Towards Carotenoid Dendrimers: Carotenoid Diesters and Triesters with Aromatic Cores

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For the evaluation of the synthesis of dendritic esters from carotenoids, various carotenols or their succinates were reacted with bi- and trifunctional aromatic acids and alcohols. Several methods were tested, from which the *Steglich* esterification was found to give the best yields. Apocarotenoids showed higher reactivity in most of the reactions and served as model compounds for C_{40} carotenoids and their derivatives. The synthesized triesters can be regarded as first generation dendrimers.

Introduction. – Carotenoids are naturally occuring antioxidants having various biological effects including anti-cancer and cardioprotective, and have been in the focus of food biochemists recently [1]. Trimers or first generation dendrimer-like structures of these compounds have not been synthesized until now, although they can have interesting physicochemical properties, especially with respect to their carotenoid nature. These compounds may have enhanced biological activity [2] and could be used for supramolecular aggregation studies [3]. Synthesis of carotenoid esters is not unknown in the literature, there are examples including enzymatic reactions with carotenoid acids [4], reactions between carotenoid acids and carotenols [5], and in our previous article, the syntheses of some carotenoid homo- and heterodimers were described [6]. The fine tuning of this kind of reactions were of a great help for the reactions shown in our present article.

Results. – Herein, we describe the synthesis of some carotenoid di- and triesters with aromatic core molecules. The apocarotenoids retinol and 8′-*β*-apocarotenol (*Fig. 1*), being not too expensive and easily accessible, were chosen as model compounds for other carotenoids. The simplest method for esterification would be the reaction of commercially available aromatic di- or triacyl chlorides with the corresponding carotenol (*Fig. 1*) in excess. Many experiments have been performed with different bases and solvents, but none of them gave the desired product in acceptable yields, most of the pigments were decomposed under the reaction conditions.

Activated carboxylic acid derivatives (*e.g.*, di- or triimidazoles) proved to be unreactive, which led us to investigate the use of the *N*,*N*′-dicyclohexylcarbodiimide (DCC)/4-(dimethylamino)pyridine (= *N*,*N*-dimethylpyridin-4-amine; DMAP) coupling used before for diesters [6][7]. Interestingly, aromatic di- or tricarboxylic acids proved to be rather unreactive, whereas non-aromatic acids like benzenediyl- or

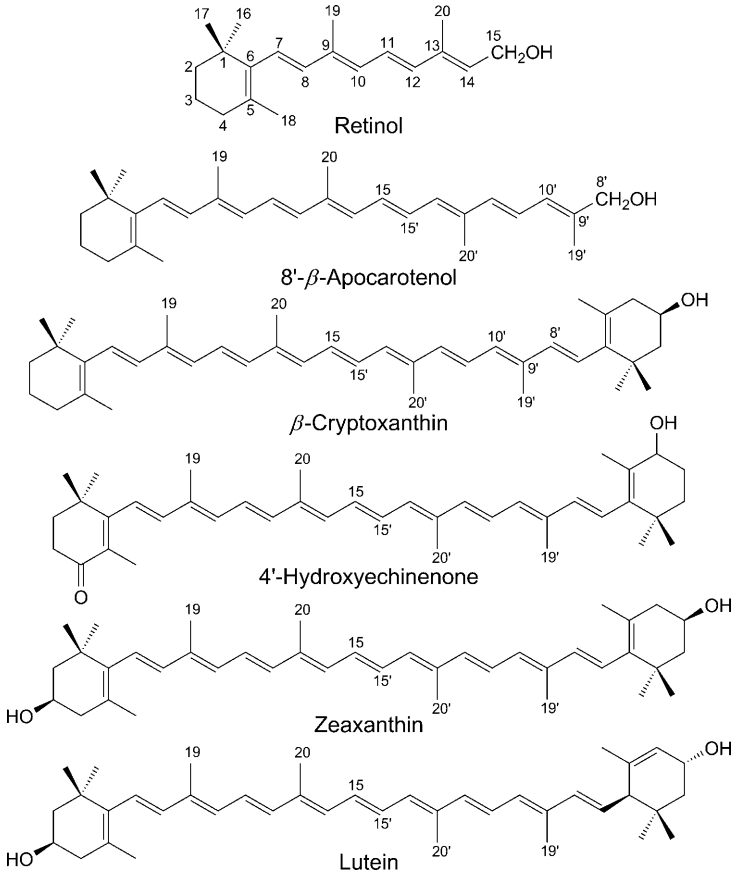
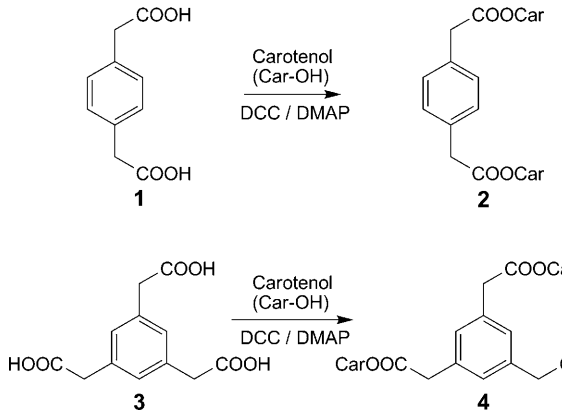


Fig. 1. *Structures of the carotenoid starting materials*

triylacetic acids gave promising results (*Scheme 1*). Most probably, both stereoelectronic and steric factors impede the reactions with aromatic carboxylic acids. Unfortunately, C_{40} carotenoids bearing secondary OH groups (*i.e.*, β-crytoxanthin, 4′-hydroxyechinenone, zea xanthin, and lutein) gave poor yields or none of the desired products, which may be a consequence of steric hindrance. The utilization of some kind of spacer for the secondary OH groups (*e.g.*, zeaxanthin) could bring the required result. If the alcohol to be esterified is cheap, it can be used in large excess to complete the reaction. Unfortunately, the carotenoid alcohols are much more valuable than the core molecules and could be applied only in a 20–30% molar excess. This also contributed to the relatively low yields of the reactions.

Another approach for the synthesis of such kind of trimers is reverse functionalization, *i.e.*, reacting carotenoid succinates with polyphenols. Once more, these attempts were unsuccessful, but reactions with the corresponding benzene-1,4-diyldimethanol (**5**) provided the products in acceptable yields, although only with retinol and 8′-β-apocarotenol succinates (*Scheme 2*). Surprisingly, the reaction of triol **7**

Scheme 1. *Synthesis of Di- and Tricarotenoid Esters of Benzenediyl- and Benzenetriylacetic Acid*

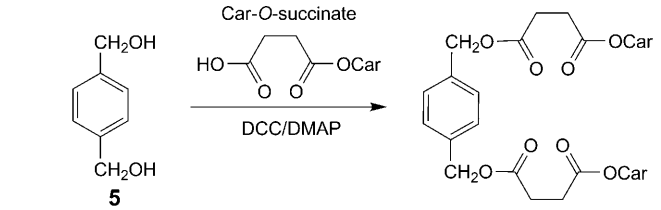


	Carotenol used	Yield [%]
2a	Retinol	72
2b	8'-β-Apocarotenol	45
2c	β-Cryptoxanthin	14
2d	4'-Hydroxyechinenone	–
2e	Zeaxanthin	–
2e	Lutein	–

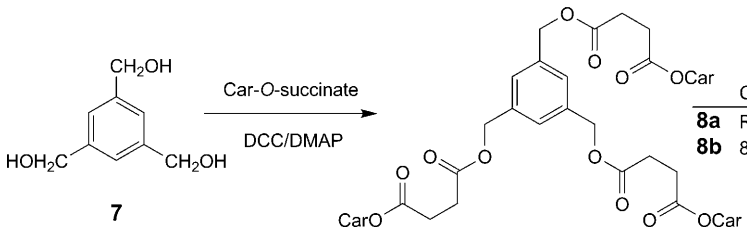
	Carotenol used	Yield [%]
4a	Retinol	76
4b	8'-β-Apocarotenol	30
4c	β-Cryptoxanthin	–
4d	4'-Hydroxyechinenone	–
4e	Zeaxanthin	–
4e	Lutein	–

(which was synthesized from benzene-1,3,5-tricarboxylic acid in two steps [8]) with carotenoid succinates gave low yields under identical conditions. In the case of retinol succinate, the yield was acceptable (**8a**), but with 8′-β-apocarotenol succinate, only trace amounts of the trimer (based on MS and NMR) could be detected even after several trials (**8b**).

Scheme 2. *Synthesis of Carotenoid Di- and Triesters with Succinic Acid as a Spacer*



	Carotenol used	Yield [%]
6a	Retinol	45
6b	8'-β-Apocarotenol	32
6c	β-Cryptoxanthin	<3
6d	4'-Hydroxyechinenone	–
6e	Zeaxanthin	–
6e	Lutein	–



	Carotenol used	Yield [%]
8a	Retinol	25
8b	8'-β-Apocarotenol	<5

In both approaches, *Mitsunobu*-type esterification was also tested, but the results confirmed that DCC coupling is superior in yields and reactivity. The structures of the carotenoid di- and triesters were proven on the basis of NMR, MALDI-TOF, and UV/VIS data. The purity of the compounds (> 95%) was determined by HPLC.

Conclusions. – Our results confirm that even the simplest reaction such as esterification can have unusual outcome among carotenoids, and carotenoids with close structural resemblance can react rather differently under the same conditions. The

synthesized tricarotenoid esters can be regarded as first generation dendrimers of carotenoids, although their size exceeds to a great extent the usual size of such kind of molecules (*Fig. 2*). The lack of reactivity is still problematic in the cases of bifunctional carotenoids (*e.g.*, zeaxanthin), most probably for steric reasons, although succinates bearing a relatively large spacer on the molecule proved also to be unreactive.

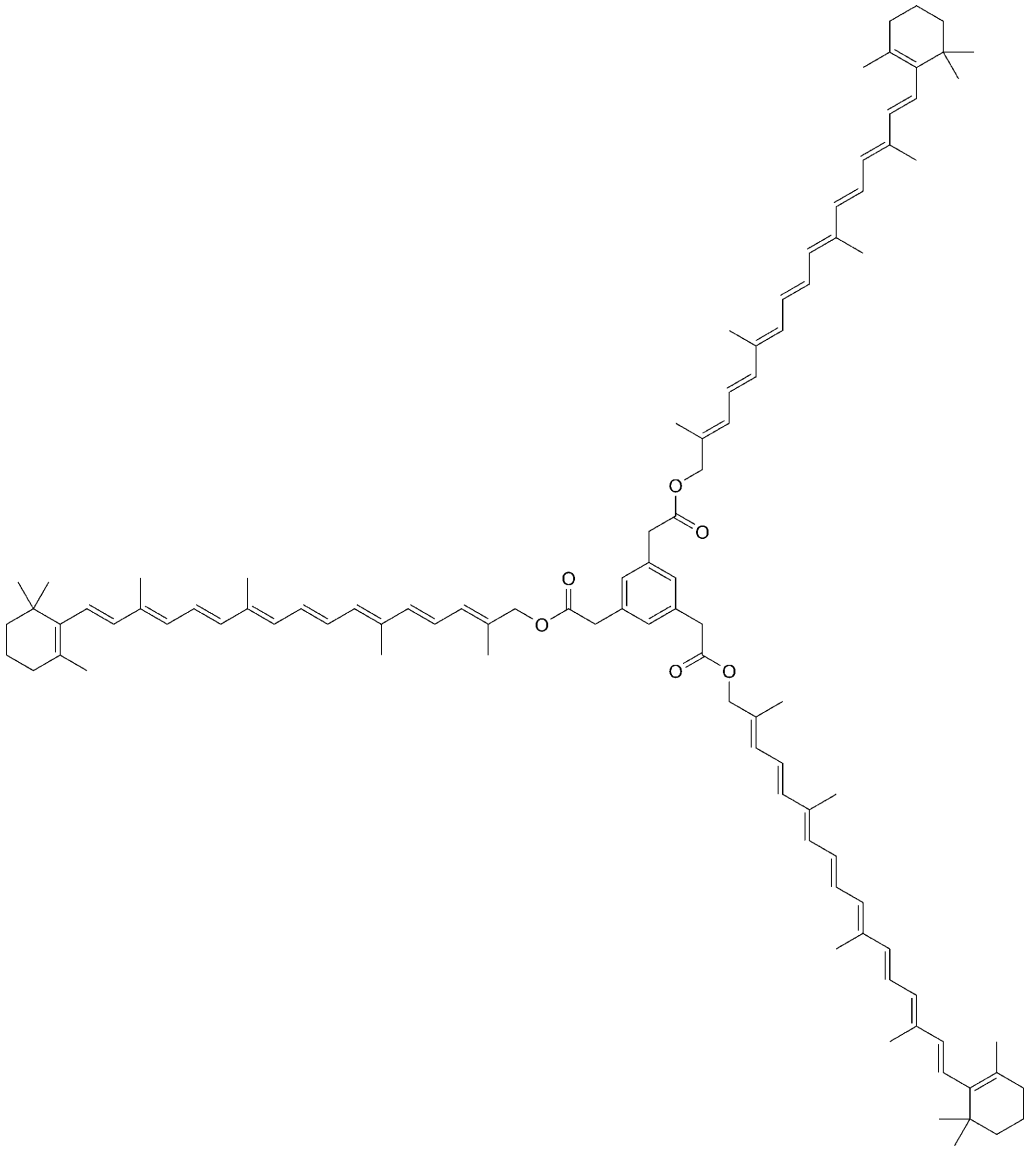


Fig. 2. *Fan-like structure of carotenoid triester* **4b**

Preliminary test results with liver cell lines showed excellent antioxidant activity for the new compounds in H_2O_2-induced oxidative stress assays.

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Experimental Part

General. All solvents were of analytical grade, dry solvents were stored over 4 Å molecular sieves. TLC and prep. TLC: *DC-Kieselgel 60 F_{254}* (*Merck*). HPLC: *Dionex P580* gradient pump, *Dionex PDA-100* detector; eluents: 12% H_2O/MeOH (*A*), MeOH (*B*), 50% acetone/MeOH (*C*), flow rate: 1.250 ml/min; column: *Chromsyl RP-18* endcapped 6 μm, detection at 450 nm; gradient program: 100% *A* 2 min, 80% *A* and 20% *B* 6 min, 50% *A* and 50% *B* 10 min, 100% *B* 7 min, 100% *C* 8 min, 100% *B* 7 min, 100% *A* 5 min. UV-VIS: *Jasco V-530* spectrophotometer; λ_{max} [nm] (ε_{mol} values); ref. values: retinol, 326 (48324); 8′-β-apocarotenol, 425 (143540); β-cryptoxanthin, 449 (131900). NMR: *Varian Unity Inova* (400/100 MHz $^1H/^{13}C$), solvent: $CDCl_3$; δ [ppm] and *J* [Hz][1]). MS: *Bruker Daltonics Autoflex II* MALDI, positive-ion mode (337 nm; N_2 laser, max. freq.: 50 Hz; laser intensity 20–30%), external calibration.

Source of Carotenoids. Carotenoids were isolated from natural sources or purchased from *Sigma-Aldrich Co.* 8′-β-Apocarotenol was synthesized from 8′-β-apocarotenal and 4′-hydroxyechinenone from canthaxanthin by $NaBH_4$ reduction.

General Procedure for the Synthesis of Di- and Tricarotenoid Esters. To a dry CH_2Cl_2 soln. (20 mg/ml) of carotenol or carotenoid succinate (1 equiv.), the corresponding di- or triacid (0.4 equiv./0.25 equiv., resp.) or di- or triol (0.4 equiv./0.25 equiv., resp.) was added under N_2. To the mixture, 1.5 equiv. of DMAP and 1.5 equiv. of DCC were added, and the mixture was stirred overnight at r.t. After completion of the reaction (TLC), the mixture was diluted with Et_2O and washed with 5% citric acid soln. and sat. NaCl soln., and finally dried with $MgSO_4$, and evaporated at 35°. The crude product was purified by prep. TLC (hexane/acetone 8:2 or 7:3). The di- and tricarotenoid esters were found in the apolar region of the chromatogram. All products are red solids. The synthesized compounds have >95% purity according to HPLC, and the UV/VIS spectra confirm the presence of the starting carotenoids.

Bis(retinyl)benzene-1,4-diacetate (= *Bis[(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl] 2,2′-Benzene-1,4-diyldiacetate*; **2a**). UV/VIS (hexane): (225), 327 (92521). ^{1}H-NMR: 1.02 (*s*, 2 Me(16), 2 Me(17)); 1.25–2.01 (*m*, 2 CH_2(2), 2 CH_2(3), 2 CH_2(4), 2 Me(18), 2 Me(19), 2 Me(20)); 3.60 (*s*, 2 CH_2); 4.74 (*d*, *J*(14,15) = 7.2, 2 CH_2(15)); 5.59 (*t*, *J*(14,15) = 7.2, 2 H–C(14)); 6.07–6.67 (*m*, 10 olefin. H); 7.23 (*s*, 4 arom. H). ^{13}C-NMR: 12.7 (C(19), C(20)); 19.2 (C(3)); 21.7 (C(18)); 28.9, 30.1 (C(16), C(17)); 33.0, 34.9 (C(4), C(1)); 39.6, 40.9 (C(2), CH_2); 61.7 (C(15)); 124.2, 125.8, 129.9, 128.3, 129.3, 129.4, 129.9, 132.8, 135.7, 136.6, 137.5, 137.8, 139.2 (polyene chain); 171.4 (2 C=O). MALDI-MS: 730 (M^+).

Bis(8′-β-apocarotenyl)benzene-1,4-diacetate (= *Bis[(2E,4E,6E,8E,10E,12E,14E,16E)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohex-1-en-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaen-1-yl] 2,2′-Benzene-1,4-diyldiacetate*; **2b**). UV/VIS (hexane): (224), 402, 425 (252340), 450. ^{1}H-NMR: 1.03–1.97 (*m*, 2 CH_2(2), 2 CH_2(3), 2 CH_2(4), 2 Me(17), 2 Me(18), 2 Me(16), 2 Me(19), 2 Me(19′), 2 Me(20), 2 Me(20′)); 3.60–3.68 (*m*, 2 CH_2); 4.57 (*s*, 2 CH_2(8′)); 6.15–6.62 (*m*, 24 olefin. H); 7.23 (*s*, 4 arom. H). ^{13}C-NMR: 12.6–12.8 (C(19), C(19′), C(20), C(20′)); 19.2 (C(3)); 21.6 (C(18)); 29.1, 30.2 (C(16), C(17)); 33.1, 34.3 (C(1), C(4)); 39.6, 41.0 (CH_2, C(2)); 68.1 (C(8′)); 123.0–138.2 (polyene chain); 171.2 (2 C=O). MALDI-MS: 994 (M^+).

Bis(β-cryptoxanthinyl)benzene-1,4-diacetate (= *Di-(3S)-β,β-caroten-3-yl 2,2′-Benzene-1,4-diyldiacetate*; **2c**). UV/VIS (hexane): (226), 425, 450 (225200), 476. ^{1}H-NMR: 0.85–2.11 (*m*, 78 H); 2.30–2.36 (*m*, CH_2(4)); 3.60–3.65 (*m*, 2 CH_2); 5.06–5.10 (*m*, 2 H–C(3′)); 6.13–6.84 (*m*, 28 olefin. H); 7.21 (*s*, 4 arom. H). MALDI-MS: 1263 (M^+).

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[1]) Carotenoid numbering is used in all cases, see *Fig. 1*.

Tris(retinyl)benzene-1,3,5-triacetate (= *Tris[(2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl] 2,2′,2″-Benzene-1,3,5-triyltriacetate*; **4a**). UV/VIS (hexane): (225), 326 (151610). ^{1}H-NMR: 1.02–2.15 (*m*, 3 CH_2(2), 3 CH_2(3), 3 CH_2(4), 3 Me(16), 3 Me(17), 3 Me(18), 3 Me(19), 3 Me(20)); 3.58 (*s*, 3 CH_2); 4.73 (*d*, *J*(14,15) = 6.4, 3 CH_2(15)); 5.59 (*t*, *J*(14,15) = 6.4, 3 H–C(14)); 6.06–6.65 (2*m*, 18 olefin. H); 7.05 (*s*, 3 arom. H). ^{13}C-NMR: 12.7–12.8 (C(19), C(20)); 19.3 (C(3)); 21.7 (C(18)); 29.0, 30.1 (C(16), C(17)); 33.0, 34.3 (C(4), C(1)); 39.6, 41.0 (C(2), CH_2); 61.8 (C(15)); 124.3, 125.8, 126.9, 129.0, 129.3, 129.8, 129.9, 128.3, 129.3, 129.4, 129.9, 134.5, 135.8, 136.6, 137.6, 137.8, 139.2 (polyene chain); 171.4 (3 C=O). MALDI-MS: 1057 (M^+).

Tris(8′-β-apocarotenyl)benzene-1,3,5-triacetate (= *Tris[(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*,16*E*)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohex-1-en-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaen-1-yl] 2,2′,2″-Benzene-1,3,5-triyltriacetate*; **4b**). UV/VIS (hexane): (227), 402, 426 (382218), 451. ^{1}H-NMR: 1.03–1.97 (*m*, 3 CH_2(2), 3 CH_2(3), 3 CH_2(4), 3 Me(17), 3 Me(18), 3 Me(16), 3 Me(19), 3 Me(19′), 3 Me(20), 3 Me(20′)); 3.64 (*s*, 3 CH_2); 4.56 (*s*, 3 CH_2(8′)); 6.15–6.62 (*m*, 36 olefin. H); 7.16 (*s*, 3 arom. H). ^{13}C-NMR: 12.8 (C(19), C(19′), C(20), C(20′)); 19.3 (C(3)); 21.7 (C(18)); 29.1, 30.3 (C(16), C(17)); 33.9 (C(4)); 34.3 (C(1)); 39.6, 41.2 (CH_2, C(2)); 70.3 (C(8′)); 123.3–130.8; 131.6, 132.2, 132.9, 134.6, 135.7–138.4 (polyene chain); 171.0 (3 C=O). MALDI-MS: 1454 (M^+).

1,4-Di-({3-[(retinyloxy)carbonyl]propionyloxy}methyl)benzene (= *1,1′-(Benzene-1,4-diyldimethanediyl) 4,4′-Bis[(2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl] Dibutanedioate*; **6a**). UV/VIS (hexane): (227), 325 (88143). ^{1}H-NMR: 1.02–2.02 (*m*, 2 CH_2(2), 2 CH_2(3), 2 CH_2(4), 2 Me(16), 2 Me(17), 2 Me(18), 2 Me(19), 2 Me(20)); 2.62–2.70 (*m*, 2 $-CH_2CH_2-$); 4.74 (*d*, *J*(14,15) = 7.6, 2 CH_2(15)); 5.13 (*s*, 2 CH_2); 5.58 (*t*, *J*(14,15) = 7.6, 2 H–C(14)); 6.10–6.65 (*m*, 12 olefin. H); 7.35 (*s*, 4 arom. H). ^{13}C-NMR: 12.9 (C(19), C(20)); 19.3 (C(3)); 21.8 (C(18)); 25.6, 28.9–29.1, 29.8 (C(16), C(17), $-CH_2CH_2-$); 33.1, 34.3 (C(4), C(1)); 39.6 (C(2)); 61.6 (C(15)); 66.2 (CH_2); 124.2, 125.9, 129.9, 127.0, 128.3, 128.4–128.6, 129.4, 129.9, 135.7, 135.9, 136.7, 137.6, 137.8, 139.3 (polyene chain); 172.2 (2 C=O). MALDI-MS: 875 (M^+).

1,4-Di-[(3-{[(8′-β-apocarotenyl)oxy]carbonyl}propionyloxy)methyl]benzene (= *1,1′-(Benzene-1,4-diyldimethanediyl) 4,4′-Bis[(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*,16*E*)-2,6,11,15-tetramethyl-17-(2,6,6-trimethylcyclohex-1-en-1-yl)heptadeca-2,4,6,8,10,12,14,16-octaen-1-yl] Dibutanedioate*; **6b**). UV/VIS (hexane): (225), 403, 425 (226152), 450. ^{1}H-NMR: 1.02 (*s*, 12 H, 2 Me(16), 2 Me(17)); 1.23–2.04 (*m*, 2 CH_2(2), 2 CH_2(3), 2 CH_2(4), 2 Me(18), 2 Me(19), 2 Me(19′), 2 Me(20), 2 Me(20′)); 2.65–2.74 (*m*, 2 $-CH_2CH_2-$); 4.57 (*s*, 2 CH_2(8′)); 5.14 (*s*, 2 CH_2); 6.14–6.64 (*m*, 24 olefin. H); 7.35 (*s*, 4 arom. H). ^{13}C-NMR: 12.7, 12.8, 14.7 (C(19), C(19′), C(20), C(20′)), 19.3 (C(3)), 21.7 (C(18)), 24.9 ($-CH_2CH_2-$), 28.9–29.1 (C(16), C(17)), 33.1 (C(4)), 34.3 (C(1)), 39.6 (C(2)), 66.2 (CH_2) 70.4 (C(8′)), 123.3–129.6, 130.4, 130.7, 131.6, 132.2, 132.9, 135.7–138.5 (polyene chain), 171.9 (2 C=O). MALDI-MS: 1139 (M^+, $C_{76}H_{98}O_8^+$).

1,3,5-Tri-({3-[(retinyloxy)carbonyl]propionyloxy}methyl)benzene (= *1,1′,1″-(Benzene-1,3,5-triyltrimethanediyl) 4,4′,4″-Tris[(2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl] Tributanedioate*; **8a**). UV/VIS (hexane): (225), 326 (136214). ^{1}H-NMR: 0.95–2.0 (*m*, 3 CH_2(2), 3 CH_2(3), 3 CH_2(4), 3 Me(16), 3 Me(17), 3 Me(18), 3 Me(19), 3 Me(20)); 2.66 (*m*, 3 $-CH_2CH_2-$); 4.74 (*d*, *J*(14,15) = 7.2, 3 CH_2(15)); 5.13 (*s*, 3 CH_2); 5.58 (*t*, *J*(14,15) = 7.2, 3 H–C(14)); 6.10–6.65 (*m*, 18 olefin. H); 7.28 (*s*, 4 arom. H). ^{13}C-NMR: 12.7 (C(19), C(20)); 19.2 (C(3)); 21.6 (C(18)); 24.8, 25.5, 29.1, 29.9 (C(16), C(17), $-CH_2CH_2-$); 33.0, 34.2 (C(4), C(1)); 39.5 (C(2)); 61.5 (C(15)); 65.9 (CH_2); 124.1, 125.9, 129.9, 127.0, 128.3, 128.4, 129.4, 129.8, 135.7, 135.9, 136.7, 137.6, 137.8, 139.2 (polyene chain); 172.0 (2 C=O). MALDI-MS: 1273 (M^+).

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