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ORIGINAL PAPER

Structured Estolides: Control of Length and Sequence

**Jonathan A. Zerkowski · Alberto Nuñez ·
Daniel K. Y. Solaiman**

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Abstract Using ester-forming reactions such as carbodiimide coupling and a modified Yamaguchi symmetrical anhydride method, a variety of estolides based on 17-hydroxy oleic and 17-hydroxy stearic acid have been prepared. These hydroxy fatty acids are produced in good yields from hydrolysis of sophorolipids, which are in turn derived from fermentation of fats and oils. Since the estolides are formed one unit, or ester bond, at a time, their length and sequence can be precisely controlled. The key to this control is the use of protecting groups at either the carboxylic or hydroxy end of the starting hydroxy fatty acids. Two mono-protected dimers, for example, when combined in a fragment-condensation approach, give a tetramer with no "contamination" from estolides of other lengths. This methodology opens the way to functionalized estolides, and several variants were prepared: hybrid estolides, containing non-fatty acid moieties such as amino acids; polymerizable estolides, containing a norbornene unit; and non-linear estolides that extend from a branched core such as glycerol or pentaerythritol. With the benzoyl chloride-mediated symmetrical anhydride method, yields for individual coupling steps ranged from 75 to 93%.

Keywords Hydroxy fatty acids · Estolides · Sophorolipids · Oligoesters · Glycerol

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J. A. Zerkowski (✉) · A. Nuñez · D. K. Y. Solaiman
USDA, ARS, Eastern Regional Research Center,
600 E. Mermaid Lane, Wyndmoor, PA 19038, USA
e-mail: jonathan.zerkowski@ars.usda.gov

Introduction

Estolides, which are intermolecular esters of fatty acids, appear to have significant promise as functional fluids. They possess good properties for serving as lubricants [1] and have the benefit of being biodegradable [2]. A more specialized use is as a lipid-solubilizing auxiliary for drug delivery [3]. They can be made from a number of vegetable oils, such as meadowfoam, castor, and cuphea, as well as from oleic acid [4–6]. Preparation of estolides has been achieved principally in two ways. The more widely investigated route uses acid catalysis, either liquid or solid, to form the ester unit. Lipases have also been used [7]. Both these methods are capable of inexpensively preparing large amounts of product, but they do have limitations. With acid catalysis, the harsh conditions (perchloric or sulfuric acid) will destroy a great many functional groups that could be of use if inserted into an estolide chain. These same functional groups might also be incompatible with enzymatic routes, for example if they hinder access to active sites or are themselves substrates. Furthermore, both kinds of routes are in principle subject to equilibria that regenerate monomer, if water is not removed (although studies have shown that the back reaction is slow [8]). In short, the methods used to make estolides to date do not allow for precise control of the length of the products (they are not monodisperse) or for control of their sequence, and many moieties cannot be included.

The aim of the work presented in this paper is twofold. First, we wished to apply simple, known esterification methods and synthetic strategies (Fig. 1) to fatty acids that would permit a wide range of functionalized estolides, of precisely defined length and sequence, to be formed. Second, we wished to demonstrate the utility of an abundant hydroxy fatty acid, compound **1**, that is obtained from

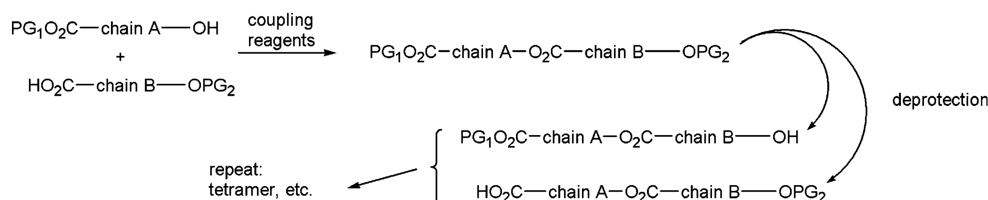


Fig. 1 Generalized scheme for the fragment-condensation approach to estolides of defined length and sequence. "Chain" could be a fatty acid unit or, in principle, a wide variety of moieties. PG_1 and PG_2 (protecting groups) need to be removable independently of each other ("orthogonal")

sophorolipids [9]. This compound is an isomer of ricinoleic acid, without (to the best of our knowledge) any of the toxicity issues associated with that molecule's source, and we expect that it should be useful in as many situations as ricinoleic acid has been. The position of the hydroxy group at the far end of the chain ($\omega - 1$) from the carboxy group may, in addition, impart novel properties to these estolides by contrast to those prepared previously, where the ester linkage is located mid-chain. We propose that the resulting estolides will find use in studies on the fundamental properties of this class of molecules as well as in broadening the scope of applications where they can be employed, such as in functionalized thin films, coatings, or macromolecular surfactants. Alternative uses are as GPC standards or as model compounds for studies of processes such as crystallization. By analogy with lipids of defined sequence, we propose the name "structured estolides" for these compounds.

Experimental Procedures

General

Compound **1** was prepared as previously reported from sophorolipids [9]. Simple esters of **1** were prepared using the esterification methods described below, and silyl ethers of **1** were prepared as previously described [9]. Chemicals and reagents were obtained commercially from Sigma-Aldrich (St Louis, MO, USA) and Lancaster Synthesis (Alfa Aesar, Ward Hill, MA, USA). All solvents and reagents were used as received. Silica gel (Grade 60 Å, Mesh 230–400, particle size 40–63 µm) used for column chromatography was obtained from Fisher Scientific (Fairlawn, NJ, USA). NMR spectra were recorded at room temperature in $CDCl_3$ on either a Varian Associates (Walnut Creek, CA, USA) Gemini 200 MHz or Inova 400 MHZ instrument and are referenced to $Si(CH_3)_4$. LCMS data were recorded on a Waters/Micromass (Milford, MA, USA) ZMD instrument with APCI, using a) 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, or b) direct injection without an LC column. Matrix-Assisted Laser Desorption/Ionization mass spectra with automated tandem time of flight fragmentation of selected ions (MALDI-TOF/TOF) were acquired with a 4700 Proteomics Analyzer mass spectrometer (Applied Biosystems, Framingham, MA, USA) in the positive reflectron mode. Masses were determined as sodiated adducts of the products $[M + Na]^+$, using 2,5-dihydroxybenzoic acid as matrix in a concentration of 10 mg/mL in acetonitrile/water (50:50) with 0.1% TFA. Approximately 0.7 µL of matrix was spotted on the MALDI plate and allowed to dry. The sample (0.5–1 µL) dissolved in chloroform or acetone at a concentration of 1–2 mg/mL was spotted on the top of the matrix crystals. Averages of 1,000–2,000 spectra were acquired for optimal signal to noise ratio.

Carbodiimide-Mediated Ester Formation

The general procedure is to dissolve roughly equimolar amounts of the hydroxy component and the carboxylic acid component with approximately 1.5 equiv of *N*-(3-dimethylaminopropyl)-*N*′-ethyl carbodiimide hydrochloride (EDC) and 0.2–0.5 equiv 4-dimethylaminopyridine (DMAP) in CH_2Cl_2. A specific example follows: The benzyl (Bn) ester of the dimer of **1a** ("Bn-oleic-oleic-OH", 216 mg, 0.32 mmol), the dimer of **1b**, protected as its *tert*-butyl diphenylsilyl (tBDPS) ether ("HO_2C-stearic-stearic-tBDPS", 225 mg, 0.27 mmol), EDC (80 mg, 0.4 mmol), and DMAP (20 mg, 0.16 mmol) were dissolved in 15 mL CH_2Cl_2 and stirred under an N_2 atmosphere at room temperature. After 24 h, another 40 mg of EDC and 20 mg DMAP were added, and the reaction continued. After 36 h, the solvent was removed on the rotary evaporator and the material chromatographed on silica gel with 3:1 hexane/ethyl acetate to afford the tetramer, Bn-oleic-oleic-stearic-stearic-tBDPS, 310 mg, 78%. Yields for individual coupling steps ranged from 66 to 90%, except for formation of the octamer **2** (see "Results and Discussion").

Benzoyl Chloride-Mediated Ester Formation

The general procedure [10] is to dissolve equimolar amounts (although other ratios can be used) of the alcohol and carboxylic acid in THF with at least 2 equiv of diisopropylethylamine (DIEA), then add 1 equiv benzoyl chloride, then 20 mol% DMAP. A specific example follows: The 2,2,2-trichloroethyl ester of **1a** (600 mg, 1.4 mmol), the *tert*-butyl dimethylsilyl (tBDMS) ether of **1a** (free acid, 495 mg, 1.2 mmol), and DIEA (522 μL, 3.0 mmol) were dissolved in 15 mL THF. Benzoyl chloride (139 μL, 1.2 mmol) was then added dropwise. After 5 min, DMAP (30 mg, 0.2 mmol) was added. The reaction was stirred overnight. Solvent was removed on the rotary evaporator, the residue applied to a silica gel column, and eluted with 3:1 hexane/ethyl acetate to afford the tBDMS trichloroethyl dimer (923 mg, 1.1 mmol, 93%). Unreacted trichloroethyl ester was also recovered. Yields for individual coupling steps ranged from 75 to 93%.

Spectroscopic and Physical Data

2. ^{1}H: 1.09 (s, 9H, Si–*t*-Bu), 1.21 (d, 6.5 Hz, 24H, C(18)H_3), 1.24–1.42 (br s, 176H), 1.49–1.75 (m, 32H), 1.83–2.11 (m, 8H, allylic H), 2.22–2.42 (m, 16H, C(O)CH_2), 3.84 (hex, 6.1 Hz, 1H, terminal C(17)H-OSi), 4.91 (hex, 6.4 Hz, 7H, internal C(17)H), 5.13 (s, 2H, benzylic H), 5.35 (m, 4H, olefinic H), 7.33–7.51 (m, 11H, $SiPh_2$ and benzyl Ar), 7.70 (m, 4H, $SiPh_2$). ^{13}C: 20.1 (C18), 25.2, 25.5, 27.1, 27.3, 29.2–29.8 (numerous peaks), 34.9 (C2), 36.1 (C16), 66.1 (C17-OSi), 69.7 (benzyl CH_2), 70.8 (C17 esterified), 127.4, 128.2, 128.3, 128.6, 129.6, 129.9, 132.7, 135.9, 173.6. MALDI: calculated 2623.2 for $C_{167}H_{294}O_{17}Si \cdot Na^+$, found 2623.4 $[M + Na]^+$.

3. ^{1}H: 0.05 (s, 24H, $SiCH_3$), 0.89 (m, 39H, Si(*t*-Bu) and propionamide CH_3), 1.13 (d, 6.0 Hz, 12H, C(18)H_3 adjacent to OtBDMS), 1.20 (d, 6.5 Hz, 9H, C(18)H3 adjacent to esters), 1.19–1.44 (m, 152H), 1.44–1.72 (m, 40H), 2.18 (t, 6.5 Hz, 2H, N-C(O)CH_2), 2.22–2.45 (m, 12H, O-C(O)CH_2), 3.23 (q, 6.9 Hz, 2H, CH_2N), 3.71–3.85 (m, 4H, C(17)H-OSi), 4.79–4.97 (m, 6H, esterified C(8)H, C(11)H, C(17)H), 5.56 (br s, NH). ^{13}C: −4.5 and −4.2 ($SiMe_2$), 14.4 (propionamide CH_3), 18.4 (C18 adjacent to OSi), 20.2 (C18 adjacent to esterified C17), 22.9, 23.1, 24.0, 25.4, 25.5, 26.0, 26.1, 29.4–29.9 (numerous peaks), 34.3, 34.4, 34.9, 36.1, 40.0, 41.4 (CH_2-NH), 60.6 (C17-OSi), 68.8, 70.8, 74.1, 173.4, 173.9. MALDI: calculated 2562.2 for $C_{153}H_{303}NO_{17}Si_4 \cdot Na^+$, found 2562.3 $[M + Na]^+$.

4. ^{1}H: 1.04–1.22 (m, 21H, C(18)H_3 and terminal propionic CH_3), 1.22–1.44 (br s, 123H), 1.44–1.76 (m, 24H), 1.89–2.11 (m, 14H, allylic H and C(3)H_2 from hydroxyproline), 2.20–2.37 (m, 14H, C(O)CH_2 including propionic), 2.56 (m, 2H, C(O)CH_2 3-hydroxybutyrate fragment), 3.50–3.78 (m, 2H, hydroxyproline N-CH_2), 3.67 (s, 3H, Me ester), 4.43 (q, 7.7 Hz, 1H, hydroxyproline C(α)H), 4.90 (hex, 6.4 Hz, 7H, C(17)H and hydroxyproline CH-O), 5.15 (m, 2H, benzylic), 5.22–5.44 (m, 7H, olefinic H and 3-hydroxybutyrate CH-O), 7.35 (m, 5H, Ar). ^{13}C: 9.3 (terminal propionic CH_3); 19.7, 19.9, and 20.1 (C18's); 24.9, 25.0, 25.2, 25.4, 25.5, 27.3, 28.1; 29.2–29.8 (numerous peaks); 34.2, 34.3, 34.6, and 34.9 (C2's); 35.8, 36.0, 36.1 (C16's); 41.4 (C2 of 3-hydroxybutyrate); 51.5 (Me ester), 52.3 (hydroxyproline C(5)-N), 58.0 (hydroxyproline Cα); 67.3 and 67.4 (benzyl CH_2 and C3 of 3-hydroxybutyrate); 70.8, 70.9, 71.5, 71.7, and 72.4 (C17's); 127.9, 128.1, 128.5, 128.6, 129.9, 136.5, 154.8 (hydroxyproline N-C(O)), 170.0, 171.7, 171.9, 173.0, 173.3, 173.7, 174.2, 174.4. MALDI: calculated 2131.7 for $C_{129}H_{225}NO_{20} \cdot Na^+$, found 2131.8 $[M + Na]^+$.

5. ^{1}H: 0.9 (t, 6.5 Hz, 3H, terminal CH_3), 1.21 (d, 6.2 Hz, 6H, internal C(18)H_3), 1.30 (br s, 68H), 1.41–1.52 (m, 4H), 1.52–1.63 (m, 7H), 1.92–2.11 (m, 5H, allylic and norbornene), 2.21–2.42 (m, 6H, C(O)CH_2), 2.95 (m, 1H, norbornene), 3.00–3.17 (m, 2H, norbornene), 3.46 (m, 2H, piperazine), 3.54–3.73 (m, 6H, piperazine), 4.91 (hex, 6.2 Hz, 2H, C(17)H), 5.36 (m, 2H olefin), 6.06 (dd, 2.9 and 5.5 Hz, 1H) and 6.21 (dd, 3.0 and 5.5 Hz, 1H) both norbornene olefinic. ^{13}C: 14.2 (terminal C18), 20.1 (internal C18), 22.8, 25.2, 25.4, 25.5, 27.3, 29.2–29.8 (numerous peaks), 31.1, 32.0, 33.4, 34.8, 36.0 (C16), 41.8 (broad, piperazine), 42.2 and 42.7 (norbornene), 45.5 (broad, piperazine), 45.8 and 49.6 (norbornene), 70.8 (C17), 129.9 (oleic C = C), 133.0 and 136.9 (norbornene C = C), 172.1, 173.4, 173.6. APCI: calculated 1035.9 for $C_{66}H_{116}N_2O_6$ H^+, found 1035.2 (MH^+).

6. ^{1}H: 1.14–1.23 (m, 9H, C(18)H_3), 1.23–1.44 (m, 48H), 1.51 (s, 9H, Boc) 1.53–1.74 (m, 15H), 1.80–2.15 (m, 13H, allylic and norbornene), 2.22–2.39 (m, 6H, C(O)CH_2), 2.87–3.00 (m, 2H, norbornene), 3.22 (m, 1H, norbornene), 3.37–3.51 (m, 6H, piperazine), 3.55–3.66 (m, 2H, piperazine), 4.75–5.00 (m, 3H, C(17)H), 5.34 (m, 6H, olefinic H), 5.93 (m, 1H) and 6.19 (m, 1H), both norbornene olefinic. ^{13}C: 20.1 (C18), 25.2, 25.4, 25.5, 27.3, 28.5, 29.1–29.7 (numerous peaks), 33.5, 34.8, 36.0, 41.4 (piperazine), 42.7 and 43.6 (norbornene), 43.8 (broad, piperazine), 45.5 (piperazine), 45.9 and 49.8 (norbornene), 70.7 and 70.8 (C17), 80.3 (Boc quaternary C), 129.9 (oleic C = C), 132.3 and 137.7 (norbornene C = C), 154.7 (Boc C = O), 172.0, 173.6, 174.4. APCI: calculated 1147.9 for $C_{71}H_{122}N_2O_9 \cdot H^+$, found 1147.5 ($MH^+$).

7. ^{1}H: 0.95 (d, 6.5 Hz, 18H, Leu CH_3), 1.20 (d, 6.2 Hz, 18H, C(18)H_3), 1.25–1.41 (br s, 96H), 1.48 (s, 27 H, Boc), 1.50–1.81 (m, 33H), 1.89–2.12 (m, 24H, allylic H), 2.21–2.48 (m, 12H, C(O)CH_2), 4.03–4.41 (m, 7H, glycerol CH_2 and Leu C(α)H), 4.72–5.01 (m, 9H, C(17)H and NH), 5.22–

5.47 (m, 13H, olefinic H and glycerol CH). [13]C: 20.0 and 20.1 (C18), 24.9, 25.1, 25.4, 25.5, 27.2, 28.4, 29.2–29.7 (numerous peaks), 34.1, 34.8, 35.9, 36.0, 42.1, 52.4 (Leu Cα), 62.2 (glycerol CH), 69.0 (glycerol CH_2), 70.7 and 72.2 (C17), 79.6 (Boc quaternary), 128.5, 129.9, 133.4, 155.4 (Boc C = O), 172.9, 173.2, 173.3, 173.5. MALDI: calculated 2435.9 for $C_{144}H_{257}N_3O_{24}{\cdot}Na^+$, found 2435.8 $[M + Na]^+$.

8. [1]H: 0.89 (t, 6.9 Hz, 9H, terminal CH_3), 1.21 (d, 6.4 Hz, 9H, $C(18)H_3$), 1.24–1.42 (br s, 132H), 1.48 (s, 18H, Boc and OtBu), 1.50–1.73 (m, 20H), 1.85–2.16 (m, 12H, allylic H), 2.22–2.49 (m, 14H, $C(O)CH_2$ including Glu side chain), 4.14 (s, 6H, pentaerythritol CH_2 esterified with estolide), 4.21 (s, 2H, pentaerythritol CH_2 esterified with Glu), 4.23–4.38 (m, 1H, C(α)H), 4.91 (hex, 6.3 Hz, 3H, C(17)H), 5.11 (d, 8.3 Hz, 1H, NH), 5.36 (m, 6H, olefinic H). [13]C: 14.2 (terminal C18), 20.1 (internal C18), 22.8, 24.9, 25.2, 25.5, 27.3, 28.1, 28.4, 29.2–29.8 (numerous peaks), 32.0, 34.2, 34.9, 36.1 (C16), 53.3 (Glu Cα), 62.0 (pentaerythritol), 70.8 (C17), 80.9 (Boc quaternary), 129.9, 130.1, 155.4 (Boc C = O), 172.0, 173.3, 173.7. MALDI: calculated 2083.7 for $C_{127}H_{233}NO_{18} \cdot Na^+$, found 2083.6 $[M + Na]^+$.

Concerning the physical appearances of the products, compounds **2**, **3**, **6**, and **7** were thick syrups or glasses at room temperature. Compounds **4** and **8** were sticky waxes that melted in the range of 35–40 °C, while compound **5** was a more friable solid that melted at 50–55 °C.

Results and Discussion

Carbodiimide Couplings

Our initial efforts at forming estolides from **1** focused on the well-known reagents for forming ester or amide bonds, the carbodiimides [11, 12, and references therein]. To form estolides of a specific length, the strategy was to use protecting groups on either the carboxy or hydroxy ends to limit reaction to one ester unit, as outlined in Fig. 1. These procedures are totally analogous to those used commonly in peptide chemistry, and they have in fact been applied to oligoesters [13]. In principle, any of a number of pairs of protecting groups could be employed; for these initial experiments we chose benzyl esters and tBDPS ethers. Coupling of the benzyl ester of **1** and the tBDPS ether of **1** led to the diestolide, protected at both ends. Splitting this material into two portions and treating them with selective deprotecting reagents (hydrogenation over Pd/C to remove the Bn ester, or fluoride to remove the silyl ether) gave (a) the benzyl diestolide with a free hydroxy group and (b) the tBDPS diestolide with a free carboxy group. These two dimers could then be recoupled with EDC to give the tetramer, which was in turn used to prepare octamer **2**. While we chose to use "symmetrical" fragment condensations, e.g. dimer plus dimer to give tetramer, then tetramer plus tetramer to give octamer, this method should be perfectly applicable to other combinations, such as dimer plus trimer to give pentamer. Although we have not prepared odd-numbered linear oligomers through fragment condensation in this work, the next example demonstrates a 3 + 3 + 1 fragment condensation to give a heptamer.

Using dihydroxy fatty acids with this reaction scheme should lead to branched estolides. We used the allylically hydroxylated versions of **1a** reported previously [9], after separating the C8, C17 and C11, C17 dihydroxy isomers and hydrogenating the olefin. First, a trimer was prepared from the benzyl ester of the C11, C17 diol and two equivalents of the *tert*-butyl dimethylsilyl (tBDMS) ether of **1b** (see Fig. 2). Meanwhile the propyl amide of the C8, C17 diol was prepared—this amide serves as a convenient NMR tag. Coupling of two equivalents of the free acid (after deprotective hydrogenation) of the trimer with the propyl amide diol led to the desired heptamer **3**. Two tetramers, monoacylated at either the C8 or C17 hydroxy groups, were also isolated. Interestingly, these isomers could be separated from each other with silica gel chromatography.

Overall, while the EDC route did give estolide products, it had the shortcoming of being sluggish: especially with larger molecules such as tetramers, even after 3 days starting material remained. Octamer **2** was formed in only 25% yield. Steric hindrance is presumably partially responsible for this poor reactivity. Adding more EDC did not solve the problem. Nonetheless, EDC-based preparation of short estolides is simple to perform, and column chromatography suffices to remove impurities.

Benzoyl Chloride Activation

Our second attempt at finding a coupling method led to better results. We opted for a variation on the Yamaguchi coupling, where a carboxylate anion initially couples with benzoyl chloride (see Fig. 3), then with another equivalent to give the symmetrical anhydride [10]. This route was more successful for forming estolides (Fig. 4). By way of comparison to the EDC method, we repeated the synthesis of **2** using the recovered starting materials from the EDC run. With the benzoyl chloride route, the reaction gave a yield of 75% after 3 days of reaction. More significantly, we chose to explore the flexibility of this reaction by forming a "hybrid" estolide, the reaction sequence for which is sketched in Fig. 5. In principle, any hydroxy carboxylic acid can be used with this route to build a chain. We therefore chose to incorporate two non-fatty hydroxy

Fig. 2 Estolides prepared using the EDC coupling method from starting material **1a/b**. The shorthand "oleic/stearic" nomenclature is shown in the *upper right corner*: a residue runs from the carbonyl group at one terminus to the hydroxyl at the other. Whenever this shorthand is used, the carbonyl is positioned at the *left* and the hydroxyl at the *right*. In heptamer **3**, a trimer unit is *boxed*. See text for abbreviations

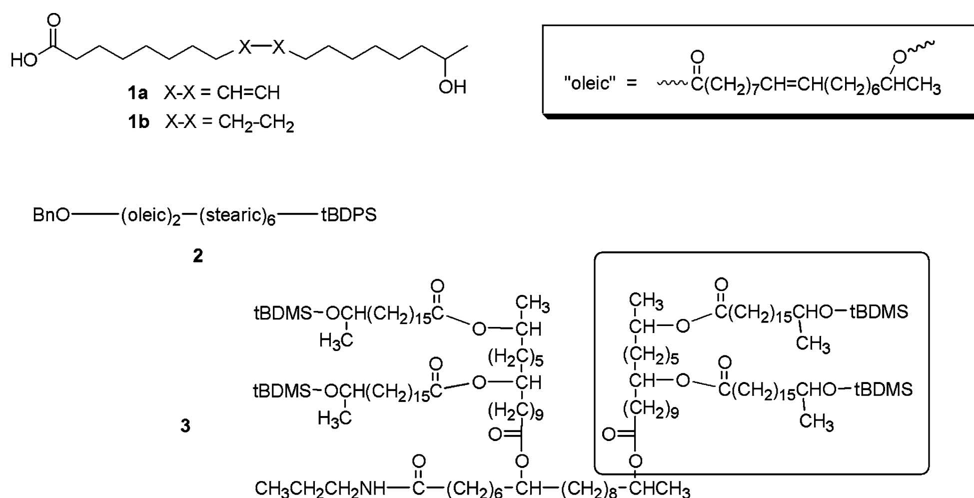
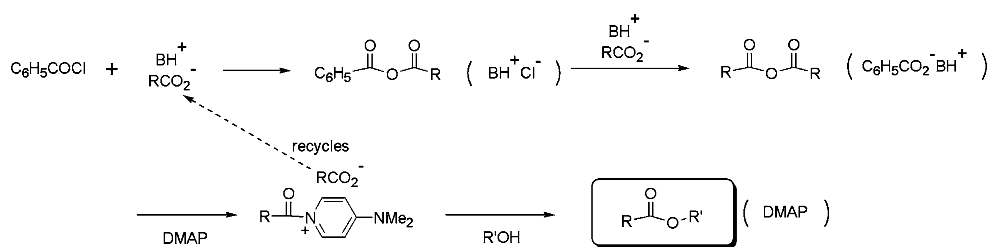


Fig. 3 Outline of the proposed mechanism for benzoyl chloride-mediated ester formation (see [10]). Side products are shown *parenthetically*; *B* is a trialkylamine base



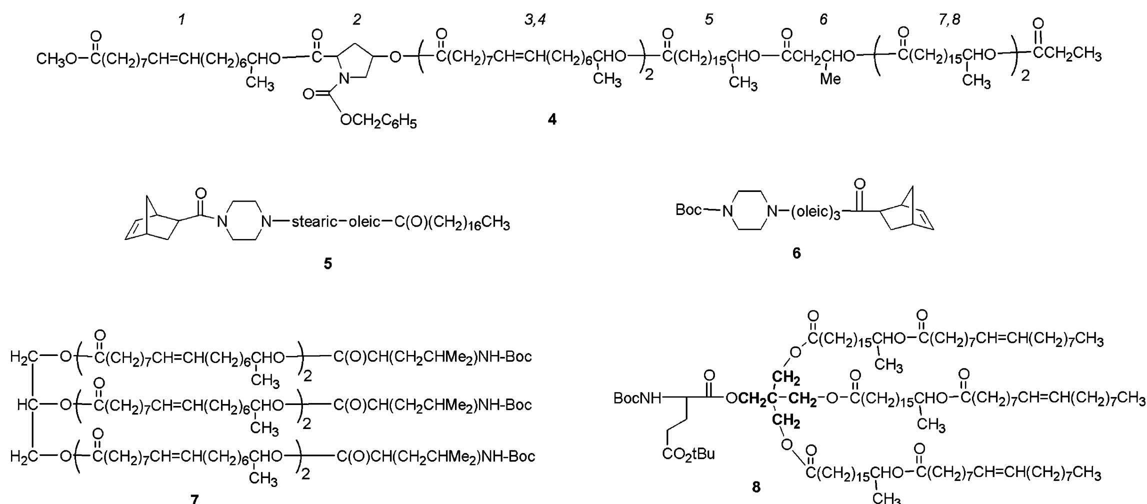


Fig. 4 Functionalized and branched estolides prepared using the benzoyl chloride route. The eight residues of **4** are *numbered*, and the pentaerythritol core of **8** is *in bold*

acids—3-hydroxybutyrate and 4-hydroxyproline—into a chain otherwise composed of 17-hydroxy C18 chains. In addition, we planned to segregate the C18 units by type along the chain, so that oleic residues were clustered on one half and stearic on the other. The protecting groups had to be chosen carefully, therefore, since benzyl esters would be incompatible with oleic acid. Instead, the trichloroethyl ester (removed with Zn dust [14]) was used for the oleic congener, and benzyl for the stearic. These esters of **1** were also prepared using the same benzoyl chloride methodology. For the hydroxy protecting group, tBDMS removed with trifluoroacetic acid was used throughout. A similar fragment condensation strategy as for the homooctamer **2** was used, that is, dimer plus dimer gives tetramer (Fig. 5), and tetramer plus tetramer (Fig. 6) gives the hybrid octamer **4**. We noted a marked improvement over the EDC

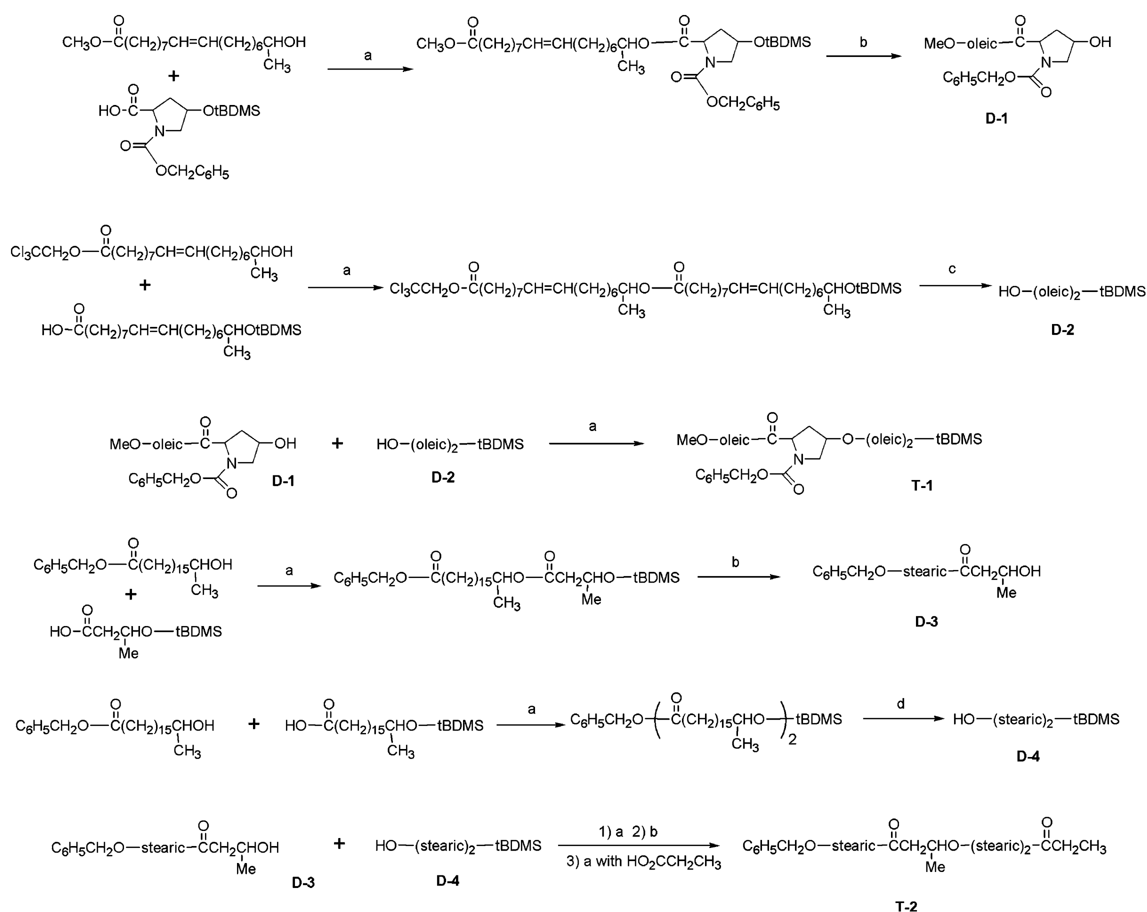


Fig. 5 Early and middle steps of the synthetic route to heterooctamer estolide **4**. To conserve space, the explicit molecular formula is drawn out for monomers (at *left*) and a condensed nomenclature (see Fig. 2) is employed for products. *D* and *T* refer to dimer and trimer, respectively. Reagents and conditions. **a** Benzoyl chloride, DIEA, DMAP; **b** 9:1 CF_3CO_2H:H_2O; **b** Zn dust, pH 7.2 phosphate buffer; **d** H_2 over Pd/C in MeOH

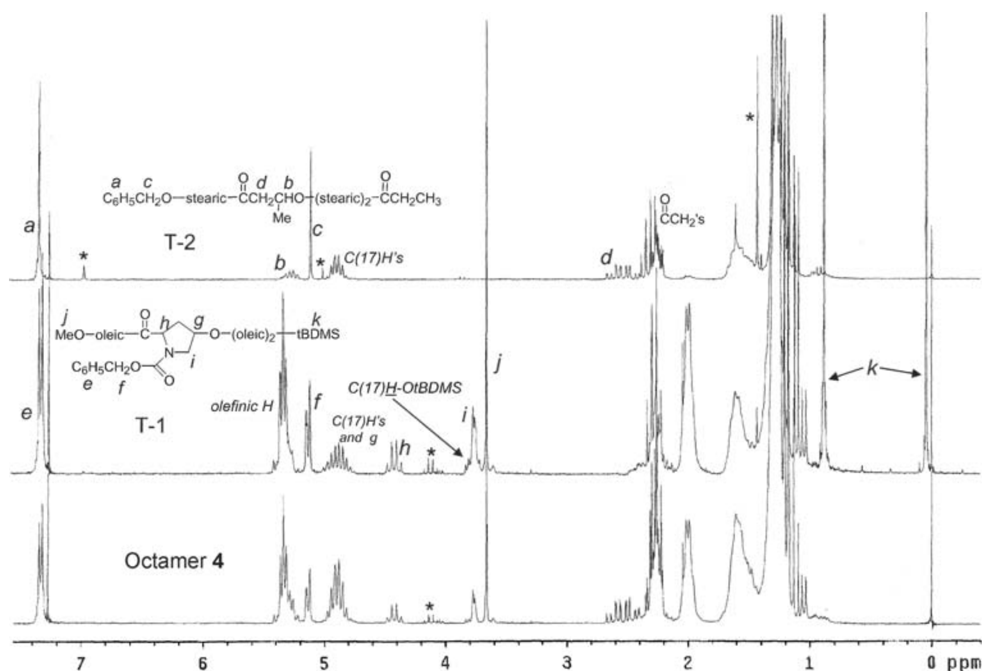


Fig. 6 Comparison of the ^{1}H-NMR spectra of the two precursor tetramers (see Fig. 5) and their coupling product **4**. Prior to coupling, T-1 is deprotected at its hydroxy terminus with aqueous CF_3CO_2H, T-2 is deprotected at its carboxy terminus by hydrogenation over Pd/C, then the deprotected tetramers are coupled using benzoyl chloride as described in the text. The *asterisk* indicates BHT or ethyl acetate impurities

couplings: most of the reactions were essentially complete in about 2 h. On the other hand, the reactions did appear to be slower for coupling of longer chains, just as for the EDC route, although we have not studied the kinetics in a systematic way. The cleanliness of the reaction was a further benefit: product, hydroxy starting material, and carboxylic starting material were readily separable from each other by column chromatography on silica gel, using hexane/ethyl acetate mixtures (R_f's high, mid, and low, respectively). This feature allowed the reaction to be driven to complete usage of one component if desired, such as by using an excess of the hydroxy component to be sure to consume all of a valuable carboxylic component, while still being able to recover the unreacted hydroxy component by chromatography. Yields of the individual coupling steps for all the benzoyl chloride reactions ranged from 75 to 93%.

With the successful synthesis of heterooctamer **4**, we turned out attention to other sorts of estolides. A desirable feature to include in an estolide is the possibility for further reactivity, such as polymerization. The norbornene unit has been used extensively with ring-opening metathesis polymerization [15], so we chose to incorporate this building block in estolides. Molecules **5** and **6**, both trimers, were synthesized analogously to **4**, using trichloroethyl ester protection. Molecule **6** shows that, with the polymerizable unit at the hydroxy terminus, the carboxy terminus can be used to append other functionality of interest; here, the Boc group could be removed to afford a basic site. Polymerization of **5** or **6** would afford a comb-type polymer, where the length and functionality of the side chains could be controlled by altering the structure of **5** or **6**.

Brief mention should be made of the concept of "estolide number", which has previously been used to characterize estolides. For oligomers of the sort monomer-(monomer)$_n$-monomer, EN is defined as n + 1. Compounds **2** and **4** would therefore have EN = 7, and compounds **5** and **6** EN = 2. In the heterodisperse estolides that have been reported previously, EN values in the range of 10–12 can be attained [1], although these usually comprise a small percentage of the bulk samples, which have an average EN around 2–3 [5, 6]. To the best of our knowledge, EN has not been defined for branched estolides, so it would be ambiguous to assign a value to compound **3** (or **7** or **8**, see below). Alternative definitions, for example the number of ester linkages in the molecule, could be proposed, but with functionalized heterooligomers the question of which ester bonds to count may lead to complications.

Finally, this methodology allows construction of nonlinear estolides. Two cores were investigated. First, glycerol, by analogy to triacylglycerols, was an obvious choice. We coupled it with an excess of a functionalized estolide, namely the dimer from two units of **1a** capped at its hydroxy terminus with Boc-Leucine, to give **7**. The second core was pentaerythritol, which was first monoacylated with BocGlu(OtBu) (this reaction required use of *N*,*N*-dimethylformamide as solvent for the pentaerythritol), then reacted with excess stearic-oleic diestolide. This 3:1 ratio of substituents in **8** was chosen to aid in NMR identification, as well as to demonstrate the range of functionalizability, but the fully symmetric quadruply substituted variant should also be accessible. In both these examples, as well as **4**, there is nothing privileged about protected amino acids, but they simply are representative of the kinds of reactive groups that can be incorporated into non-traditional estolide structures.

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