 h, after which it was concentrated on the rotary evaporator, stirred with concentrated citric acid solution (caution: gas evolution), and extracted with 100 mL EtOAc. Column chromatography on silica gel with 2:1 hexane/ethyl acetate (R_f = 0.8) yielded **11** (262 mg, 33% from the *t*BDPS free acid (57% allowing for recovered starting material 347 mg, 0.7 mmol), or 17% from **1**).

Monoprotected diol **11** (150 mg, 0.3 mmol), AD-mix-α (406 mg) and methanesulfonamide (27 mg, 0.3 mmol) were combined in 10 mL 1:1 *t*BuOH/H_2O and worked up as for **5** above, then purified by column chromatography in ethyl acetate to give the singly-protected tetrol (151 mg, 94%, R_f = 0.6). A portion of this material (50 mg, 0.1 mmol) was reacted with 1 mL 2,2-dimethoxypropane and 25 mg PPTS in 5 mL CH_2Cl_2 to give triprotected tetrol **12** after chromatography in 2:1 hexane/ethyl acetate (44 mg, 83%, R_f = 0.6).

Tetrol 13

Molecule **8** (200 mg, 0.5 mmol) was reacted with 0.8 g AD-mix-α and 50 mg methanesulfonamide and worked up as for **5** to afford the tetrol. TLC in 9:1 $CHCl_3$/MeOH indicated one principal product and two minor ones, which could be separated from each other by chromatography on silica gel (**13a**, **b**, and **c** R_f's = 0.5, 0.45, 0.4) for yields of 116, 47, and 26 mg (88% total).

Results and Discussion

Synthesis of Diols

Three methods were used to introduce a second hydroxy group to the skeleton of **1** (Fig. 1). The first was to perform allylic hydroxylation with selenium dioxide and *t*-butyl hydroperoxide [13]. This route gave two isomers in equal amounts as expected, the C8,C17 and the C11,C17 diols, which could be separated from each other by column chromatography on silica gel. Their identity was determined by mass spectrometry of fragments resulting from oxidative cleavage with $KMnO_4/NaIO_4$. With LCMS, mono-methyl nonanedioate was observed only in the cleavage products from molecule **2b**, and hydroxynonanoic acid from **2a**. Portions of the crude cleavage products were also dissolved in methanol and treated with EDC and DMAP to form the methyl esters, which were analyzed by GCMS, showing dimethyl nonanedioate for **2b** and methyl hydroxynonanoate from **2a**. Although in our hands this allylic hydroxylation route always left some starting material unreacted, it gave reasonable yields and was straightforward, and the possibility of separating the two resulting diol isomers may be useful.

Concerning these compounds, as well as most of the ones which follow, a note about stereochemistry is warranted. Compound **1** is chiral, with L configuration [12, 14, and references therein]. The added hydroxy groups at C8 and C11 in **2a** and **2b** are not introduced stereoselectively, so diastereomers are created. Separation of these diastereomers could well be difficult, requiring extensive HPLC usage, and we have not attempted to do so. NMR spectra of these compounds do not appear to be made more complicated by the existence of the compounds as diastereomers. We propose that this is because the chiral centers are widely spaced, from the chain midsection to near its end, and the flexibility of the methylene chain precludes favored or fixed conformations of one diastereomer versus another. Regioisomers, on the other hand, such as **2a** and **2b** and others below, have been separated.

The second route converted the carboxylic unit of **1** to a primary alcohol by reduction of an activated ester formed in situ [15]. Other methods such as using $LiAlH_4$ for reduction of a methyl ester may be feasible, but we sought milder and easier-to-handle reagents that would be com-

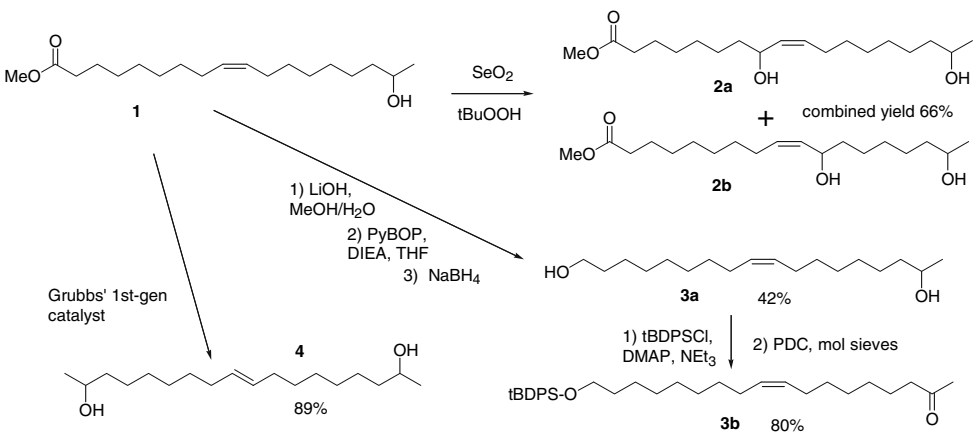

Fig. 1 Synthetic routes used to convert molecule 1 into dihydroxy compounds. See text for abbreviations

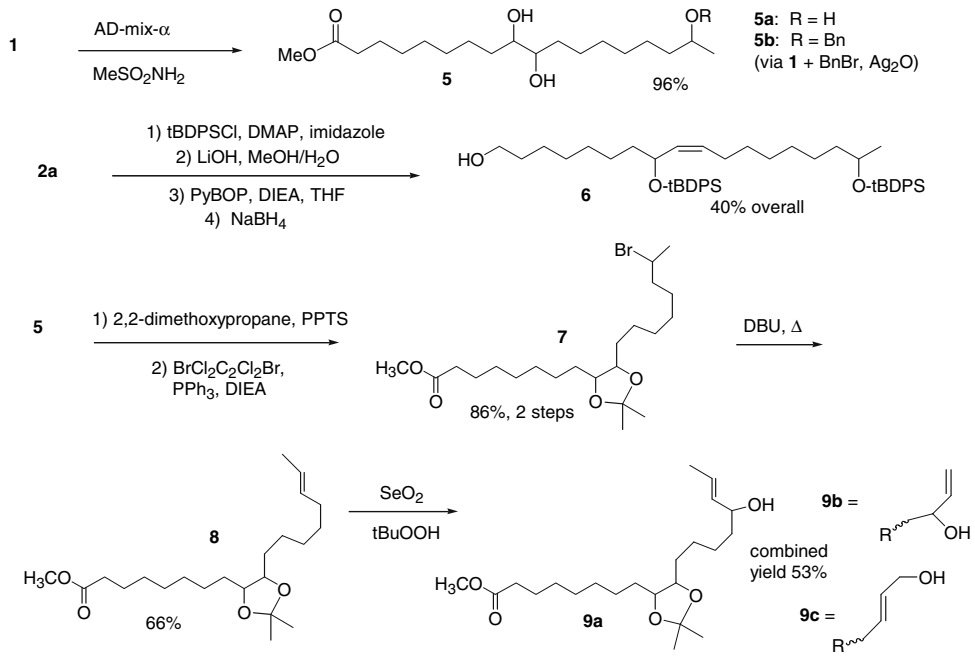

Fig. 2 Synthetic routes used to obtain trihydroxy compounds. See text for abbreviations

patible with other functional groups in the molecule. Using the protocols outlined in Ref. [15], for example, reduction of a carboxylic acid (as its activated ester) in the presence of an alkyl ester is possible. The reagent used to form an HOBt ester, PyBOP, is not cheap, but the conversion to the primary alcohol was conveniently performed with $NaBH_4$. The alkene was not reduced, and the 17-OH did not need to be protected. To give an example of the kind of further manipulations that can be performed with a diol like **3a**, we selectively protected the primary alcohol as a *t*BDPS ether and oxidized the secondary alcohol to a ketone. By analogy with some polyhydroxylated fatty acids that have been produced microbially, a ketone of this sort could be a useful intermediate in studying the formation of bicyclic compounds that may have anti-cancer activity [16, 17]. Had the 17-OH been protected prior to reduction, the opposite pattern of monoprotection of the diol could have been obtained (as it was for the preparation of **6** and **12**, below). The third route to a diol was olefin metathesis of **1** using Grubbs' first generation catalyst [18]. This route affords a mixture of *trans* and *cis* isomers in the ratio of about 5:1 as determined by both LCMS and NMR. The other possible metathesis products—dimethyl 1,18 octa-9-decenoate and the ''reconstituted'' HFA methyl ester **1**—are also obtained, but are easily separated from the diol by column chromatography. In fact, this method can be thought of as an alternative ''elaidization'' route for **1** that avoids toxic selenium or thiophenol. While **3** and **4** are structurally similar in that their hydroxy groups are at the far ends of the chain from each other, it is worth noting that the primary hydroxy group of **3** can be chemoselectively derivatized.

Synthesis of Triols

Several strategic options exist for introducing two new hydroxy groups to **1** in a variety of geometries (Fig. 2). The simplest is to use a dihydroxylation catalyst. We chose to work with Sharpless' asymmetric dihydroxylation (AD) reagents for ease, since the conditions are mild and the materials are conveniently commercially available [19]. The asymmetric aspect is not of course particularly relevant to oleic acid derivatives, since *cis* olefins are known to give poor stereoselectivity [19, 20]. Enantioenriched 9,10-dihydroxystearates have however been obtained from methyl oleate through HPLC separation of diastereomers formed with chiral auxiliaries after osmium-catalyzed dihydroxylation [20]. Molecule **5a** was produced in essentially quantitative yield. The C17-OH does not need to be protected, but we have also performed the reaction on the C17 benzyl ether (giving **5b**).

Another likely strategy is to combine the individual steps that were used for production of diols. For example, we intended to subject the allylicly hydroxylated **2a** to the reduction conditions used to produce **3**. Protection of both hydroxy groups of **2a** seemed judicious, however, since the possibility for cyclization existed if either the C8 or C17 groups were to intramolecularly react with the HOBt ester. Intermolecular esterification was also a possibility. The bis-*t*BDPS ether was prepared, then hydrolyzed to the free carboxylic acid, and reduced to give doubly-protected primary alcohol **6**.

Finally, more elaborate multistep schemes can be employed to generate triols with added functionality. A key

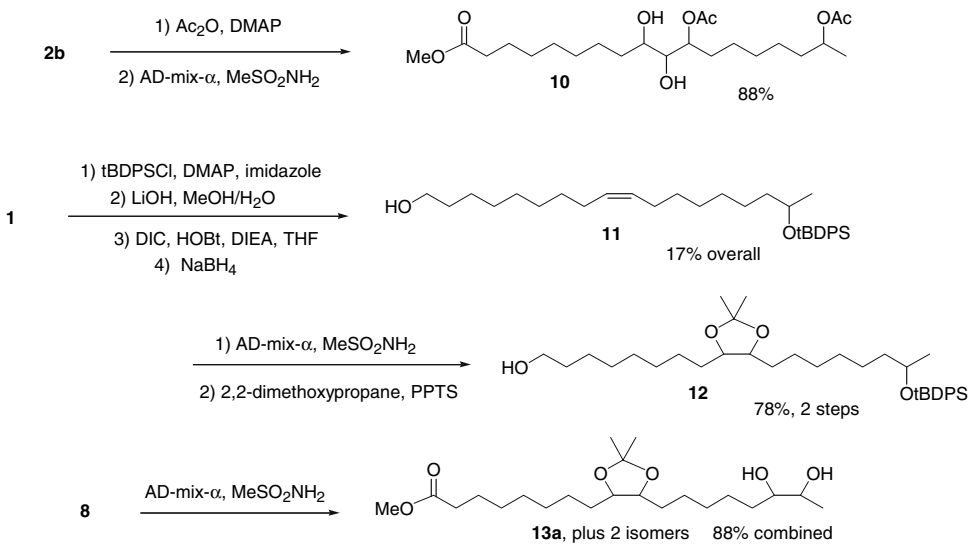

Fig. 3 Synthetic routes used to obtain tetrahydroxy compounds. See text for abbreviations

motivation was to attain the ability to place hydroxy groups widely spaced throughout the molecule. Molecule **5a** was protected as the acetonide, then converted to the C17 bromide. Elimination with strong base DBU gave the olefin in several isomeric forms: GCMS showed three closely eluting peaks, one major and two minor, all with correct mass for **8**, and NMR of the crude mixture indicated the presence of terminal alkene. We propose that **8** is formed predominantly as *trans* $\omega - 1$ alkene along with some *cis* $\omega - 1$ and terminal isomers, although they could not be preparatively separated with column chromatography. However, when olefin **8** was hydroxylated with selenium dioxide as in the preparation of **2**, three isomeric products were obtained in the ratio of 64:20:16, and this time they could be separated on silica gel and identified by NMR. In accord with our proposal about **8**, the major product **9a** was the *trans* $\omega - 1$ C15-OH product. Its identity was determined based on 1H-1H coupling ($J_{H16/H17}$ = 15.4 Hz), but in addition the calculated value of the chemical shift for C18 (17.3 ppm from ChemDraw version 5.0) agrees with the observed (17.8 ppm), while that calculated for the *cis* version is 11.3 ppm. The ω-olefin C16-OH (**9b**) and the *trans* $\omega - 1$ olefin C18-OH (**9c**) were minor products. The former is clearly identified by its terminal alkene pattern in 1H NMR, and the latter by its lack of a terminal methyl group. The *trans* stereochemistry of **9c** is harder to be sure about, but again calculated ^{13}C chemical shifts agree better with *trans* (C15: 33.3 ppm, observed 32.2) than with *cis* (C15: calculated 27.3). We cannot explain why no *cis* isomers were observed in the product distribution of **9**, except to suggest that since *cis* **8** was present in minor relative amounts, derivatives of it would afford only trace amounts that could not be observed on the scale we used.

Synthesis of Tetrols

Combining the individual steps used to prepare diols and triols is again a productive strategy for obtaining higher hydroxylated molecules (Fig. 3). AD was relied upon heavily since it efficiently introduces two hydroxy groups. Diol **2b** was diprotected as the acetate, then subjected to AD conditions to give **10**. Two isomers were isolated in roughly 1:1 ratio (53:47). There should of course be several diastereomers of **10**, since the stereocenter at C11 could exist in either configuration, and stereoselectivity of the AD reagent at the *cis* olefin will be poor. We tentatively propose that the two isomers obtained could be one with an all-syn arrangement of the oxygens at C9, C10, and C11, and another with C9 and C10 oxygens anti to the one at C11. In any case, the existence of these polyhydroxy compounds as a mixture of stereoisomers should not be deleterious in most polymer or materials applications.

For the next tetrol, borohydride reduction of the HOBt ester derived from **1** was performed after first protecting the C17 hydroxy group with a silyl group. For this example of the reduction, the HOBt ester was prepared without the more expensive PyBOP reagent by using a carbodiimide and HOBt. Yields were not as good as with the PyBOP reagent. This diol was then subjected to AD conditions, and finally protected as the acetonide to give **12**. The olefinic diol **8** is also a useful starting point; AD gives the tetrol **13**. As with the preparation of **9**, hydroxylation allows separation of the isomers, in a ratio of 61:25:14. The predominant isomer **13a** is the C16/C17 diol, and by analogy with our analysis of the isomers of compound **9**, since this is the major isomer we assume it to be *trans*. The second in abundance, **13b**, is the C17/C18 diol arising from the terminal alkene component of **8**, identified by the presence of

five CH–O protons (C9, C10, C17, and two on C18), the absence of a terminal CH_3 group, and the peak at 67 ppm for C18. The least abundant isomer **13c** is also a C16/C17 diol, based on NMR data, and we propose that it arose from the *cis* alkene, although detailed NMR experiments to ascertain stereochemistry have not been performed.

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