Organic Process Research & Development **2004**, *8,* 796–801

Technical Notes

The Efficient Synthesis of Disodium Disuccinate Astaxanthin (Cardax)

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Abstract:

A practical procedure is described for the multigram preparation of disodium disuccinate derivatives of synthetic astaxanthin (Cardax) [from all-*trans*-(all-*E*)-3*S*,3′*S*-, *meso*-(3*R*,3′*S*)-, and 3*R*,3′*R*-dihydroxy-*β*,*β*-carotene-4,4′-dione) in a 1:2:1 statistical mixture of stereoisomers, as well as from the individual component stereoisomers]. Process development eliminated chromatographic separations, controlled geometric isomerization, and improved the overall yield of the two-step process, with significant improvements in both the yield and purity of Cardax. Bulk chromatographic separation of the diastereomeric dicamphanic acid ester of synthetic astaxanthin was performed by modifications of the published procedure to subsequently generate multigram quantities of each stereoisomer of disodium disuccinate of astaxanthin.

Introduction

The synthetic preparation of disodium disuccinate astaxanthin **3** at multigram scale (200 g to 1 kg) was necessary as part of the proof-of-concept portion of a drug development program (patent application pending).[1] Synthetic modifications of carotenoids, with the goal of increasing aqueous solubility and/or dispersibility, have been sparingly reported in the literature.[2] At the time process development began, surveys of the peer-reviewed and patent literature indicated that neither a synthetic sequence nor an efficient process for the synthesis of **2** or **3** had been reported. Therefore, the bench-scale synthetic sequence and later the scale-up to multigram scale were optimized to improve both the yield and purity of the desired compound, shown in Scheme 1.

The disodium disuccinate derivatives (Cardax) of synthetic astaxanthin were successfully synthesized in gram amounts and at high purity (>90%) area under the curve (AUC) by HPLC. The novel compound in "racemic" form demonstrated water "dispersibility" of 8.64 mg/mL, a significant improvement over the parent compound astaxanthin, which is insoluble in water.[3,4] Initial biophysical characterization demonstrated that Cardax derivatives (as both the statistical mixture of stereoisomers and as individual stereoisomers) were potent direct scavengers of superoxide anion in the aqueous phase,[5] the first such description in this model system for a C40 carotenoid. Plasma-protein binding studies in vitro revealed that the *meso*-(3*R*,3′*S*)-disodium disuccinate astaxanthin derivative bound immediately and preferentially to human serum albumin (HSA) at a novel binding site, suggesting that beneficial ligand-binding associations might take place in vivo after parenteral administration of the compound.[6] The single- and multiple-dose pharmacokinetics of an oral preparation of the racemic compound (in lipophilic emulsion) were then investigated in a murine model, and significant plasma and tissue levels of nonesterified astaxanthin were achieved.[7] Proof-of-concept studies in ischemia−reperfusion injury performed in rodents subsequently revealed that intravenous pretreatment with Cardax was significantly cardioprotective and achieved myocardial salvage in this experimental infarction model up to 56% at the highest dose tested.[8] The test material for three of the published studies described above was obtained from a single pilot batch of compound (>200 g single batch at >97% purity by HPLC).[5,7,8]

Results and Discussion

Process Development. In the initial synthetic route, the disuccinic acid ester of astaxanthin was formed by reacting astaxanthin with an excess of succinic anhydride (6 equiv), 4-(dimethylamino)pyridine (DMAP) (8 equiv) in chloroform (10 mL/g) at 55 °C for 14 h. The geometric isomerization of the all-*trans*-astaxanthin to 13- and 9-*cis* made chromatography necessary to make the initial reference standards of the disuccinic acid ester of astaxanthin. Thus, efforts were

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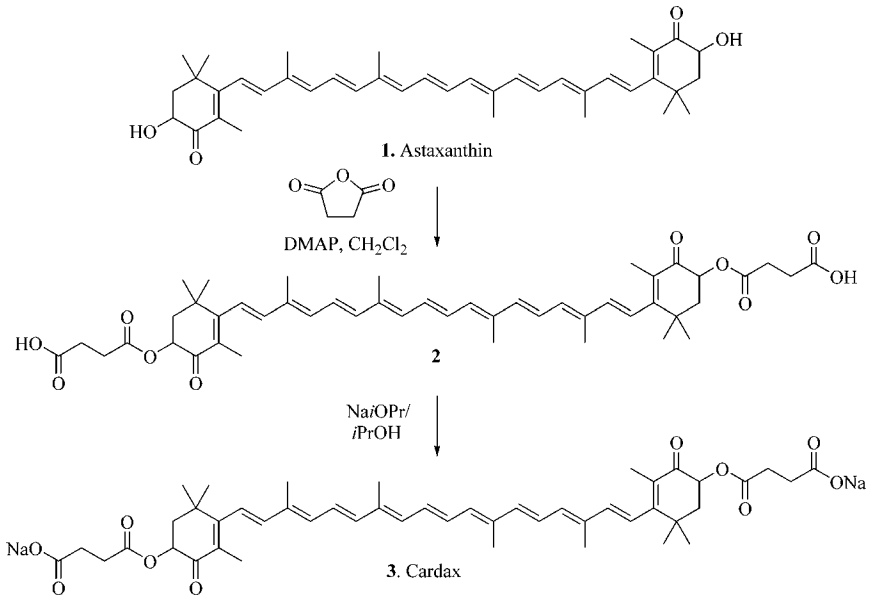
Published on Web 08/12/2004

10.1021/op049909i CCC: $27.50

Scheme 1



focused on controlling isomerization. Although the rate of isomerization for *trans*-astaxanthin to *cis*-isomers is higher in methylene chloride (22% in 10 h at 35 °C) than in chloroform (6% in 10 h at 35 °C),[9] the solubility of astaxanthin in methylene chloride (33 mL/g) is much higher than that in chloroform (100 mL/g).[10] The limited solubility of astaxanthin in chloroform necessitated the higher temperature. The use of methylene chloride permitted more concentrated solutions of astaxanthin in the reaction and allowed the temperature of the reaction to be lowered to room temperature; the reaction was complete after 4 h with less than 3% *cis*-isomer formation by HPLC. With the *cis*-isomerization minimized, the use of excess DMAP (8 equiv) and succinic anhydride (6 equiv) were addressed. It was found that 2.5 equiv of DMAP and 3 equiv of succinic anhydride yielded the same result in 14−16 h. The succinylation reaction was also attempted with catalytic amounts of DMAP and Hunig's base in methylene chloride, but the temperature had to be increased to 35 °C to keep the reaction times to 14−16 h.

The initial workup of the reaction consisted of the following sequence: (1) concentration of the reaction mixture to a minimum volume, (2) chromatography through a plug of silica gel to separate the DMAP (2−5% MeOH/DCM), and (3) precipitation of the excess succinic anhydride from isopropyl acetate/heptane. This procedure worked well on a 5-g scale, producing 4.95 g (74% yield, 99.4% purity by HPLC), but on a 60-g scale the yields were diminished by hydrolysis of the ester (43% yield, 99.2% purity by HPLC). Although ester derivatives of astaxanthin are known to be more stable to *cis*-isomerization,[11,12] we were reluctant to use extraction with weak acids to remove the excess DMAP and succinic anhydride/acid because of Chen's observation of fast degradation of both *trans*- and *cis*-astaxanthin in solutions of acid.[9] After screening aqueous extraction conditions using brine, water, phosphate buffer solutions (pH 4−6), 10% aqueous ammonium chloride, and 5% citric acid, only aqueous ammonium chloride and citric acid were found to be effective at removing the excess of both DMAP and succinic anhydride/acid without isomerization. Ammonium salts of the disuccinic acid ester of astaxanthin reduced solubility in the salt formation reaction; therefore, citric acid was chosen for the aqueous extraction. The organic layer was concentrated under vacuum, and isopropyl acetate was used to azeotrope the residual water. The procedure was scaled up with 240 g of astaxanthin and afforded a quantitative yield of product with 92.3% purity by HPLC.

The sodium salt of the disuccinic acid was initially formed by adding 2 equiv of sodium hydroxide in water and lyophilizing the sample. On a 100-mg scale this procedure worked well, but on gram scale hydrolysis of the ester became a problem. Attempts at making the salt using sodium hydride in tetrahydrofuran (THF) were also not reproducible. It was found that predrying the disuccinic acid ester solution using triethylorthoformate reduced hydrolysis of the succinic acid esters significantly. After screening alkoxides in alcohols under anhydrous conditions, the hydrolysis of the succinic ester was subsequently minimized by using sodium isopropoxide/2-propanol (4−10%). Although the throughput of the reaction was limited by the solubility of the disuccinic acid ester of astaxanthin in 2-propanol (45−50 mL/g), it was necessary to limit the reaction time. Attempts at performing the salt formation at higher concentrations (10 and 20 mL/g) were not reproducible. Hydrolysis of the disuccinic acid ester was significant (>10%) at all concentrations studied (10−50 mL/g) if stirred for longer than 4 h on small scale. Salt formation performed at higher concentrations for less than 4 h produced solids with poor water solubility characteristics. The optimum salt formation was performed by predrying the disuccinic acid ester of astaxanthin using 3 equiv of triethylorthoformate in 30 mL/g of anhydrous

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2-propanol for 30 min, followed by addition of a premade solution of 3 equiv of sodium isopropoxide in 15 mL/g of 2-propanol over 1 h. The reaction was stirred for 3−4 h, and the salt was filtered under nitrogen and dried under vacuum to afford crude product.

The main impurities from the salt formation reaction were the monosuccinic acid ester of astaxanthin, astaxanthin, and the 13- and 9-*cis* isomers of the disuccinic acid ester of astaxanthin. As impurities were all found to be more soluble than the all-*trans* disodium disuccinate ester of astaxanthin in methylene chloride, the crude product was reslurried in 10 mL/g of methylene chloride until a purity of >95% by HPLC was obtained. A final reslurry in 2-propanol was used to wash out any residual methylene chloride in the disodium disuccinate ester of astaxanthin, and the reslurry also provided a method for purging more polar impurities to obtain 97% purity by HPLC. The optimized procedure was scaled-up to 240 g of astaxanthin to give 189 g of Cardax, representing a 59% overall yield from astaxanthin (97% purity by HPLC).

Preparation of Gram-Scale Quantities of 3*S*,3′*S*-, *meso*-(3*R*,3′*S*)-, and 3*R*,3′*R*-Astaxanthin. Bulk chromatographic separation of the diastereomeric dicamphanic acid ester(s) of synthetic astaxanthin at preparative chromatography scale was performed with modifications of a published procedure to subsequently make gram-scale quantities of each stereoisomer of disodium disuccinate ester astaxanthin.[13]

A total of 135 g of astaxanthin dicamphanate esters (ASTA-DCE) prepared by derivatization of racemic astaxanthin with (−)-camphanic acid chloride were fractionated by preparative HPLC (using a 77 mm i.d. × 25 cm column formed by packing 550 g of 10 μm Kromasil 60 Å silica; Eka Chemicals, Marietta, GA) into a Varian RamPak column packing station. After the dry column packing material was mixed with 1200 mL of toluene/2-propanol (50/50) and the resulting slurry was transferred to the 77 mm i.d. column packing chamber, the column bed was formed using the dynamic axial compression of the RamPak unit. The packing solvent was flushed from the column bed for 50 min at a flow rate of 150 mL/min using the preparative HPLC mobile phase consisting of 95% toluene and 5% methyl ethyl ketone (MEK). The preparative HPLC system consisted of a Waters Prep 4000 solvent delivery system and a Waters model 486 variable UV detector fitted with a prep cell (3 mm path length). Sample solution was injected directly through the pump, detection was at 580 nm, and the chromatogram was recorded on a strip chart recorder. At the preparative flow rate of 280 mL/min, the system backpressure was 840 psi. The laboratory was equipped with yellow lights, and the windows were covered to avoid any effects of light on the sample.

A sample solution for preparative HPLC was prepared by dissolving 30 g of ASTA-DCE in 90 mL of methylene chloride and diluting the solution with 210 mL of toluene. A portion of the resulting solution (272 mL) was further diluted with 688 mL of preparative HPLC mobile phase to generate the sample solution that was subsequently injected onto the preparative HPLC system. The preparative HPLC injection consisted of pumping 120 mL of this ASTA-DCE sample solution (~3.4 g of ASTA-DCE) through the pump and onto the preparative column.

The preparative loading was selected to optimize sample throughput, and the resulting chromatogram consisted of three slightly overlapping peaks with the 3*R*,3′*R* ester eluting at 14 min, the *meso*-(3*R*,3′*S*) ester at 16.5 min, and the 3*S*,3′*S* ester at 21.5 min. To take advantage of the blank section of the chromatogram for the first 10 min, subsequent injections were made 20 min into the previous run at the valley between the *meso* and 3*S*,3′*S* peaks. Heart cuts of each of the three peaks were collected in addition to the mixed fractions at the overlap of the 3*R*,3′*R*/*meso* and the *meso*/3*S*,3′*S* peaks. A total of 40 preparative injections were processed using 84 L of mobile phase. Thirty-six (36) L of effluent were collected among the five fractions. The preparative system was flushed with 100 mL of methylene chloride approximately every 6−8 injections or whenever the chromatographic separation deteriorated due to effects from mixing with mobile phase in the pump heads during the injection process. Purified materials were recovered by removing the solvents in a rotary evaporator protected from light to afford 25.4 g of 3*R*,3′*R* ester, 47.8 g of *meso*-(3*R*,3′*S*) ester, and 24.9 g of 3*S*,3′*S* ester.

The purified astaxanthin dicamphanate esters were saponified using Chen's method to afford 8.5 g (79.8% purity by HPLC) of 3*R*,3′*R*-astaxanthin, 18.2 g (90.1% purity by HPLC) of *meso*-astaxanthin, and 9.4 g (82.0% purity by HPLC) of 3*S*,3′*S*-astaxanthin.[11] The major impurities of the saponification reaction were the 13- and 9-*cis* isomers of astaxanthin, identified by HPLC using Turujman's method.[14] The *cis*-isomers were thermally isomerized to all-*trans* by refluxing in heptane to afford 8.5 g (87.3% purity by HPLC) of 3*R*,3′*R*-astaxanthin, 18.2 g (92.5% purity by HPLC) of *meso*-astaxanthin, and 9.4 g (86.8% purity by HPLC) of 3*S*,3′*S*-astaxanthin.

Conclusions

In summary, we reported the novel multigram-scale preparation of Cardax from a 1:2:1 statistical mixture of stereoisomers. In addition, this enhanced synthesis was applied successfully to the individual stereoisomers. The key improvements to the synthesis were found in (1) the removal of time-consuming and difficult chromatographic separations and replacement with an aqueous extraction and (2) the control of geometric isomerization which was achieved by increasing reaction times and lowering reaction temperatures. The improvements significantly increased both the yield and purity of Cardax.

Experimental Section

General Procedures. Unless otherwise noted, all reagents and solvents were purchased and used as received without further purification, from commercial suppliers. Proton and carbon nuclear magnetic resonance (NMR) spectra were

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obtained on a Bruker AMX 500 spectrometer at 500 MHz for proton NMR (^{1}H NMR) and 125 MHz for carbon NMR (^{13}C NMR). Chemical shifts are given in ppm (δ), and coupling constants, *J*, are reported in hertz (Hz). Tetramethylsilane (TMS) was used as an internal standard for proton spectra, and the solvent peak was used as the reference peak for carbon spectra. High-resolution mass spectra were obtained by M-Scan (West Chester, PA) using either fast atom bombardment (FAB) analysis carried out using M-Scan's VG Analytical ZAB 2-SE high field mass spectrometer or positive ion electrospray (ESI+ mode) high-resolution mass spectroscopy carried out with a Perkin-Elmer Sciex Q-Star hybrid quadrupole/time-of-flight mass spectrometer. High performance liquid chromatography (HPLC) analysis for in-process control (IPC) was performed on a Varian Prostar series 210 liquid chromatograph with a PDA detector using an Alltech Rocket, Platinum-C18, 100 Å, 3 μm, 7 mm $\times$ 53 mm, PN 50523 column; temperature: 25 °C; mobile phase: A = water w/ 0.025% trifluoroacetic acid (TFA); B = acetonitrile w/ 0.025% TFA, 70% A/30% B (start); hold 40 sec; linear gradient to 50% B over 4 min 20 sec; linear gradient to 100% B over 1 min 30 sec; hold 100% B over 4 min 50 sec, linear gradient to 70% A/30% B over 20 sec; flow rate: 2.5 mL/min; UV detector wavelength: 474 nm.

***rac*-(Succinic acid mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester) (2).** A 12-L three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. "Racemic" astaxanthin (as the commercially available statistical mixture of stereoisomers is commonly known; Divi's Laboratories, Limited, India) (240 g, 402 mmol) was charged to the flask followed by methylene chloride (2.40 L), succinic anhydride (121 g, 1.21 mol), and DMAP (123 g, 1.01 mol). After 14 h, the reaction was found to be complete by HPLC. A citric acid solution (5% aq, 2.90 L) was added to the reaction and stirred for 30 min. The layers were separated, and the organic layer was extracted two more times with the citric acid solution (5% aq, 2.90 L) then concentrated. The residue was taken up in isopropyl acetate (IPAC) (1000 mL), and the resulting solution was concentrated again to azeotrope any residual water from the washes. The material was dried under high vacuum to afford 344 g of a red solid (107% of theoretical). ^{1}H NMR ($CDCl_3$) δ 7.68−6.18 (14 H, m), 5.54 (2 H, dd, J = 13.3, 6.1), 2.85−2.73 (8 H, m), 2.07−1.98 (16 H, m), 1.89 (6 H, s), 1.35 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 193.9, 177.2, 171.6, 160.8, 142.3, 139.7, 136.7, 135.2, 134.5, 133.8, 130.7, 128.3, 124.6, 123.1, 71.5, 67.7, 42.5, 37.1, 30.4, 28.9, 26.3, 21.8, 21.4, 14.0, 12.8, 12.6; Anal. Calcd for [$C_{48}H_{60}O_{10}$ + Na]: 819.4084, ESI MS *m*/*z* 819.4108 [$C_{48}H_{60}O_{10}$ + Na]$^+$; HPLC 92.3% AUC, t_R = 7.80 min.

***rac*-(Sodium succinate mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester) (3).** A 22-L three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. Racemic disuccinic acid ester of astaxanthin **2** (344 g, theoretically 320 g, 0.402 mol, stoichiometry based on theoretical, 92.3% purity by HPLC) was charged to the flask followed by anhydrous 2-propanol (10.5 L) and triethylorthoformate (128 mL, 1.21 mol), and the reaction was stirred for 30 min. A premade solution of sodium isopropoxide using sodium (27.7 g, 1.21 mol) in anhydrous 2-propanol (5.3 L) was added over 60 min. After 4 h, the reaction mixture was filtered, rinsed with 620 mL of anhydrous 2-propanol, and dried under high vacuum overnight to afford to 536 g of a red solid (54.2% AUC by HPLC). The solid was reslurried in 4.0 L of methylene chloride for 45 min; the reaction mixture was filtered, rinsed with 1.5 L of methylene chloride, and dried under high vacuum (93.5% AUC by HPLC). The solid was reslurried a second time in 3.0 L of methylene chloride for 1 h, and the reaction mixture was again filtered, rinsed with 1.0 L of methylene chloride, and dried under high vacuum (94.6% AUC by HPLC). A final reslurry was performed with anhydrous 2-propanol (2.0 L) for 30 min, and the mixture was again filtered, subsequently washed with 2-propanol (1.0 L), and finally dried under high vacuum to afford 189 g of an amorphous red solid (59% yield). ^{1}H NMR ($CDCl_3$) δ 7.68−6.18 (14 H, m), 5.54 (2 H, dd, J = 13.3, 6.1), 2.85−2.73 (8 H, m), 2.07−1.98 (16 H, m), 1.89 (6 H, s), 1.35 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 194.8, 178.9, 178.8, 161.9, 142.3, 139.5, 136.4, 135.0, 134.2, 133.6, 130.5, 127.5, 124.3, 122.8, 70.8, 67.4, 63.2, 42.2, 36.7, 32.0, 31.9, 30.3, 29.3, 25.0, 23.8, 20.5, 12.8, 11.3, 11.0; DSC one exothermic event at 149.9 °C; Anal. Calcd for [$C_{48}H_{60}O_{10}$ + Na]: 819.4084, ESI MS *m*/*z* 819.4073 [$C_{48}H_{60}O_{10}$ + Na]$^+$; HPLC 97.0% AUC, t_R = 7.80 min.

(3*S*,3′*S*)-(Succinic acid mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester). A 250 mL three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. 3*S*,3′*S*-Astaxanthin (8.43 g, 14.1 mmol) was charged to the flask followed by methylene chloride (84 mL), succinic anhydride (4.24 g, 42.4 mmol), and DMAP (4.31 g, 35.3 mmol). After 16 h, the reaction was complete by HPLC. A citric acid solution (5% aq, 85 mL) was added to the reaction and stirred for 30 min. The layers were separated and the organic layer was extracted 2 more times with the citric acid solution (5% aq, 85 mL) and concentrated. The residue was taken up in IPAC (43 mL) and the resulting solution was concentrated again to azeotrope any residual water from the washes. The material was dried under high vacuum to afford 11.84 g of a red solid (105% of theoretical). ^{1}H NMR ($CDCl_3$) δ 6.67−6.19 (14 H, m), 5.54 (2 H, dd, J = 13.4, 6.1), 2.80−2.74 (8 H, m), 2.07−1.99 (16 H, m), 1.89 (6 H, s), 1.35 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 193.9, 177.1, 171.6, 170.7, 160.8, 142.3, 139.7, 136.7, 135.1, 134.5, 133.8, 130.7, 128.3, 124.6, 123.1, 71.5, 67.7, 42.5, 37.1, 30.4, 28.9, 26.3, 21.8, 21.4,

14.0, 12.8, 12.6; Anal. Calcd for [$C_{48}H_{60}O_{10}$]: 796.4186, FAB MS *m*/*z* 796.4184 [$C_{48}H_{60}O_{10}$]$^+$; HPLC 89.6% AUC, t_R = 7.80 min.

(3*S*,3′*S*)-(Sodium succinate mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nona-enyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester). A 1 L three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. Disuccinic acid ester of 3*S*,3′*S*-astaxanthin (11.6 g, 14.6 mmol) was charged to the flask followed by anhydrous 2-propanol (384 mL) and triethylorthoformate (7.3 mL, 43.8 mmol) and the reaction was stirred for 30 min. A pre-made solution of sodium isopropoxide using sodium (1.00 g, 43.8 mmol) in anhydrous 2-propanol (87 mL) was added over 30 min. After 3 h, the reaction mixture was filtered, then rinsed with 25 mL of anhydrous 2-propanol, and dried under high vacuum overnight (78.4% AUC by HPLC). The solid was reslurried in 110 mL of methylene chloride for 1 h, the mixture was filtered, rinsed with 50 mL of methylene chloride, and then dried under high vacuum (86.4% AUC by HPLC). The solid was reslurried a second time in 60 mL of methylene chloride, the mixture filtered, rinsed with 30 mL of methylene chloride, and dried under high vacuum (93.4% AUC by HPLC). The solid was reslurried a third time in 60 mL of methylene chloride, the mixture was filtered, rinsed with 30 mL of methylene chloride, and again dried under high vacuum to afford 4.8 g of a red solid (95.8% AUC by HPLC). A final reslurry was performed with anhydrous 2-propanol (50 mL) for 15 min, the mixture was filtered, washed with 2-propanol (25 mL), and then dried to afford 4.60 g of an amorphous red solid (37% yield). ^{1}H NMR (Methanol-d_4) δ 6.75−6.29 (14 H, m), 5.53 (2 H, dd, J = 13.2, 6.2), 2.70−2.44 (8 H, m), 2.08−1.92 (16 H, m), 1.89 (6 H, s), 1.37 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 196.3, 180.7, 180.2, 174.5, 163.3, 143.8, 140.9, 137.9, 136.5, 135.7, 135.1, 131.9, 129.0, 125.8, 124.3, 72.3, 64.7, 43.7, 38.2, 34.3, 33.4, 31.8, 30.8, 26.5, 25.3, 14.2, 12.8, 12.5; DSC one exothermic event at 135.6 °C; Anal. Calcd for [$C_{48}H_{60}O_{10}$ + Na]: 819.4084, ESI MS *m*/*z* 819.4072 [$C_{48}H_{60}O_{10}$ + Na]$^+$; HPLC 95.6% AUC, t_R = 7.80 min.

***meso*-(Succinic acid mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nonaenyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester).** A 250 mL three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. *meso*-(3*R*,3′*S*)-Astaxanthin 6.00 g, 10.1 mmol) was charged to the flask followed by methylene chloride (60 mL), succinic anhydride (3.02 g, 30.2 mmol), and DMAP (3.07 g, 25.1 mol). After 14 h, the reaction was complete by HPLC. A citric acid solution (5% aq, 60 mL) was added to the reaction and stirred for 30 min. The layers were separated and the organic layer was extracted two more times with the citric acid solution (5% aq, 60 mL) and concentrated. The residue was taken up in IPAC (30 mL) and the resulting solution was concentrated again to azeotrope any residual water from the washes. The material was dried under high vacuum to afford 8.49 g of a red solid (106% of theoretical). ^{1}H NMR ($CDCl_3$) δ 6.68−6.19 (14 H, m), 5.54 (2 H, dd, J = 13.4, 6.1), 2.82−2.68 (8 H, m), 2.07−1.99 (16 H, m), 1.89 (6 H, s), 1.35 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 193.9, 177.2, 171.6, 160.8, 142.3, 139.7, 136.7, 135.1, 134.5, 133.8, 130.7, 128.3, 124.6, 123.1, 71.5, 67.6, 42.5, 37.1, 30.4, 28.9, 28.4, 26.3, 21.8, 21.4, 14.0, 12.8, 12.6; Anal. Calcd for [$C_{48}H_{60}O_{10}$]: 796.4186, FAB MS *m*/*z* 796.4180 [$C_{48}H_{60}O_{10}$]$^+$; HPLC 92.2% AUC, t_R = 7.80 min.

***meso*-(Sodium succinate mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nona-enyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester).** A 1 L three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. Disuccinic acid ester of *meso*-astaxanthin (8.39 g, 9.74 mmol) was charged to the flask followed by anhydrous 2-propanol (277 mL) and triethylorthoformate (3.4 mL, 31.6 mmol) and the reaction was stirred for 30 min. A pre-made solution of sodium isopropoxide (0.5 M, 63.2 mL, 31.6 mmol) was added over 30 min under nitrogen. After 3 h, the reaction mixture was filtered, rinsed with 30 mL of methylene chloride, and dried under high vacuum overnight to afford 4.11 g of a red solid (83.7% AUC by HPLC). The solid was reslurried in 40 mL of methylene chloride for 2 h, the mixture was filtered, rinsed with 20 mL of methylene chloride, and then dried under high vacuum (96.0% AUC by HPLC). The solid was reslurried a second time in 40 mL of methylene chloride for 2 h, the mixture was filtered, rinsed with 20 mL of methylene chloride, and then dried under high vacuum (98.4% AUC by HPLC). A final reslurry was performed with anhydrous 2-propanol (40 mL) for 30 min, the mixture was filtered, rinsed with 2-propanol (20 mL), and again dried under high vacuum to afford 3.95 g of an amorphous red solid (45% yield). ^{1}H NMR (Methanol-d_4) δ 6.76−6.30 (14 H, m), 5.53 (2 H, dd, J = 13.2, 6.2), 2.70−2.48 (8 H, m), 2.10−1.99 (16 H, m), 1.90 (6 H, s), 1.37 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 196.3, 181.1, 180.3, 180.2, 174.9, 174.6, 163.3, 143.8, 141.0, 137.9, 136.5, 135.7, 135.0, 131.9, 129.0, 125.8, 124.3, 72.3, 68.9, 433.7, 38.2, 34.6, 33.4, 33.4, 32.3, 31.8, 30.8, 28.5, 26.5, 25.3, 22.0, 14.3, 12.8, 12.5; DSC one exothermic event at 135.4 °C; Anal. Calcd for [$C_{48}H_{60}O_{10}$ + Na]: 819.4084, ESI MS *m*/*z* 819.4045 [$C_{48}H_{60}O_{10}$ + Na]$^+$; HPLC 99.0% AUC, t_R = 7.80 min.

(3*R*,3′*R*)-(Succinic acid mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nona-enyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester). A 250 mL three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. 3*R*,3′*R*-astaxanthin (6.00 g, 10.1 mmol) was charged to the flask followed by methylene chloride (60 mL), succinic anhydride (3.02 g, 30.2 mmol), and DMAP (3.07 g, 25.1 mol). After 16 h, the reaction was complete by HPLC. A citric acid

solution (5% aq, 60 mL) was added to the reaction and stirred for 30 min. The layers were separated and the organic layer was extracted 2 more times with the citric acid solution (5% aq, 60 mL) and concentrated. The residue was taken up in IPAC (30 mL) and the resulting solution was concentrated again to azeotrope any residual water from the washes. The material was dried under high vacuum to afford 7.88 g of a red solid (98% yield). ^{1}H NMR ($CDCl_3$) δ 6.67−6.19 (14 H, m), 5.54 (2 H, dd, J = 13.4, 6.0), 2.81−2.74 (8 H, m), 2.07−1.98 (16 H, m), 1.90 (6 H, s), 1.35 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 193.9, 171.6, 160.8, 142.3, 139.7, 136.7, 135.1, 134.5, 133.8, 130.7, 128.3, 124.6, 123.1, 71.5, 67.6, 42.5, 37.1, 30.4, 28.9, 28.7, 26.3, 21.8, 21.4, 14.0, 12.8, 12.6; Anal. Calcd for [$C_{48}H_{60}O_{10}$]: 796 4186, FAB MS m/z 796.4185 [$C_{48}H_{60}O_{10}$]$^+$; HPLC 93.3% AUC, t_R = 7.80 min.

(3*R*,3′*R*)-(Sodium succinate mono-(4-{18-[4-(3-carboxy-propionyloxy)-2,6,6-trimethyl-3-oxo-cyclohex-1-enyl]-3,7,12,16-tetramethyl-octadeca-1,3,5,7,9,11,13,15,17-nona-enyl}-3,5,5-trimethyl-2-oxo-cyclohex-3-enyl) ester). A 1.0-L three-neck round-bottom flask wrapped with aluminum foil was equipped with an overhead stirrer and a digital thermocouple under nitrogen at room temperature. Disuccinic acid ester of 3*R*,3′*R*-astaxanthin (7.76 g, 9.74 mmol) was charged to the flask followed by anhydrous 2-propanol (256 mL) and triethylorthoformate (3.1 mL, 29.2 mmol), and the reaction was stirred for 30 min. A premade solution of sodium isopropoxide (0.5 M, 58.4 mL, 29.2 mmol) was added over 30 min under nitrogen. After 3 h, the reaction mixture was filtered, rinsed with 25 mL of anhydrous 2-propanol, and dried under high vacuum to afford 4.80 g of a red solid (75.0% AUC by HPLC). The solid was reslurried in 48 mL of methylene chloride for 2 h; the mixture was filtered, rinsed with 25 mL of methylene chloride, and then dried under high vacuum to afford 4.00 g (76.7% AUC by HPLC). The solid was reslurried a second time in 40 mL of methylene chloride for 2 h; the mixture was filtered, rinsed with 20 mL of methylene chloride, and then dried under high vacuum to afford 3.15 g (82.4% AUC by HPLC). The solid was reslurried a third time in 31 mL of methylene chloride for 2 h, and the mixture was filtered, rinsed with 15 mL of methylene chloride, and then dried under high vacuum to afford 2.80 g (83.2% AUC by HPLC). The solid was reslurried a fourth time in 28 mL of methylene chloride for 2 h, and the mixture was filtered, rinsed with 14 mL of methylene chloride, and then dried under high vacuum to afford 2.48 g (90.3% AUC by HPLC). A final reslurry was performed with anhydrous 2-propanol (25 mL) for 30 min, and the mixture was filtered, washed with 2-propanol (10 mL), and then dried under high vacuum to afford 2.3 g of an amorphous red solid (28% yield). ^{1}H NMR (methanol-d_4) δ 6.75−6.29 (14 H, m), 5.53 (2 H, dd, J = 13.1, 6.3), 2.70−2.36 (8 H, m), 2.09−1.98 (16 H, m), 1.91 (6 H, s), 1.37 (6 H, s), 1.24 (6 H, s); ^{13}C NMR ($CDCl_3$) δ 196.3, 181.1, 180.3, 180.2, 174.9, 174.6, 163.3, 143.8, 141.0, 137.9, 136.5, 135.7, 135.0, 131.9, 129.0, 125.8, 124.3, 72.3, 68.9, 433.7, 38.2, 34.6, 33.4, 33.4, 32.3, 31.8, 30.8, 28.5, 26.5, 25.3, 22.0, 14.3, 12.8, 12.5; DSC one exothermic event at 127.9 °C; Anal. Calcd for [$C_{48}H_{60}O_{10}$ + Na]: 819.4084, ESI MS m/z 819.4045 [$C_{48}H_{60}O_{10}$ + Na]$^+$; HPLC 91.0% AUC, t_R = 7.80 min.

Received for review May 6, 2004.

OP049909I