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Synthesis, characterization, and direct aqueous superoxide anion scavenging of a highly water-dispersible astaxanthin-amino acid conjugate

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Abstract—The aqueous solubility and/or dispersibility of synthetic carotenoid analogs can be improved by varying the chemical structure(s) of the esterified moieties. In the current study, a highly water-dispersible astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) derivative was synthesized by esterification to the amino acid L-lysine, and subsequently converted to the tetrahydrochloride salt. Deep violet, evenly colored aqueous suspensions were obtained with addition of the novel derivative to USP purified water up to a maximum of 181.6 mg/mL. These aqueous suspensions were obtained without the addition of heat, detergents, co-solvents, or other additives. At higher concentrations (above 181.6 mg/mL), the dispersion became turbid and viscous. There was no saturation point up to 181.6 mg/mL. The direct superoxide scavenging ability of the tetrahydrochloride dilysine astaxanthin salt was also evaluated by electron paramagnetic resonance (EPR) spectroscopy in a well-characterized in vitro isolated human neutrophil assay. The novel derivative was an extremely potent (micromolar concentration) aqueous-phase scavenger, with near-complete suppression of the superoxide anion signal (as detected by spin-trap adducts of DEPMPO) achieved at 100 μM. To the authors' knowledge, this novel carotenoid derivative exhibits the greatest aqueous dispersibility yet described for a natural and/or synthetic C40 carotenoid, and as such, will find utility in those applications for which aqueous-phase singlet oxygen quenching and direct radical scavenging are required.

1. Introduction

Reactive oxygen species (ROS) such as the hydroxyl radical ($HO^{\cdot}$), superoxide anion ($O_2^{-\cdot}$), and peroxyl radicals ($ROO^{\cdot}$) are key intermediates in the free radical-mediated damage associated with oxidative stress. Increasing evidence suggests that the pathogenesis of a number of disease states, including cancer and cardiovascular disease, can be attributed at least in part to oxidative stress.[1] Humans have evolved a number of intrinsic (i.e., endogenous) systems to deal with the oxidative stressors that we are subjected to in our daily lives. Enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase, and catalase work in concert to protect against oxidative damage. Diet can also provide a diverse array of antioxidants including vitamins E and C, flavonoids, and carotenoids. Numerous epidemiological studies suggest that diets enriched in these exogenous antioxidants can be associated with a reduced risk for various cancers as well as cardiovascular disease.[2] Studies in model systems have also shown carotenoids to be potent singlet oxygen quenchers,[3] as well as direct radical scavengers.[4] The association of distinct carotenoids with specific tissues in humans and other primates ('tissue tropism') also suggests a targeted evolutionary role for these compounds in protection against various ROS.[5] As a class (>700 naturally occurring carotenoids described to date),[6] the inherent lipophilicity of the vast majority of these compounds is a potential hurdle that must be overcome if they are to be utilized clinically as parenteral and/or aqueous-phase antioxidants.

In the current study, the principles of retrometabolic drug design were utilized to produce a novel soft drug

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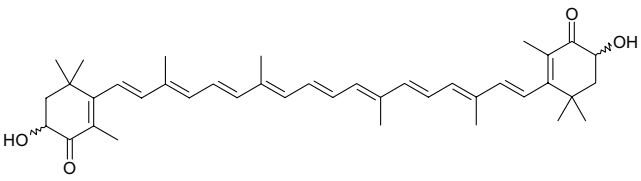


Figure 1. Chemical structure of astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) utilized as a synthetic scaffold for retrometabolic synthesis in the current study.

(i.e., active in the derivatized form) from the parent C40 carotenoid scaffold astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione).[7] Astaxanthin (Fig. 1) possesses key characteristics which make it an ideal starting platform for retrometabolic syntheses, including synthetic handles for conjugation and a benign safety profile for the parent compound.[8] Astaxanthin is also available commercially in bulk as the 'racemic' mixture of stereoisomers [3*S*,3′*S*, *meso*(3*R*,3′*S*), and 3*R*,3′*R* in a 1:2:1 ratio; Buckton Scott, India]. Previously, Hawaii Biotech, Inc. (HBI) successfully synthesized a novel astaxanthin derivative with improved aqueous dispersibility, the disodium salt disuccinate diester (dAST) of astaxanthin (**1**)[21] (Fig. 2). This novel derivative exhibited water 'dispersibility' of approximately 8.6 mg/mL, and was utilized in biophysical,[4,9] pharmacokinetic,[10] and proof-of-principle studies in cardiovascular disease and cancer.[11,12] The aqueous dispersibility achieved with this novel derivative (dAST) represented a significant improvement over the parent compound, as nonesterified, free astaxanthin is insoluble in water.[12,13] The recognition that the dAST formed supramolecular assemblies ('aggregates') in aqueous solution—as reported for other novel carotenoid derivatives—suggested that evaluation of its biophysical behavior in solution was appropriate prior to evaluation in model systems.[9,14,15] We herein report the synthesis, characterization, and aqueous-phase superoxide anion scavenging ability of the tetrahydrochloride salt of the dilysinate ester of astaxanthin (Fig. 2). To our knowledge, the aqueous dispersibility achieved with the current soft drug of astaxanthin (181.6 mg/mL) is the most significant increase in aqueous dispersibility reported for either a naturally occurring or synthetic C40 carotenoid. In addition, potent direct superoxide scavenging ability (at micromolar concentration) is documented for the novel compound.

2. General methods

All reactions were carried out under nitrogen at ambient temperature. Reactions were shielded from light by covering the reaction vessels with aluminum foil. Unless otherwise noted, reagents and solvents were utilized as received from commercial suppliers. Synthetic astaxanthin was obtained as a statistical mixture of stereoisomers [3*S*,3′*S*, *meso*(3*R*,3′*S*), and 3*R*,3′*R* in a 1:2:1 ratio] in all-*trans* (all-*E*) form from Buckton Scott, USA, Inc. TLC was performed on HLF 250 μm plates (Analtech) using reagent grade solvents. Flash chromatography was performed using Natland Int. Corp. silica gel (230–400 mesh).

2.1. Physical methods

HPLC analysis was performed on a Waters system with Symmetry C18 3.5 μm column (4.6 mm × 150 mm); temperature: 25 °C; mobile phase: (A = 0.025% TFA in H_2O; B = 0.025% TFA in MeCN); conditions for **2**: 70% A/30% B (start); linear gradient to 100% B over 12 min, hold for 11 min; linear gradient to 95% B/5% A over

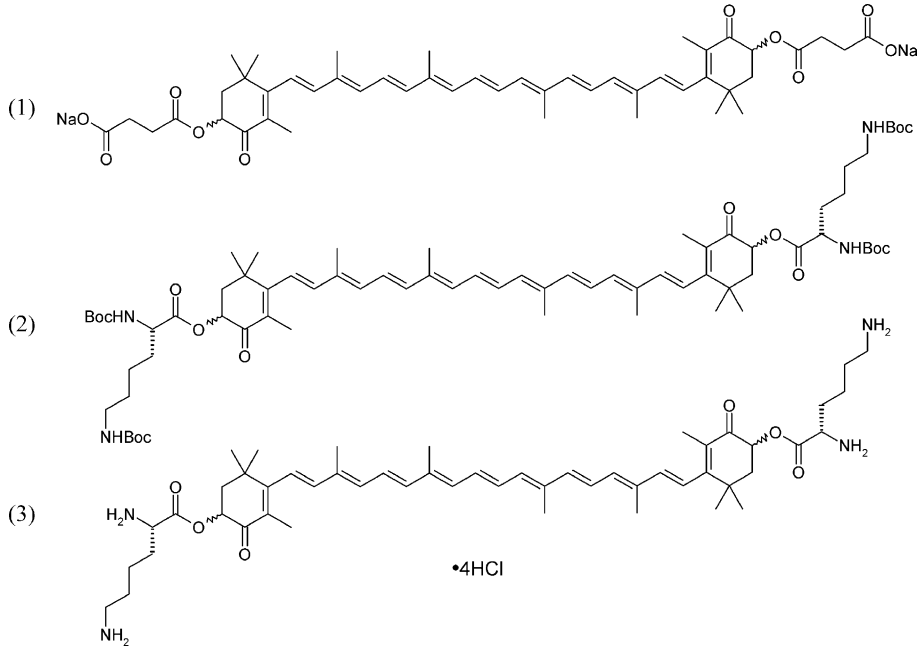


Figure 2. Structures of the disodium salt disuccinate diester of astaxanthin (**1**) (dAST; Cardax™), the Boc-protected dilysinate diester of astaxanthin (**2**), and the tetrahydrochloride salt dilysinate diester of astaxanthin (**3**).

5 min; linear gradient to 95% A/5% B over 2 min; flow rate: 1.0 mL/min; detector wavelength: 474 nm; conditions for **3**: 95% A/5% B (start); linear gradient to 100% B over 12 min, hold for 4 min; linear gradient to 95% B/5% A over 2 min; linear gradient to 95% A/5% B over 4 min; flow rate: 1.0 mL/min; detector wavelength: 474 nm. LC/MS analysis was performed on an Agilent 1100 LC/MSD VL ESI system with Zorbax Eclipse XDB-C18 Rapid Resolution 3.5 μm column (4.6 mm × 75 mm), USUT002736; temperature: 25 °C; mobile phase: (%A = 0.025% TFA in H_2O; %B = 0.025% TFA in MeCN), 70% A/30% B (start); linear gradient to 50% B over 5 min, linear gradient to 98% B over 3 min, hold at 98% B for 17 min; flow rate: 1.0 mL/min; starting pressure: 108 bar; PDA detector 470, 373, 214 nm. LRMS: + mode, ESI. Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity INOVA 500 spectrometer operating at 500.116 MHz (megahertz) for proton and 125.767 MHz for carbon. Electronic absorption spectra were recorded on a Shimadzu BioSpec-1601 UV/visible spectrophotometer. All EPR measurements were performed using a Bruker ER 300 EPR spectrometer operating at X-band with a TM_{110} cavity. The microwave frequency was measured with a Model 575 microwave counter (EIP Microwave, Inc., San Jose, CA).

2.2. Synthesis of the BocLys(Boc)OH ester of astaxanthin (2)

To a mixture of astaxanthin (11.5 g, 19.3 mmol) and $N^{\alpha,\varepsilon}$-bis-(*tert*-butoxycarbonyl)-L-lysine (BocLys(Boc)OH) (20.0 g, 57.7 mmol) in methylene chloride (500 mL) were added 4-dimethylaminopyridine (DMAP) (10.6 g, 86.6 mmol) and 1,3-diisopropylcarbodiimide (DIC) (13.4 g, 86.7 mmol). The round-bottomed flask was covered with aluminum foil and the mixture was stirred at ambient temperature under nitrogen overnight. After 16 h, the reaction was incomplete by HPLC and TLC. An additional 1.5 equiv of DMAP and DIC were added to the reaction and after 2 h, the reaction was complete by HPLC. The mixture was then concentrated to 100 mL and a white solid (1,3-diisopropylurea) was filtered off. The filtrate was flash chromatographed through silica gel (10–50% heptane/EtOAc) to give the desired product as a dark red solid (28.2 g, >100% yield). ^{1}H NMR (DMSO-d_6) δ 7.24 (2H, t, $J = 6.3$ Hz), 6.78 (2H, d, 5.0 Hz), 6.57–6.27 (14H, m), 5.50–5.41 (2H, m), 3.99–3.97 (2H, d, 6.0 Hz), 2.90 (4H, m), 2.03 (4H, m), 2.00 (6H, s), 1.97 (6H, s), 1.82 (6H, s), 1.70–1.55 (4H, m), 1.39–1.33 (36H, m), 1.24–1.13 (8H, m), 1.01–0.99 (6H, m), 0.86–0.83 (6H, m). HPLC: 21.3 min (24.6% AUC); 22.0 min (48.1% AUC); 22.8 min (20.6% AUC). TLC (1:1 heptane/EtOAc: R_f 0.41; R_f 0.5; R_f 0.56. MS+ESI, $m/z = 1276.1$ ($M+Na^+$).

2.3. Synthesis of the tetrahydrochloride salt of the dilysinate ester of astaxanthin (3)

A mixture of diBocLys(Boc) ester of astaxanthin (20.0 g, 16.0 mmol) and HCl in 1,4-dioxane (4.00 M, 400 mL, 1.60 mol, 100 equiv) was stirred at ambient temperature under a nitrogen atmosphere. The round-bottomed flask was covered with aluminum foil and the reaction was stirred for 1 h, at which time the reaction was complete by HPLC. The title compound precipitated and was collected by filtration, washed with ether (3 × 100 mL) and dried (14.7 g, 92%, 91.6% purity by HPLC). A portion (13.5 g) of the crude solid was dissolved in 500 mL of a 1:2 methanol/methylene chloride mixture and stirred under nitrogen. Diethyl ether (168 mL) was then added dropwise and the precipitated solid was collected by filtration to afford the desired product as a dark red solid (8.60 g, 63.7% yield). ^{1}H NMR (DMSO-d_6) δ 8.65 (6H, s), 8.02 (6H, s), 6.78–6.30 (14H, m), 5.59–5.51 (2H, m), 4.08 (2H, m), 2.77 (4H, m), 2.09–2.07 (4H, m), 2.01 (6H, s), 1.97 (6H, s), 1.90–1.86 (4H, m), 1.84 (6H, s), 1.61–1.58 (8H, m), 1.37 (6H, s), 1.22 (6H, s). HPLC: 7.8 min (97.0% AUC). MS+ESI, $m/z = 853.9$ ($M+H^+$), $m/z = 875.8$ ($M+Na^+$).

2.4. UV/visible spectroscopy

For spectroscopic sample preparations, **3** was dissolved in the appropriate solvent to yield a final concentration of approximately 0.01 mM. The solutions were then added to a rectangular cuvette with 1 cm pathlength fitted with a glass stopper. The absorption spectrum was then registered between 250 and 750 nm. All spectra were accumulated one time with a bandwidth of 1.0 nm at a scan speed of 370 nm/min. Spectra were obtained at baseline (immediately after solvation; time zero) up to and including 72 h post-solvation for some samples (see Figs. 4 and 5).

2.5. Determination of aqueous solubility/dispersibility

Portions of **3** were added to 2 mL of USP purified water in 50–160 mg increments. After each addition of solute, the sample was sonicated for 5 min and then centrifuged. The sample was visually inspected between additions to determine if any undissolved material remained. After a total of 908.4 mg of **3** were added, residual undissolved solid was detected. A 1.4 mL aliquot of the resulting solution was then diluted to a final volume of 3.5 mL (181.6 mg/mL) and filtered through a 0.45 μm polyvinylidene fluoride (PVDF) membrane. Solid was not detected upon the membrane or within the resulting solution.

2.6. Leukocyte purification and preparation

Leukocytes were prepared essentially as described.[4] Briefly, human polymorphonuclear leukocytes (PMNs) were isolated from freshly sampled venous blood of a single volunteer (S.F.L.) by Percoll density gradient centrifugation, which yielded PMNs with a purity of >95%. Each 10 mL of whole blood was mixed with 0.8 mL of 0.1 M EDTA and 25 mL of saline. The diluted blood was layered over 9 mL of Percoll at a specific density of 1.080 g/mL. After centrifugation at 400*g* for 20 min at 20 °C, the plasma, mononuclear cell, and

Percoll layers were removed. Erythrocytes were lysed by addition of 18 mL of ice-cold water for 30 s, followed by 2 mL of 10× PIPES buffer (25 mM PIPES, 110 mM NaCl, and 5 mM KCl, titrated to pH 7.4 with NaOH). Cells were pelleted at 4 °C, the supernatant was decanted, and the procedure was repeated. After the second hypotonic lysis, cells were washed twice with PAG buffer (PIPES buffer containing 0.003% human serum albumin and 0.1% glucose). Afterward, PMNs were counted by light microscopy on a hemocytometer. The final pellet was then suspended in PAG-CM buffer (PAG buffer with 1 mM $CaCl_2$ and 1 mM $MgCl_2$).

2.7. EPR measurements

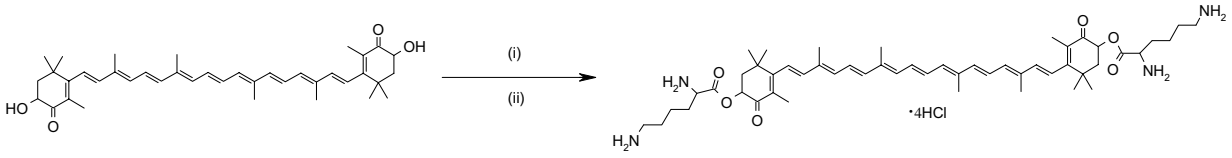
To measure superoxide anion ($O_2^{\cdot-}$) generation from phorbol-ester (PMA)-stimulated PMNs, EPR spin-trapping studies were performed using the spin trap DEPMPO (Oxis, Portland, OR) at 10 mM. PMNs (1×10^6) were stimulated with PMA (1 ng/mL) and loaded into capillary tubes for EPR measurements. To determine the radical scavenging ability of **3** in aqueous formulation, PMNs were pre-incubated for 5 min with test compound, followed by PMA stimulation as previously described. The instrument settings used in the spin-trapping experiments were as follows: modulation amplitude, 0.32 G; time constant, 0.16 s; scan time, 60 s; modulation frequency, 100 kHz; microwave power, 20 mW; and microwave frequency, 9.76 GHz. The samples were placed in a quartz EPR flat cell, and spectra were recorded. The component signals in the spectra were identified and quantified as reported.[16]

2.8. Synthesis

Facile preparation of the ester-linked dilysine conjugate of astaxanthin was accomplished in two steps using standard carbodiimide methods as illustrated in Scheme 1. In a typical reaction, 4.5 equiv each of DMAP and DIC were added to a stirred methylene chloride solution of 4.5 equiv of di-Boc-protected lysine and one (1) equivalent of astaxanthin. Reactions were shielded from light to minimize *Z* isomerization and decomposition. Purification of **2** (Fig. 2) was accomplished by flash chromatography on silica gel. The three diastereomeric intermediates (Fig. 3) of **2** could be separated using an ethyl acetate/heptane gradient on normal silica gel. NMR and MS analyses of the individual compounds confirmed their identities as diastereomers. Separation of the diastereomers was not observed using methylene chloride/methanol mixtures as eluent. Removal of the *N*-Boc protecting groups was initially attempted using TFA/methylene chloride mixtures but only decomposition products were isolated. Methanolic and ethanolic solutions of HCl resulted in hydrolysis of the ester linkages and recovery of a mixture of di- and mono-lysine astaxanthin conjugates along with free astaxanthin. Deprotection was ultimately accomplished using anhydrous HCl in dioxane. The tetrahydrochloride salt **3** precipitated from solution at >90% purity. Dissolution of the crude material in a methanol/methylene chloride mixture and precipitation with diethyl ether yielded analytically pure **3**.

2.9. UV/vis spectral properties in organic and aqueous solvents

The absorption spectra of **3** in ethanol (EtOH), dimethyl sulfoxide (DMSO), and water are shown in Figure 4. Derivatization of astaxanthin, as expected, had minimal affects on the polyene chromophore (and thus the λ_{max}). The UV/vis spectrum of astaxanthin in ethanol ($\lambda_{max} = 478$ nm) is virtually identical to the spectrum of **3** in ethanol ($\lambda_{max} = 484$ nm).[17] Previous studies of



Scheme 1. Synthesis of the tetrahydrochloride salt of the dilysinate diester of astaxanthin (**3**). Reagents and conditions: (i) BocLys(Boc)OH, DIC, DMAP, methylene chloride; (ii) HCl, dioxane.

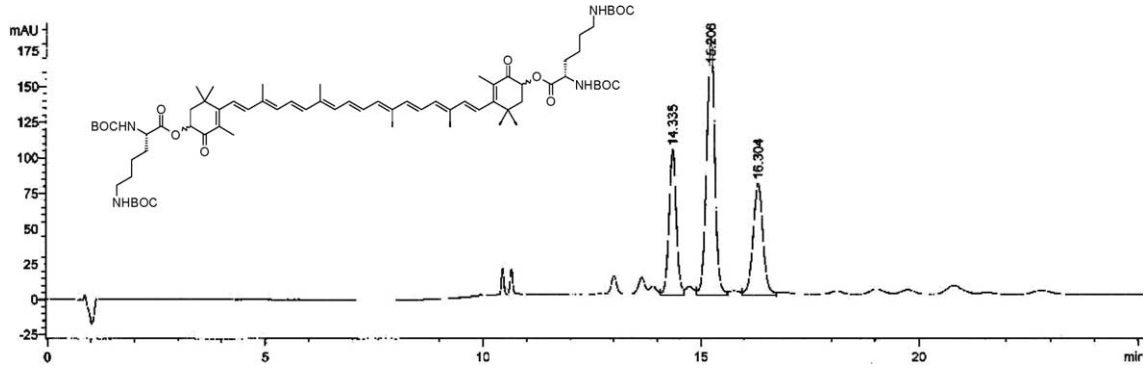


Figure 3. Time course of LC separation of tetra Boc-protected dilysinate diester astaxanthin (**2**) diastereomers.

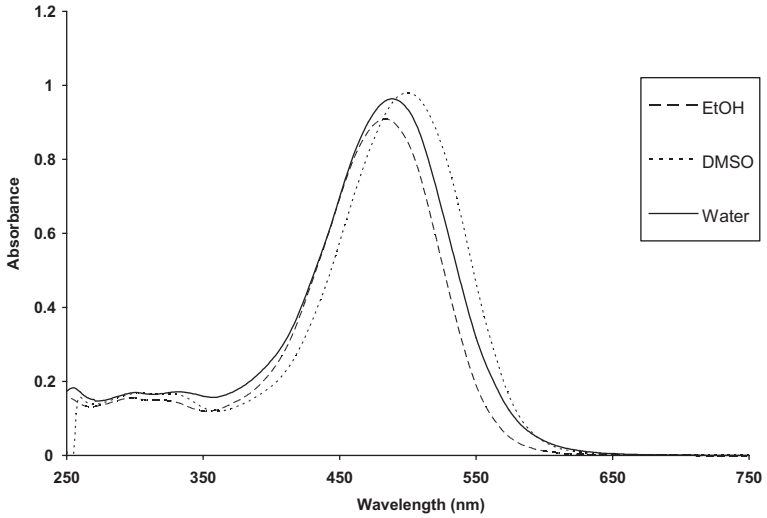


Figure 4. UV/vis absorption spectra of **3** in ethanol ($\lambda_{max} = 484$ nm), DMSO ($\lambda_{max} = 500$ nm), and water ($\lambda_{max} = 488$ nm). Spectra were obtained at time zero.

astaxanthin in 10% acetone–water solutions demonstrated that aggregate formation occurred over the course of several hours.[18] Time series of the UV/vis spectra of solutions of **3** in EtOH, DMSO, and water were observed to determine if aggregates formed in a time-dependent fashion. In EtOH over 72 h a maximum blue-shift of 10 nm ($\lambda_{max} = 484$ nm to $\lambda_{max} = 474$ nm) and a 1% decrease in absorbance intensity were observed. Similarly, in DMSO over 72 h a maximum blue-shift of 4 nm ($\lambda_{max} = 500$ nm to $\lambda_{max} = 496$ nm) and a 6% decrease in absorbance intensity was observed. However, the most dramatic changes were observed in aqueous formulation. In water over a 24 h time period a maximum blue-shift of 28 nm ($\lambda_{max} = 488$ nm to $\lambda_{max} = 460$ nm) and a 54% decrease in absorbance intensity was observed (Fig. 5). These spectral changes were highly suggestive of supramolecular assembly in aqueous formulation, with formation of H-type (or 'card-pack') aggregates in aqueous solution.[9,19]

2.10. Direct superoxide scavenging by EPR spectroscopy

Dose-dependent increases in superoxide scavenging were observed as the concentration of **3** was increased in the test assay (Table 1; Fig. 6). Significant scavenging was seen at micromolar concentrations; at 10 μM, nearly 47% of the superoxide signal (as detected by the DEPMPO spin-trap adduct) was eliminated. At 100 μM, near-complete suppression of superoxide signal was achieved (Table 1; Fig. 6). This scavenging potency approaches that observed previously with superoxide dismutase mimetics (e.g. from Metaphore, Inc., Atlanta, GA),[4] which are capable of complete elimination of

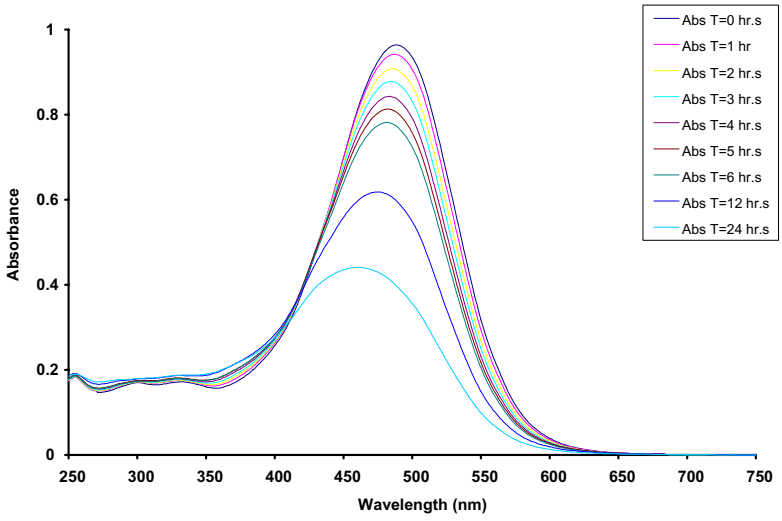


Figure 5. UV/vis absorption spectra of **3** in water. The λ_{max} blue-shifted from 488 to 460 nm over the course of 24 h, and the spectra became hypochromic (i.e., decreased in absorbance intensity), indicating time-dependent supramolecular assembly (aggregation). Supramolecular assembly is believed to be of the H-('card-pack') type. Addition of less polar solvent (e.g., ethanol) can produce a solution of monomers (a molecular solution; data not shown).

Table 1. Descriptive statistics for mean percent (%) inhibition of aqueous superoxide anion production for increasing concentrations of aqueous formulations of the tetrahydrochloride salt dilysinate astaxanthin derivative tested in the current study

Concentration (μM)	*N*	Mean percent (%) inhibition	SD	SEM	Min	Max	Range
1.0	3	10.3	4.6	2.7	5	13	8
10	3	46.7	11.8	6.8	33	54	21
50	3	86.0	1.7	1.0	84	87	3
100	3	95.7	1.2	0.7	95	97	2

Percent inhibition in control samples without addition of test compound were set at 0% inhibition. A sample size of 3 was evaluated at each concentration of test compound. Dose-dependent increases in direct superoxide scavenging were observed for the tetrahydrochloride salt dilysinate astaxanthin derivative in aqueous formulation. At 100 μM, near-complete mean inhibition (95.7%) of superoxide signal was seen. *N* = sample size; SD = standard deviation; SEM = standard error of the mean.

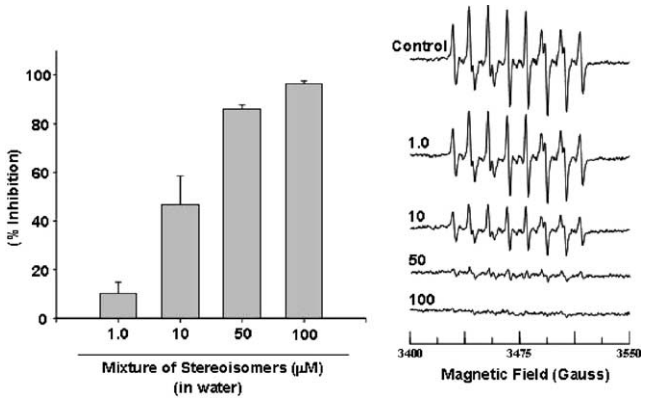


Figure 6. Mean percent (%) inhibition of superoxide anion signal as detected by DEPMPO spin-trap adducts by the mixture of stereoisomers of the tetrahydrochloride salt dilysinate astaxanthin derivative (in aqueous formulation). The mixture of stereoisomers contains 3*S*,3′*S*, *meso*(3*R*,3′*S*), and 3*R*,3*R* in a 1:2:1 ratio. As the concentration of the derivative increases in solution, mean % inhibition increases in a dose-dependent manner. At 100 μM, near-complete inhibition of superoxide anion signal is seen (95.7% inhibition).

superoxide signal in this test system at 10 μM. Other novel carotenoid derivatives tested in this system [disodium disuccinate astaxanthin (Cardax™) and C30 carotenoid-phosphotidylcholine conjugates][4,20] required mM concentrations for near-complete suppression of superoxide signal, as well as addition of ethanol co-solvent to maintain the stock solutions in monomeric form prior to introduction into the assay. *Significantly, potent micromolar scavenging by **3** was achieved without the addition of co-solvent.* It is postulated that supramolecular assembly—in which the size and stability of the aggregates is enhanced as the ionic strength of the aqueous solution increases—can contribute significantly to the differences in aqueous-phase scavenging observed among these derivatives.[22] This complicated process requires additional evaluation to elucidate the biophysical mechanism(s), which may be responsible for this phenomenon.

A facile, two-step synthesis of the tetrahydrochloride salt of dilysinate astaxanthin was accomplished, producing a soft drug with increased utility as a potential aqueous-phase and/or in vivo therapeutic antioxidant and radical scavenger. *Aqueous dispersibility (>181.6 mg/mL) was achieved without addition of heat, detergents, co-solvents, or other additives; to the authors' knowledge, this represents the greatest improvement in aqueous dispersibility yet reported for a natural or synthetic C40 carotenoid.* UV/visible spectral properties in solution were consistent with the production of H-type, or 'card-pack', aggregates; this phenomenon, described previously for carotenoid derivatives, can potentially limit the interaction of the compound with radical species in solution. Potent direct superoxide scavenging was observed, with near-complete suppression of superoxide signal achieved at 100 μM concentration. This compound may find utility in those biological and chemical applications in which aqueous-phase radical scavenging is desired.

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References and notes

1. Cross, C. E.; Halliwell, B.; Borish, E. T.; Pryor, W. A.; Ames, B. N.; Saul, R. L.; McCord, J. M.; Harman, D. *Ann. Int. Med.* **1987**, *107*, 526–545.
2. Tapiero, H.; Townsend, D. M.; Tew, K. D. *Biomed. Pharmacother.* **2004**, *58*, 100–110.
3. Miki, W. *Pure Appl. Chem.* **1991**, *63*, 141–146.
4. Cardounel, A. J.; Dumitrescu, C.; Zweier, J. L.; Lockwood, S. F. *Biochem. Biophys. Res. Commun.* **2003**, *307*, 704–712.
5. Khachik, F.; Bernstein, P. S.; Garland, D. L. *Invest. Ophth. Vis. Sci.* **1997**, *38*, 1802–1811.
6. *Carotenoids Handbook*; Britton, G.; Liaaen-Jensen, S.; Pfander, H.; Mercadante, A. Z.; Egeland, E. S., Eds.; Birkhäuser: Basel, 2004.
7. Bodor, N.; Buchwald, P. *Mol. Biotechnol.* **2004**, *26*, 123–132.
8. Spiller, G. A.; Dewell, A. *J. Med. Food* **2003**, *6*, 51–56.
9. Zsila, F.; Simonyi, M.; Lockwood, S. F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4093–4100.
10. Showalter, L. A.; Weinman, S. A.; Østerlie, M.; Lockwood, S. F. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.* **2004**, *137*, 227–236.

11. Gross, G. J.; Lockwood, S. F. *Life Sci.* **2004**, *75*, 215–224.
12. Hix, L. M.; Lockwood, S. F.; Bertram, J. S. *Cancer Lett.* **2004**, in press.
13. *Carotenoids: Spectroscopy*; Britton, G.; Liaaen-Jensen, S.; Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. Ia, p 82.
14. Foss, B. J.; Naess, S. N.; Sliwka, H. R.; Partali, V. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 5237–5240.
15. Simonyi, M.; Bikadi, Z.; Zsila, F.; Deli, J. *Chirality* **2003**, *15*, 680–698.
16. Lee, C.; Miura, K.; Liu, X.; Zweier, J. L. *J. Biol. Chem.* **2000**, *275*, 38965–38972.
17. *Carotenoids: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. Ib, p 48.
18. Mori, Y.; Yamano, K.; Hashimoto, H. *Chem. Phys. Lett.* **1996**, *254*, 84–88.
19. Bikádi, Z.; Zsila, F.; Deli, J.; Mady, G.; Simonyi, M. *Enantiomer* **2002**, *7*, 67–76.
20. Foss, B. J.; Sliwka, H. R.; Partali, V.; Cardounel, A. J.; Zweier, J. L.; Lockwood, S. F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2807–2812.
21. Frey et al., submitted for publication.
22. Foss et al., submitted for publication.