Synthesis of Polyfunctional Fatty Amines from Sophorolipid-Derived 17-Hydroxy Oleic Acid

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ABSTRACT: A series of mono- and diamines and one triamine have been prepared using methyl 17-hydroxy oleate as the common starting material. The 17-hydroxy oleate is an abundant bioderived material obtained from acid alcoholysis of sophorolipids, which in turn are produced by fermentation of agricultural by-products. Incorporation of the amino unit(s) can be selectively performed at either end of the chain or at its middle. The chief synthetic reactions used are allylic bromination, Curtius rearrangement, and the Mitsunobu reaction. These fatty amines also possess functionality such as hydroxy groups, carboxylic acids, and C–C double bonds. The amines are isolated in protected form using a variety of protecting groups, the identities of which can be selected on the basis of the intended use of the amine. These novel compounds will be of interest in the preparation of highly functionalized polymers and surfactants, among other areas.

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KEY WORDS: Biorenewables, fatty amines, hydroxy fatty acids, sophorolipids.

The conversion of bulk agricultural products and co-products, such as vegetable oils, tallow, restaurant greases, bioglycerol, and soy molasses, into value-added materials is an area of burgeoning scientific and commercial interest. Proposed uses for such value-added materials include surfactants, gelators, drug-delivery adjuvants, and building blocks for polymers (1,2). Microbial, enzymatic, and chemical routes have been used, and a wide range of molecular structures may result (3). One promising class of compounds is glycolipids, particularly sophorolipids, which can be produced abundantly from renewable feedstocks (4–6). The crude sophorolipids as obtained from a fermentation run consist of a variety of lactonic and free acid forms in various states of acetylation (hence the pluralized name). Although the sophorolipid molecule offers much potential for derivatization in its own right (7,8), it can also be subdivided into the carbohydrate and FA components. The latter, which are hydroxylated predominantly at the ω-1 position, are attractive raw materials for many purposes. A good comparison is afforded by the hydroxy-containing ricinoleic acid, which has been the subject of many synthetic modifications (1,9).

As Figure 1 shows, this starting material is a trifunctionalized hydrocarbon. We hypothesized that each of its three functional groups (carboxyl, olefin, and hydroxy) could be addressed individually or sequentially for purposes of further derivatization. The roughly equidistant spacing of the three functional groups along the alkyl chain presents a unique opportunity; ricinoleic acid, for example, is not subject to ready derivatization at its alkane terminus. The class of derivatives we have chosen to investigate initially are amines. A great deal of work has appeared on oxygenation of FA, but fewer studies have been devoted to amination. We report here some preliminary synthetic manipulations that convert 17-hydroxy oleic acid derived from sophorolipids into several classes of polyfunctional fatty amines (Fig. 2). For ease of purification and characterization, the amines are generated as derivatives with common, readily removable protecting groups attached (10), but to reduce wordiness these are termed "amines" in the rest of this paper.

The rationale behind our focus on amines is their use in a wide range of important technological situations. Since the compounds depicted in Figure 2 are novel and have not themselves been used in such situations, some justification may be in order. We expect that the most likely applications of these multifunctional fatty amines will be in polymer chemistry, where they will be useful for preparing new types of polyamides or polyureas, and in surfactant chemistry, where the ability to position two charges at opposite ends of an alkyl

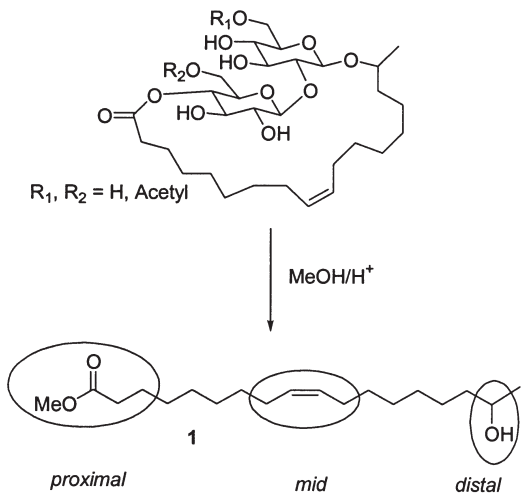

FIG. 1. The 17-hydroxy oleic acid that is the precursor for new compounds discussed in this work is obtained from acid alcoholysis of sophorolipids, which are predominantly acetylated macrolactones. Molecule **1** can be derivatized at its carboxyl, olefin, or hydroxy group.

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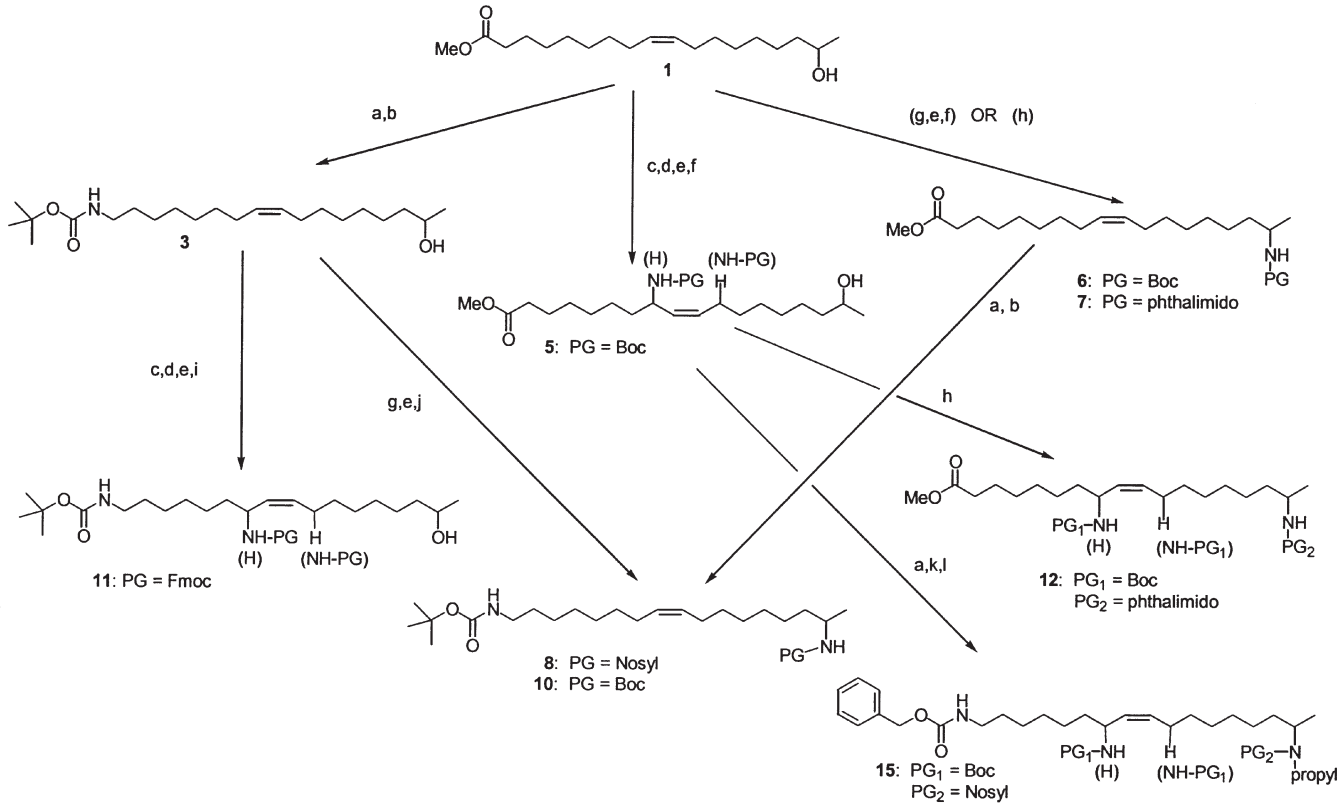


FIG. 2. Synthetic transformations used to introduce amine units into 17-hydroxy oleate. Reagents and conditions: a: LiOH, MeOH/H_2O. b: diphenylphosphoryl azide (DPPA) in *t*-BuOH, NEt_3, Δ. c: N-bromosuccinimide in CCl_4, Δ. d: NaN_3, Bu_4NBr, dimethylformamide/toluene, Δ. e: (1) triphenylphosphine (PPh_3), Δ. (2) H_2O, Δ. f: di-*t*-butyl dicarbonate (Boc_2O)/NEt_3. g: DPPA, di-isopropylazodicarboxylate (DIAD), PPh_3. h. phthalimide, PPh_3, DIAD. i: N-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu), NEt_3. j: *o*-nitrobenzenesulfonyl (Nosyl)-chloride, NEt_3. k: DPPA in BnOH, NEt_3, Δ. l:*o*-$NO_2C_6H_4SO_2NH$-propyl, DIAD, PPH_3. PG, protecting group. For a discussion of the regioisomerism of the allylic substituents in **5**, **11**, **12**, and **15** (indicated in the figure), see the text.

chain could be harnessed to make bola-amphiphiles (11). Biological applications are also compelling. α-Amino oleic acid has been used as an anticalcification agent in prosthetic heart valves (12). Isomeric amino oleic acids of the sort prepared in this paper will shed light on structure/function relationships in that application, as well as perhaps finding use as coatings in other biomaterials-type devices. Long-chain fatty amines also constitute the core of ceramide analogs, which are under investigation as inducers of apoptosis (13). Fluorescent derivatives of amino FA have been used as probes to study enzymes involved in lipid regulation and biosynthesis (14,15). Finally, there are a number of newer technologies where fatty amines are being used: as matrices for nucleation of inorganic nanocrystals (16); as surface-bound monolayers for anchoring cells (17) or immobilizing enzymes (18); as cross-linking agents for composite materials (19); and as anticorrosion or antioxidation units in lubricant formulations (20). In regard to this last area, some of the molecules shown in Figure 2 could be converted into amino versions of estolides (21). In any of these fields, the opportunity to incorporate some extra functionality of choice (e.g., a fluorescent or metal-chelating unit) or to alter the hydrophobicity profile by, for example, hydroxylating the olefin, would open up rich new avenues of exploration.

EXPERIMENTAL PROCEDURES

General. Sophorolipids were prepared as previously reported, using oleic acid and glucose as carbon sources (5). 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 on a Varian Associates Gemini 200 MHz instrument. LC-MS data were recorded on a Waters/Micromass ZMD instrument with atmospheric pressure CI (APCI), using an elution gradient of 20:80 water/methanol 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. See Table 1 for spectroscopic data.

*Methyl 17-hydroxy oleate (**1**)*. Crude sophorolipids (20 g) were dissolved in 1% H_2SO_4/MeOH (250 mL) and heated to 60–65°C for 16–20 h. Methanol was removed on the rotary evaporator, and the resulting brown syrup was diluted with 100 mL of water and extracted with ethyl ether (3 × 100 mL). The organic fractions were condensed to give 8.5 g material. Yields can be calculated only approximately, since the starting mater-

TABLE 1
Spectra of Products

Compound	^{13}C NMR[a]	^{1}H NMR	Mass spectra[b]
3	23.6, 25.9, 26.9, 27.3, 28.5, 29.2, 29.3, 29.6, 29.7, 30.2, 39.5, 40.7, 68.2, 79.1, 129.9, 130.0, 156.1	1.20 (*d*, 3H, 7.5 Hz), 1.24–1.48 (*br s*, 16 H), 1.48–1.56 (*br s*, 11 H), 1.56–1.74 (*m*, 2 H), 1.90–2.16 (*m*, 4H), 3.10 (*q*, 2H, 7.3 Hz), 3.72–3.90 (*m*, 1H), 4.55 (*br s*, 1H), 5.24–5.45 (*m*, 2H)	370, 314 (loss of *t*-Bu), 270 (loss of Boc), 252 (loss of Boc + H_2O)
4	23.6, 25.9, 27.0, 27.3 (*m*), 28.5, 29.2, 29.3, 29.6, 29.7, 30.3, 39.5, 41.1, 43.6 (*br*), 68.2, 80.3, 129.9, 130.0, 154.8, 157.8	1.20 (*d*, 3H, 7.5 Hz), 1.24–1.43 (*br s*, 16 H), 1.43–1.65 (*br s*, 11 H), 1.65–1.79 (*m*, 2 H), 1.93–2.17 (*m*, 4H), 3.24 (*q*, 2H, 7.2 Hz), 3.30–3.41 (*m*, 4H), 3.41–3.57 (*m*, 4H), 3.71–3.90 (*m*, 1H), 4.48 (*t*, 1H, 7.0 Hz), 5.26–5.47 (*m*, 2H)	482, 426 (loss of *t*-Bu), 382 (loss of Boc), 278, 270 (loss of Boc-piperazine-CO), 252
5	23.6, 25.0, 25.8 (*m*), 28.5, 29.2 (*m*), 29.5 (*m*), 32.3 (*m*), 34.2, 35.8 (*m*), 39.4 (*m*), 51.6, 52.5, 68.2, 79.2, 130.8, 130.9, 131.1, 131.3, 155.5, 174.4	1.21 (*d*, 3H, 7.4 Hz), 1.24–1.48 (*br s*, 16 H), 1.48–1.56 (*br s*, 11 H), 1.56–1.78 (*m*, 2 H), 1.93–2.14 (*m*, 2H), 2.32 (*t*, 2H, 7.2 Hz), 3.68 (*s*, 3H), 3.74–3.89 (*m*, 1H), 3.91–4.11 (*m*, 1H), 4.40 (*br s*, 1H), 5.22–5.37 (*m*, 1H), 5.46–5.65 (*m*, 1H)	428, 383 (loss of CH_3CHOH), 372 (loss of *t*-Bu), 354 (loss of *t*-Bu and H_2O), 328 (loss of Boc), 311, 293, 278
6	21.4, 25.0, 26.1, 27.3 (*m*), 28.5, 29.2 (*m*), 29.3, 29.5, 29.8, 34.2, 37.5, 46.7, 51.6, 79.0, 129.9, 130.0, 155.5, 174.4	1.12 (*d*, 3H, 7.5 Hz), 1.20–1.43 (*br s*, 16 H), 1.43–1.52 (*br s*, 9 H), 1.52–1.78 (*m*, 4H), 1.92–2.15 (*m*, 4H), 2.33 (*t*, 2H, 7.3 Hz), 3.53–3.80 (*m*, 1H), 3.66 (*s*, 3H), 4.34 (*br s*, 1H), 5.26–5.44 (*m*, 2H)	412, 356 (loss of *t*-Bu), 312 (loss of Boc)
7	18.8, 25.0, 26.9, 27.2, 29.2 (*m*), 29.7, 33.8, 34.2 (*m*), 47.6, 51.5, 123.1, 129.9 (*m*), 132.1, 133.9, 168.7, 174.4	1.14–1.43 (*br s*, 16 H), 1.47 (*d*, 3H, 7.5 Hz), 1.55–1.80 (*m*, 4 H), 1.91–2.16 (*m*, 4H), 2.31 (*t*, 2H, 7.2 Hz), 3.67 (*s*, 3H), 4.24–4.44 (*m*, 1H), 5.26–5.45 (*m*, 2H), 7.66–7.77 (*m*, 2H), 7.77–7.92 (*m*, 2H)	442
8	21.9, 25.6, 26.9, 27.2, 27.3, 28.5, 29.2 (*m*), 29.3, 29.7 (*m*), 30.2, 37.5, 40.7, 51.3, 79.1, 125.4, 129.8, 130.0, 130.7, 132.9, 133.4, 135.3, 147.9, 156.1	1.12 (*d*, 3H, 7.4 Hz), 1.15–1.41 (*m*, 16 H), 1.41–1.76 (*br s*, 13 H), 1.89–2.15 (*m*, 4H), 3.13 (*q*, 2H, 7.6 Hz), 3.42–3.63 (*m*, 1H), 4.54 (*br s*, 1H), 5.16 (*br d*, 1H), 5.25–5.44 (*m*, 2H), 7.70–7.82 (*m*, 2H), 7.83–7.97 (*m*, 1H), 8.14–8.21 (*m*, 1H)	554, 498 (loss of *t*-Bu), 454 (loss of Boc)
10	21.4, 26.1, 26.9, 27.3, 28.5, 29.3 (*m*), 29.5, 29.6, 29.7, 29.8, 30.1, 37.4, 41.0, 46.9, 79.3, 129.9, 130.0, 151.1, 151.3	1.13 (*d*, 3H, 7.4 Hz), 1.22–1.44 (*br s*, 16 H), 1.44–1.68 (*br s*, 22 H), 1.94–2.14 (*m*, 4H), 3.14 (*q*, 2H, 7.2 Hz), 3.53–3.75 (*m*, 1H), 4.33 (*br s*, 1H), 4.56 (*br s*, 1H), 5.31–5.44 (*m*, 2H)	469, 413 (loss of *t*-Bu), 369 (loss of Boc), 357 (loss of 2 *t*-Bu), 313, 295, 269 loss of 2 Boc), 252
11	23.6, 25.8 (*m*), 26.8 (*m*), 28.5, 29.2 (*m*), 29.4, 30.1, 32.3, 35.6, 39.3 (*m*), 40.7, 47.4, 53.0, 66.5, 68.2, 79.1, 120.1, 125.2, 127.1, 127.7, 130.5, 131.8 (*m*), 141.4, 144.1, 155.9, 156.1	1.19 (*d*, 3H, 7.4 Hz), 1.23–1.40 (*m*, 16 H), 1.40–1.58 (*s*, 13H), 1.95–2.08 (*m*, 2H), 3.10 (*t*, 2H, 7.3 Hz), 3.71–3.86 (*m*, 1H), 4.24 (*m*, 1H), 4.42 (*t*, 1H, 8.0 Hz), 4.53 (*br s*, 1H), 4.59 (*d*, 2H, 7.9 Hz), 4.73 (*br s*, 1H), 5.22–5.40 (*m*, 1H), 5.44–5.68 (*m*, 1H), 7.30–7.53 (*m*, 4H), 7.58–7.68 (*m*, 2H), 7.78 (*d*, 2H, 6.5 Hz)	607, 507 (loss of Boc), 440, 385 (loss of Fmoc), 267
12	18.8, 25.0 (*m*), 25.7, 25.8, 26.7, 26.8, 28.5, 28.8, 29.0 (*m*), 29.1, 29.3 (*m*), 32.3 (*m*), 33.8, 34.2, 35.7, 47.5, 51.5, 52.4, 79.2, 123.1, 130.8, 130.9, 131.0, 131.2, 132.1, 133.9, 155.4, 168.7, 174.4	1.10–1.38 (*br s*, 16 H), 1.38–1.53 (*br s*, 11 H), 1.46 (*d*, 3H, 7.6 Hz), 1.53–1.69 (*m*, 2 H), 1.90–2.16 (*m*, 2H), 2.30 (*t*, 2H, 7.2 Hz), 3.66 (*s*, 3H), 3.87–4.14 (*m*, 1H), 4.23–4.45 (*m*, 1H), 4.41 (*br s*, 1H), 5.16–5.36 (*m*, 1H), 5.42–5.62 (*m*, 1H), 7.64–7.76 (*m*, 2H), 7.76–7.90 (*m*, 2H)	557, 501 (loss of *t*-Bu), 457 (loss of Boc), 440, 408
13	21.1, 23.6, 25.0, 25.8 (*m*), 26.0 (*m*), 28.5, 29.0, 29.2 (*m*), 29.4, 32.2 (*m*), 34.2, 35.7, 37.0, 45.4, 51.6, 52.4, 79.2, 130.8, 131.0, 131.1, 131.3, 155.5, 169.4, 174.4	1.13 (*d*, 3H, 7.5 Hz), 1.18–1.39 (*br s*, 16 H), 1.39–1.54 (*br s*, 11 H), 1.54–1.73 (*m*, 2 H), 1.88–2.15 (*m*, 2H), 1.98 (*s*, 3H), 2.32 (*t*, 2H, 7.4 Hz), 3.67 (*s*, 3H), 3.85–4.11 (*m*, 2H), 4.42 (*br s*, 1H), 5.17–5.35 (*m*, 1H), 5.38 (*br s*, 1H), 5.45–5.65 (*m*, 1H)	469, 413 (loss of *t*-Bu), 369 (loss of Boc), 352, 320
14	23.6, 25.8, 26.7, 28.5, 29.2 (*m*), 29.4, 29.8, 30.0, 30.4, 32.3, 35. 7 (*m*), 39.4, 41.2, 50.0, 52.4, 66.7, 68.2, 79.2, 128.2, 128.4, 128.6, 130.9 (*m*), 131.2 (*m*), 136.8, 155.5, 156.5	1.20 (*d*, 3H, 7.4 Hz), 1.23–1.42 (*br s*, 14 H), 1.41–1.79 (*br s*, 15 H), 1.94–2.18 (*m*, 2H), 3.20 (*q*, 2H, 7.6 Hz), 3.71–3.86 (*m*, 1H), 3.93–4.10 (*m*, 1H), 4.42 (*br s*, 1H), 4.83 (*br s*, 1H), 5.10 (*s*, 2H), 5.21–5.39 (*m*, 1H), 5.45–5.65 (*m*, 1H), 7.38 (*m*, 5H)	519, 419 (loss of Boc), 358, 311, 294
15	11.6, 19.5, 21.5, 25.1, 25.8, 26.5 (*m*), 28.5, 29.3 (*m*), 29.8, 30.0, 32.2 (*m*), 35.7 (*m*), 41.2, 45.4, 52.4, 54.4, 66.7, 79.2, 124.0, 124.2, 128.2 (*m*), 128.6, 130.8, 131.0, 131.2, 131.5, 131.6, 133.3, 134.3, 136.8, 148.1, 155.5, 156.5	0.91 (*t*, 3H, 7.4 Hz), 1.13 (*d*, 3H, 7.5 Hz), 1.18–1.42 (*br s*, 16 H), 1.42–1.77 (*br s*, 15 H), 1.92–2.12 (*m*, 2H), 3.11–3.35 (*m*, 4H), 3.70–3.83 (*m*, 1H), 3.85–4.06 (*m*, 1H), 4.52 (*br s*, 1H), 4.77 (*br s*, 1H), 5.11 (*s*, 2H), 5.23–5.36 (*m*, 1H), 5.46–5.65 (*m*, 1H), 7.39 (*br s*, 5H), 7.65–7.78 (*m*, 3H), 7.97–8.10 (*m*, 1H)	745, 645 (loss of Boc), 628, 595, 581, 538, 520, 494, 464

[a]In ^{13}C NMR spectra, "*m*" indicates overlapped peaks that are poorly resolved, with $\delta\Delta < 0.1$ ppm.
[b]The first mass listed is in all cases MH^+. BOC, *t*-butyl carbonate; Fmoc, 9-fluorenylmethoxycarbonyl.

ial is heterogeneous, but in assuming it to be pure di-acetyl oleic sophorolactone (M.W. = 688) this amount of product would represent a yield of 93%. Compound **1** was isolated out of the mixture of hydroxy methyl esters (see Results and Discussion) by column chromatography on silica gel with 2:1 hexane/ethyl acetate.

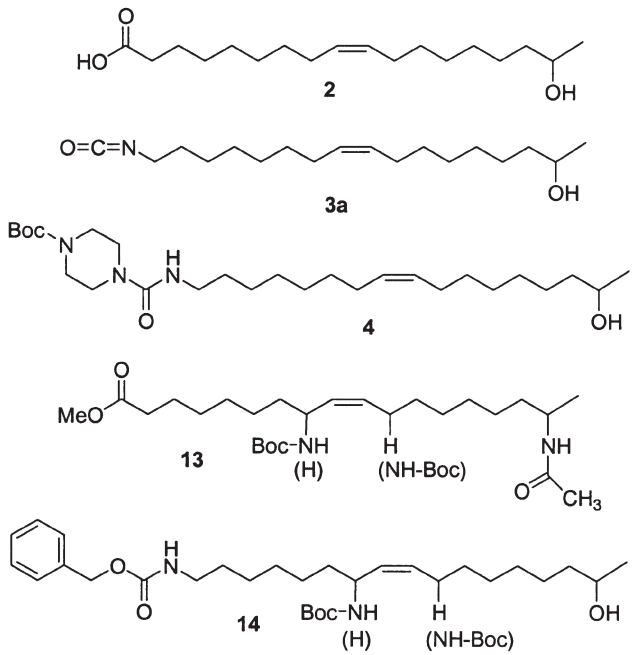
*17-Hydroxy oleic acid (**2**)*. Compound **1** (1.25 g, 4 mmol) was dissolved in 50 mL 1:1 MeOH/H_2O, and LiOH hydrate (430 mg, 10.2 mmol) was added. The mixture was heated to 40–45°C overnight. Methanol was removed on the rotary evaporator, 10% wt/vol citric acid solution (100 mL) was added to acidify the product, and the mixture was extracted twice with 50 mL ethyl ether. Evaporation of the solvent yielded an off-white solid, 1.18 g (98%).

*Synthesis of terminal amine (**3**)*. To a solution of 17-hydroxy oleic acid **2** (810 mg, 2.7 mmol) in 10 mL *t*-BuOH heated to 50–55°C under N_2 was added triethylamine (417 mL, 3 mmol) and a solution of diphenylphosphoryl azide (DPPA; 825 mg, 3 mmol) in 2 mL THF. Heating was increased to afford reflux, and the mixture was stirred for 24 h. It was then cooled and concentrated on the rotary evaporator, then applied to a silica gel column. Elution with 2:1 hexane/ethyl acetate afforded molecule **3** (408 mg, 41%). The 16-hydroxy heptadecyl isocyanate **3a** was also recovered (295 mg, 37%) and was reacted with N-butyloxycarbonyl-piperazine in THF and NEt_3 at 50°C overnight to obtain **4** (see Scheme 1).

*Synthesis of mid-chain amine (**5**)*. A solution of **1** (1 g, 3.2 mmol), N-bromosuccinimide (NBS; 571 mg, 3.2 mmol), and benzoyl peroxide (30 mg) in 30 mL CCl_4 was heated to reflux under N_2. After 90 min, TLC indicated that no **1** remained, and all of the suspended solid floated. The reaction flask was cooled to room temperature and the contents were filtered. Solvent was removed on the rotary evaporator and was further concentrated twice out of THF to remove all traces of chlorinated solvents. The resulting colorless syrup was dissolved in 50 mL 1:1 dimethylformamide/toluene, and *n*-Bu_4NBr (50 mg) and NaN_3 (520 mg, 8 mmol) were added. The solution was heated to reflux (of toluene) under N_2. Progress of the reaction was monitored by TLC to observe disappearance of the intermediate bromo compound; heating was continued for 24 h. The mixture was cooled to room temperature and poured into 300 mL H_2O, then extracted with toluene (3 × 50 mL). The organic layer was dried over $MgSO_4$ and filtered.

A sequence of steps, which was used as a general procedure in several reactions (see below), was then performed (Method A): Triphenylphosphine (PPh_3, 838 mg, 3.2 mmol) was added to the toluene solution, and it was heated to 50–55°C. Stirring under N_2 proceeded for another 24 h, following which 1 mL of water and 100 mL THF were added. Stirring at the same temperature continued for another 24 h.

At the end of this sequence, a reagent for introducing the protecting group of choice onto the newly formed amino group was added, which for this example was di-*t*-butyl dicarbonate (Boc_2O, 700 mg, 3.2 mmol), along with 3 mL NEt_3. This mixture was stirred under N_2 at room temperature for 72 h. At the end of this time, solvent was removed on the rotary evaporator,



SCHEME 1

and compound **5** (656 mg, 48%) was isolated by column chromatography on silica gel (2:1 hexane/ethyl acetate).

*Synthesis of 17-amino-oleic acid derivatives (**6** and **7**)*. To a magnetically stirred solution of **1** (1.56 g, 5 mmol), DPPA (1.38 g, 5 mmol), and PPh_3 (1.31 g, 5 mmol) in 50 mL THF under N_2 a solution of di-isopropylazodicarboxylate (DIAD, 1.01 g, 5 mmol) in 3 mL THF was added dropwise over the course of 5 min. Stirring was continued overnight for 24 h. Method A was then applied, followed by addition of Boc_2O (1.09 g, 5 mmol). Silica gel chromatography in 4:1 hexane/ethyl acetate yielded **6** (622 mg, 45% based on recovered starting material) as well as starting material **1** (510 mg).

In a variant of this reaction, **1** (1.48 g, 4.7 mmol), phthalimide (691 mg, 4.7 mmol), and PPh_3 (1.24 g, 4.7 mmol) were dissolved in 20 mL THF in a round-bottomed flask submerged in an ice-water bath, and a solution of DIAD (950 mg, 4.7 mmol) in 3 mL THF was added. The solution was stirred under N_2 and allowed to warm to room temperature overnight. Solvent was removed on the rotary evaporator, and the material was purified by column chromatography to yield 1.45 g of compound **7** (3.3 mmol, 70%).

*Synthesis of proximal/distal diamines (**8** and **10**)*. An analogous procedure to the one for synthesizing **6** was followed, using **3** (715 mg, 1.94 mmol) as starting material. Instead of protection with Boc_2O, *o*-nitrobenzenesulfonyl chloride (663 mg, 3 mmol) and NEt_3 (420 µL, 3 mmol) were added after performing Method A. Column chromatography in 3% MeOH/$CHCl_3$ afforded **8** (266 mg, 36% allowing for recovered starting material) and **3** (224 mg).

To prepare molecule **10**, molecule **6** (625 mg, 1.5 mmol) and LiOH (64 mg, 1.5 mmol) were dissolved in 10 mL 2:1 MeOH/H_2O and heated to 45°C overnight. MeOH was removed

on the rotary evaporator, and the aqueous portion was acidified with citric acid and then washed with ether (2 × 50 mL). The ether extracts were dried over Na_2SO_4 to afford a colorless oil, which was purified by column chromatography on silica gel with 9:1 $CHCl_3$/MeOH. Molecule **9**, the carboxylic acid, was obtained (250 mg, 0.63 mmol) as well as starting material (305 mg). Molecule **9** was dissolved in 10 mL *t*-BuOH and 2 mL THF, heated to 55°C, and then NEt_3 (104 μL, 0.75 mmol) and DPPA (204 mg, 0.74 mmol) were added. The heat was increased to reflux the solution for 18 h. Workup proceeded as for **3** to give **10** (107 mg, 36%).

*Synthesis of proximal/mid diamine (**11**)*. Molecule **3** (335 mg, 0.91 mmol) was treated with NBS (160 mg, 0.9 mmol) and benzoyl peroxide (20 mg) as for **5** above, then reacted with NaN_3 and processed with Method A. At the end of this sequence, N-(9-fluorenylmethoxycarbonyloxy)-succinimide (Fmoc-OSu, 300 mg, 0.92 mmol) and NEt_3 (0.5 mL) were added, and the mixture was stirred overnight. Solvent was removed on the rotary evaporator, and the residue was chromatographed on silica gel with 5% MeOH/$CHCl_3$ to afford **11** (217 mg, 39%).

*Synthesis of mid/distal diamine (**12**)*. Molecule **5** (200 mg, 0.47 mmol) was treated with phthalimide (76 mg, 0.52 mmol), DIAD (104 mg, 0.52 mmol), and PPh_3 (136 mg, 0.52 mmol) as for molecule **7** above to afford **12** (193 mg, 74%).

To remove the phthalimido protecting group, **12** (130 mg, 0.23 mmol) was dissolved in 8 mL 2-propanol/H_2O (6:1, vol/vol), and $NaBH_4$ was added in four portions of 20–25 mg each over the course of 20 min. After another 90 min, another 30 mg was added, and the reaction was stirred for another 90 min. At the end of this time, glacial acetic acid was added (cautiously—evolution of gas) to an apparent pH of 5 (approx. 1 mL was added), then the mixture was heated to 75°C for 2 h. It was cooled to room temperature and stirred overnight. The solution was evaporated to dryness on the rotary evaporator, and pyridine (5 mL) and acetic anhydride (0.5 mL) were added. The reaction was stirred at room temperature for 24 h, then concentrated under vacuum to a syrup and chromatographed on silica gel with 1:1 hexane/ethyl acetate to remove a by-product, then 100% ethyl acetate to afford **13** (66 mg, 62%; see Scheme 1).

*Synthesis of triamine (**15**)*. Molecule **5** (450 mg, 1.05 mmol) was hydrolyzed with LiOH and worked up according to the procedure above for converting **6** to **10**. The resulting carboxylic acid (375 mg, 0.91 g) was then dissolved in 5 mL THF; 5 mL benzyl alcohol and NEt_3 (280 μL, 2 mmol) were added, and the solution was heated to 55°C, at which point DPPA (275 mg, 1 mmol) was added and heating was increased to reflux the THF. Heating was continued for 18 h, then the reaction mixture was cooled to room temperature and the THF was removed on the rotary evaporator. Ether (100 mL) was added, and the solution was extracted with H_2O (5 × 100 mL). Diamine **14** was purified by chromatography on silica gel.

To add the distal amino unit, **14** (140 mg, 0.27 mmol) was dissolved in 10 mL THF, and PPh_3 (105 mg, 0.4 mmol) and N-propyl-*o*-nitrobenzenesulfonamide (99 mg, 0.4 mmol, prepared by reacting *o*-nitrophenylsulfonyl chloride with an excess of propylamine) were added, followed by the dropwise addition of DIAD (81 mg, 0.4 mmol) in 2 mL THF over 3 min. The solution was stirred at room temperature under nitrogen for 18 h, then another portion of PPh_3, DIAD, and the sulfonamide (half the previous amounts) was added. Stirring was continued for another 6 h, solvent was removed on the rotary evaporator, and the crude product was purified by column chromatography on silica gel, first with 2:1 hexane/ethyl acetate and then with 9:1 chloroform/ethyl acetate, to give **15** (107 mg, 74% based on recovered **14**).

RESULTS AND DISCUSSION

Preparation of starting material. Methyl 17-hydroxy oleate (**1**) was readily obtained by acid alcoholysis of sophorolipids that were prepared according to literature procedures using oleic acid as the feedstock. Since one of the long-term goals of this work is to convert agricultural by-products to useful materials, it might seem counterintuitive to begin by using a purified chemical as feedstock rather than a chemically complex waste. At this early stage of the project, however, it is important to validate the kinds of synthetic conversions that can be accomplished without introducing ambiguities due to chemical heterogeneity. Even using pure feedstock, the hydroxy FAME obtained consisted of a mixture of products, predominantly **1** with smaller amounts of the corresponding stearate (approximately 15%), the 18-hydroxy oleate (5%), and palmitates (5%, presumably 15- and 16-hydroxy although we did not investigate those compounds). Compound **1** could be purified away from these minor analogs by column chromatography. Yields were very good, better than 90%, but it is important to note that by discarding the disaccharide portion, 10 g of sophorolipid starting material can theoretically yield only 4.5 g of **1**. For steps where a free carboxylic acid rather than the ester was required, hydrolysis with LiOH in aqueous methanol gave excellent yields.

Synthesis of monoamines. To introduce an amino functionality at the end of the molecule proximal to the carboxylic acid, several synthetic strategies can be envisioned. α- or β- substitution through enolates or conjugate addition will be worth investigating in the longer term, but since those routes require several steps and possibly harsher conditions, we initially chose to convert the carboxylic acid functionality of **1** directly into an amine. For this we used the Curtius rearrangement as implemented with DPPA. This reagent has attained popularity as a convenient way to introduce azide groups, and when used at high temperature in an alcohol gives the carbamate-protected amine (22). Molecule **3** was obtained in acceptable yields of 30–40%. Note that the FA chain has been shortened by one carbon owing to the rearrangement mechanism. A minor by-product (approximately 10%) was the *t*-butyl ester of 17-hydroxy oleic acid, which could be recycled or used in other syntheses if desired. A more significant by-product, **3a,** was also observed, the amount of which (30–40%) could not be lessened by varying reaction conditions such as temperature, base, or

co-solvent (although see the synthesis of **14**, which follows, for a way to circumvent it). We were surprised to discover that this material was the isocyanate, as could be demonstrated by converting it to the desired carbamate **3** by further heating with *t*-butanol and NEt_3, or to the urea **4** by reaction with mono-Boc piperazine. This isocyanate appears to be less prone to hydrolysis than shorter-chain analogs: A sample of it that had been stored in ethyl acetate solution survived with little or no apparent decomposition at room temperature for several months, and samples of it were regularly chromatographed on silica gel. Equally significant was the observation that the distal hydroxy group seemingly does not need to be protected in this route: Although we did not explicitly search for them, neither the cyclic urethane nor polymeric self-condensation products were observed.

To append an amine unit at the midsection of **1**, we used allylic substitution. Again, other methods should ultimately be investigated (e.g., azide opening of an epoxide) but for now we preferred to retain the C–C double bond as a site that could be further modified. The allylic bromide was readily prepared with NBS and was then converted to the azide with NaN_3 (23). We sought to avoid isolation and purification of this potentially dangerous intermediate, so it was used in a one-pot procedure (24) by reaction with PPh_3 (the Staudinger reaction), then water to hydrolyze the iminophosphorane, and finally a reagent for introducing a protecting group to the newly generated free amine. Allylic reaction of an oleic acid derivative should, of course, give two products, one substituted at the 8-position and one at the 11-position. We observed two poorly resolved peaks in the HPLC trace of molecule **5**, both of which have the same mass. Conditions to separate these isomers from each other using column chromatography on silica were sought but not found. Carbon NMR shows four olefinic peaks. A further complication is that diastereomeric mixtures should also be produced, since allylic substitution generates a new chiral center. Although the existence of molecule **5** (or any of the other mid-chain-substituted products) as an inseparable isomeric mixture might be problematic for some applications, such as medicinal chemistry, we believe that this situation should not be a drawback in polymeric, materials, or surfactant applications. That epoxidized plant oils, for example, exist as "a vast mixture of isomers" (25) has not prevented them from finding many uses and being produced in hundreds of thousands of tons a year. This sequence of reactions (formation of an azide, reduction with PPh_3, hydrolysis with water, and protection) is one we used regularly in the synthesis of these fatty amines. It is far from optimal, since monitoring of the progress of the reactions is difficult. The intermediate iminophosphorane, for example, is probably hydrolytically unstable and incompatible with silica gel chromatography. Nonetheless, we have continued to use these steps since the likely alternative of reduction of the azide over palladium would also reduce the olefin.

To replace the 17-hydroxy group with an amino unit, we used two variants of the Mitsunobu reaction (26). First, the azide unit was directly appended to the oleate with DPPA (27), followed by the same sequence of PPh_3, water, and protection as above. This route has the advantage of avoiding use of the hazardous sodium azide. Alternatively, **1** could be subjected to the Mitsunobu reaction with phthalimide as the nitrogen carrier. This reaction is convenient to perform and proceeds in good yield. Both of these reactions should proceed with inversion of configuration at C17, although we have not investigated chirality in any of the molecules reported in this work.

Synthesis of diamines. By combining the methodologies used to generate monoamines from **1**, three positional isomers of diamines can be obtained. Using the terminology described in Figure 1, we refer to these as proximal/mid, proximal/distal, and mid/distal. Not all combinations of preparative steps are equally apt, however. For example, use of allylic bromination to derivatize the chain adjacent to the olefin is also likely to modify benzylic protecting groups, so if this combination of procedures and structural elements is to be used, the benzylic group would need to be introduced after the bromine, not vice versa. We have explored several synthetic pathways for the preparation of diamines.

To make the proximal/distal isomer, we examined two routes. First was to use **3** in a Mitsunobu-type reaction with DPPA, analogous to the preparation of **6**, presented earlier. In this case the final step was protection of the newly installed amine with the *o*-nitrobenzenesulfonyl, or Nosyl, group. This diamine therefore possesses orthogonally protected amino groups at opposite ends of the carbon chain: The Nosyl group can be removed with nucleophilic reagents (28), and the Boc group with acid. As expected, the Boc group of **3** was unaffected by the conditions needed to append this second amine. Second was to proceed in the "opposite" direction: The methyl ester **6** was hydrolyzed to the free acid and subjected to Curtius rearrangement conditions with DPPA in *t*-BuOH. Again, the NHBoc unit (at C17) was unaltered by these conditions. As in the preparation of **3**, a significant amount of a by-product was observed, which by analogy we assume to be the isocyanate.

The proximal/mid isomer was prepared by subjecting the Boc-protected **3** to NBS substitution followed by the standard sequence of reduction (Method A), then protecting with the Fmoc group. Again, the two protecting groups are orthogonal, responding to acid and base, while the unprotected hydroxy group remains open to further chemistry. The final diamine is the mid/distal isomer, synthesis of which began with allylic-substituted **5,** which was then phthalimidated using Mitsunobu conditions to give **12**. Repeatedly, this phthalimidation reaction worked in high yield and conveniently. The only foreseeable drawback was that the phthalimide protecting group has a reputation for being difficult to remove, so we wished to test a reported improvement for deprotection (29). A one-pot reduction with $NaBH_4$ and acid treatment followed by acetyl capping worked well to give **13**, without altering the methyl ester, the Boc group, or the olefin. This route looks promising as a way to introduce an acyl unit that would be more interesting than CH_3CO at the distal nitrogen.

Some comment on the choice of protecting groups (PG) in these molecules is in order. For these initial experiments, we were simply motivated by discovering what works. In the longer

term, the pattern of PG adopted will also be determined by the intended use of the compound. For example, to use a diaminocarboxylic acid such as **12** as the diamine component in the synthesis of a polyamide (in conjunction with a dicarboxylic co-monomer), the PG on the two amino units should be removable in one step by the same reagent, and the PG on the carboxylic acid should be orthogonal. By contrast, to use **12** in a head-to-tail fashion to prepare a homopolyamide, the carboxylic acid and one amine should be protected with groups that are removed simultaneously, while the second amine remains orthogonal. The steps we have used so far to introduce amines into **1** provide five classes of protecting groups: Boc (removable with acid), benzyloxycarbonyl (H_2), Fmoc (base), phthalimide ($NaBH_4$/acid), and Nosyl (nucleophile, e.g. thiophenol).

Triamine synthesis. To combine the above steps into the synthesis of a triply substituted derivative, we began with the allylic amine **5**. Again, this molecule is a likely starting point, since the free-radical bromination used to produce it is incompatible with several protecting groups. First, the methyl ester was successfully hydrolyzed. The DPPA-mediated Curtius reaction was then performed in benzyl alcohol to give benzyloxycarbonyl-protected diamine **14**, which is a second example of the proximal/mid class of diamines. It is worth noting that none of the isocyanate by-product was observed, as it was when *t*-BuOH was the solvent during the synthesis of **3**; the primary benzyl alcohol is, as would be expected, much more successful at attacking the OCN unit than the tertiary alcohol. Removal of the excess benzyl alcohol was tedious but could be accomplished by repeated extraction of an ether solution of the product with water. The final step was to replace the distal hydroxy group with an amino unit, and for this we chose Mitsunobu conditions, which are mild. The nitrobenzenesulfonamido unit has several advantages for this step: not only is the NH acidic enough for use in the Mitsunobu protocol, but also the preparation of alkyl- or aryl-substituted nitrobenzenesulfonamides is easy, starting from the sulfonyl chloride and a primary amine. In this way, a Nosyl-protected secondary amine, where the added N-substituent could in principle bear a wide variety of groups, can be introduced. We used the propyl group for ease and because its proton NMR signature is characteristic but uncomplicated. Triamine **15** was produced in acceptable yield. This molecule, along with the other polyfunctional fatty amines, amino alcohols, and amino acids described in this work, demonstrates the range of value-added compounds that can be obtained in a few synthetic steps from an abundant bioderived starting material.

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