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Introduction of click chemistry to carotenoids

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ABSTRACT

We describe the synthesis of carotenoid derivatives via the azide–alkyne click reaction and optimize the conditions for these sensitive molecules. After finding the mildest conditions possible for the reaction we were able to use the click reaction for the synthesis of PEG–carotenoid conjugates starting from carotenoid pentynoates and PEG azides.

Carotenoids are naturally occurring antioxidants and show various biological effects including anti-cancer and cardioprotective. They have also been the subject of focus of food biochemists.[1] As the synthetic chemistry of carotenoids has been in decline, new methods and modern reactions have rarely been introduced to carotenoids.

We chose the azide–alkyne click reaction to synthesize carotenoid derivatives smoothly. This reaction is typically used as a mild alternative for the coupling of a wide variety of bioactive molecules or biomolecules such as sugars or proteins.[2]

Previously, we synthesized carotenoid trimers[3] and PEG-carotenoid derivatives via esterification.[4] In this work our aim was the synthesis of similar compounds using the azide–alkyne click reaction.[5] Generally two approximations are possible: the carotenoid can bear the azide group, or an alkyne moiety can somehow be attached to the carotenoid.

As this reaction was completely unknown in carotenoid chemistry the first task was to evaluate optimal, and if possible general, reaction conditions which would result in relatively high yields and conversions.

We chose the strategy that involved attachment of the alkyne moiety to the carotenoid. The simplest way to achieve this would have been the synthesis of propargyl esters or ethers from the corresponding carotenoid, but these reactions gave very low yields. Further investigation revealed pent-4-ynoic acid to be the reagent of choice: DCC-mediated coupling with the carotenols (Fig. 1, lutein, zeaxanthin, β-cryptoxanthin and capsanthin were isolated from natural sources, the others were synthesized from purchased carotenoids) gave the expected esters in good yields (Scheme 1). These were obtained in pure form by crystallization from a toluene/methanol/water mixture. Dihydroxy-carotenoids gave dipentynoate esters; in these cases the amounts of the reagents were doubled.

To find the optimum reaction conditions, 8′-β-apocarotenol pentynoate (**1**) was reacted under various conditions with benzylic azide as model system (Table 1). The results showed that the in situ generation of the copper(I) catalyst from copper sulfate did not work well and delivered no products. The best combina-

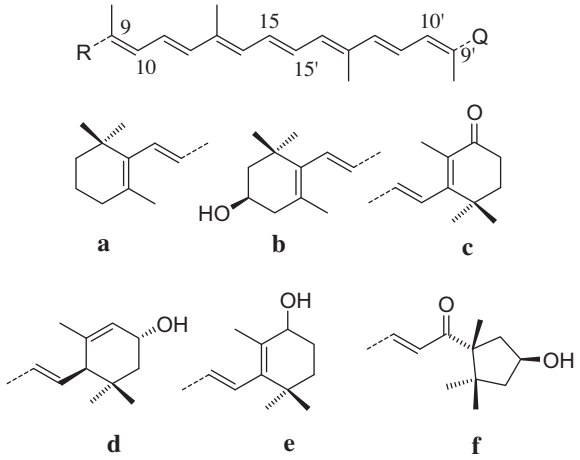

Figure 1. Structures of the carotenoid starting materials: R = **b**, Q = **a**: β-cryptoxanthin; R = **b**, Q = **d**: lutein; R = Q = **b**: zeaxanthin; R = Q = **e**: isozeaxanthin; R = **c**, Q = **e**: 4′-hydroxy-echinenone; R = **a**, Q = CH_2OH: 8′-β-apocarotenol; R = **b**, Q = **f**: capsanthin.

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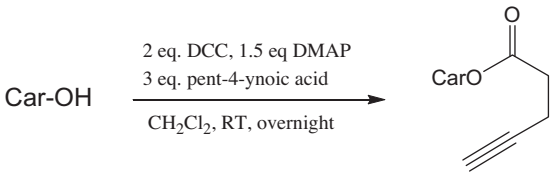
tion, with respect to reaction time and temperature needed, was found to be the use of copper(I) iodide as the catalyst with DMF as the solvent. These conditions were then applied to all the carotenoids. The application of a base (triethylamine) made no difference to the yield or conversion. The conversions of the starting carotenoids were high, although in the case of longer reaction times, considerable decomposition occurred.

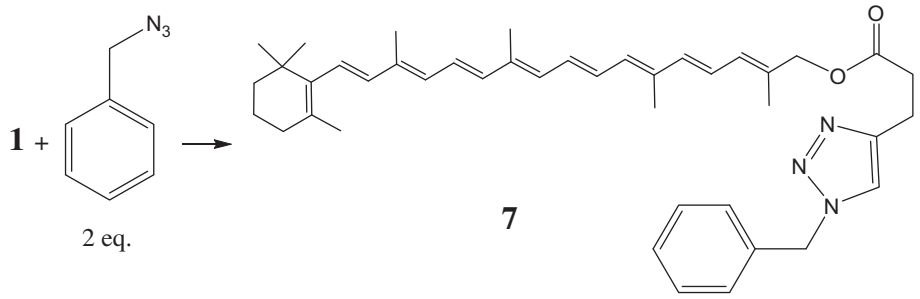
Polyethyleneglycol (PEG) conjugates of a wide range of biomolecules are known (especially for peptides).[6,7] These conjugates usually have better pharmacokinetic behavior, and



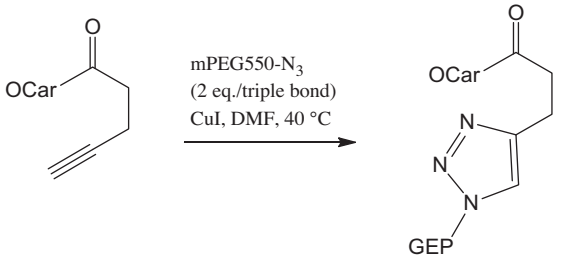
(**1**) Car: 8'-β-apocarotenol (75%, including reduction)
(**2**) Car: β-cryptoxanthin (90%)
(**3**) Car: zeaxanthin (53%)
(**4**) Car: isozeaxanthin (61%)
(**5**) Car: capsanthin (59%)
(**6**) Car: 4'-hydroxy-echinenone (52%)

Scheme 1. Synthesis of carotenoid pentynoates.

Table 1
Optimization of the conditions using benzyl azide model



Catalyst	Solvent	Time (h)	Temperature (°C)	Yield
$CuSO_4$+Na-ascorbate	*i*-BuOH, acetone, H_2O	15	40	<5%, dec.
CuI	*i*-BuOH, acetone, H_2O	15	40	<5%
CuI	DMSO	4	40–50	dec.
CuI	*i*-PrOH, H_2O, toluene	15–20	70	20%
CuI	*DMF*	*4*	*40*	*40–45%*
CuI	DMF+Et_3N	4	40	40–45%



(**8**) Car: 8'-β-apocarotenol (41%)
(**9**) Car: β-cryptoxanthin (47%)
(**10**) Car: zeaxanthin (44%)
(**11**) Car: isozeaxanthin (33%)
(**12**) Car:capsanthin (33%)
(**13**) Car: 4'-hydroxy-echinenone (<3%)

Scheme 2. Click-reaction with mPEG-550 monoazide.

water-solubility and, in general, are more efficient in drug targeting. In our previous article, we described the synthesis of PEG–carotenoid conjugates via esterification.[4] After finding optimal conditions, similar water-soluble PEG derivatives could be synthesized by the way of the click reaction. Monofunctional MeO-PEG550-OH (mPEG-550) was tosylated or mesylated and then substitution with NaN_3 in DMF delivered the PEG-azide overnight. This azide was applied in excess in the cycloaddition reaction with the mono- or dipentynoates (Scheme 2). The products were purified by preparative TLC, and were characterized by NMR, UV, HPLC and MALDI-TOF.[8]

In conclusion, as the click reaction proved to be an efficient and mild tool for the synthesis of carotenoid derivatives we plan to use it for the synthesis of other carotenoid–biomolecule conjugates.

Acknowledgments

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8. General procedure for the synthesis of carotenoid cycloadducts **8**–**12**: carotenoid pentynoate (30 mg, 1 equiv) was added to a solution of mPEG-550 (2 equiv/triple bond) in dry DMF (2 mL) under nitrogen. To the resulting red solution was added CuI (0.3 equiv) and the mixture stirred for 4 h at 40 °C in the dark. The mixture was poured into Et_2O (100 mL), dried, filtered and evaporated. The polar main product was separated by preparative TLC (Merck, Kieselgel 60, eluent *n*-hexane:acetone 2:8, or CH_2Cl_2: MeOH 95:5) to give a red oily product. According to HPLC and ^1H NMR the purity of the products was over 95%.
Spectroscopic data for **12**: UV (λ_{max} nm, EtOH): 477; MS (MALDI-TOF) *m*/*z* (most intense peaks) = 1718, 1762, 1806. ^1H NMR (400 MHz, $CDCl_3$) 1.08–2.20 (m, 36H, methyl Hs, H-2, H-2′, H-4b, H-4′b), 2.40 (m, 1H, H-4′a), 2.60–2.90 (m, 9H, CH_2-pent., H-4a), 3.40 (s, 6H, OMe), 3.50–3.80 (m, PEG-CH_2), 4.25 (m, 4H, PEG-CH_2), 5.05 (m, 1H, H-3), 5.23 (m, 1H, H-3′), 6.10–6.75 (m, 13H, olefinic), 7.33 (d, 1H, H-8′, *J* = 14.8 Hz), 7.51 (s, 2H, CH-triazole). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) = 12.7, 12.8, 21.0, 21.4, 24.7, 25.6, 28.5, 30.0, 34.0, 36.7, 38.4, 42.2, 43.3, 43.7, 44.0, 47.6, 50.1, 50.7, 58.9, 69.5, 69.9, 70.1-70.5, 71.9, 122.5-138.6, 140.8, 142.0, 146.2, 147.1, 150.0, 172.4, 202.4.