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Review

Synthesis of highly ^{13}C enriched carotenoids: access to carotenoids enriched with ^{13}C at any position and combination of positions*

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Carotenoids and their metabolites are essential factors for the maintenance of important life processes such as photosynthesis. Animals cannot synthesize carotenoids *de novo*, they must obtain them *via* their food. In order to make intensive animal husbandry possible and maintain human and animal health synthetic nature identical carotenoids are presently commercially available at the multi-tonnes scale per year. Synthetically accessible ^{13}C enriched carotenoids are essential to apply isotope sensitive techniques to obtain information at the atomic level without perturbation about the role of carotenoids in photosynthesis, nutrition, vision, animal development, etc. Simple highly ^{13}C enriched C_1, C_2 and C_3 building blocks are commercially available *via* 99% ^{13}CO. The synthetic routes for the preparation of the ^{13}C enriched building blocks starting from the commercially available systems are discussed first. Then, how these building blocks are used for the synthesis of the various ^{13}C enriched carotenoids and apocarotenoids are reviewed next. The synthetic Schemes that resulted in ^{13}C enriched β-carotene, spheroidene, β-cryptoxanthin, canthaxanthin, astaxanthin, (3*R*,3′*R*)-zeaxanthin and (3*R*,3′*R*,6′*R*)-lutein are described. The Schemes that are reviewed can also be used to synthetically access any carotenoid and apocarotenoid in any ^{13}C isotopically enriched form up to the unitarily enriched form.

Keywords: β-carotene, spheroidene, astaxanthin, canthaxanthin, zeaxanthin

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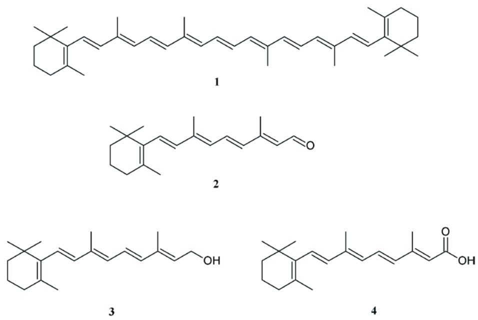
INTRODUCTION

Carotenoids are a class of coloured unsaturated tetraterpenes. Carotenoid biosynthesis takes place in the chloroplast of plant cells, cyanobacteria, algae and fungi via the methylerythritol phosphate pathway distinct from the mevalonic acid pathway for all other isoprenoids (Goodwin, 1980; Thibodeux & Liu, 2009).

The carotenoids occur in membrane carotenoid complexes in photosynthetic organism both in antennae pigments that absorb light and in the photosynthetic reaction centre that convert the electronic energy into energy rich molecules. In the latter complexes, they protect these systems against light damage (Britton, 2009; Telfer *et al*., 2009).

The products of organic photosynthesis serve as food and energy rich materials in our society. The fossil fuels that we use are the products of photosynthesis in the past. Without carotenoids oxygenic photosynthesis would be impossible and life on earth as we know it would not be possible.

Animals cannot synthesize carotenoids *de novo*. They are dependent on the plant materials in their food. A very important role of some carotenoids is their enzymatic generation of retinoids in the body. In Scheme 1 it is indicated that β-carotene **1** is enzymatically converted into all-*trans* retinal **2** which is further converted into retinol **3** and retinoic acid **4** (Britton, 2009).



Scheme 1. The enzymatic conversion of β-carotene 1 in the human (animal) body into retinal 2, which can be converted into retinol (vitamin A) 3 and retinoic acid 4.

Besides this important role as source of the retinoids, carotenoids fulfill many other important roles to maintain health in humans and animals (Carotenoids, 2009). The present day intense animal husbandry and maintenance of human health is impossible without industrially produced nature-identical carotenoids. A number of important synthetic, nature-identical carotenoids are produced by BASF and DSM at a 3000 tonnes scale a year (Paust, 1996).

In order to follow the conversions of nutrients in the body isotopically labeled systems are essential. This type of study was pioneered by Schoenheimer with stable isotope labeled macronutrients (Schoenheimer, 1942). At that time this approach for macronutrients like carotenoids would not have worked due to the low sensitivity of the isotope sensitive techniques. In the mean time the analytical techniques have greatly improved in sensitivity such that site-directed stable isotope retinoids with high isotope incorporation at at least 3 positions

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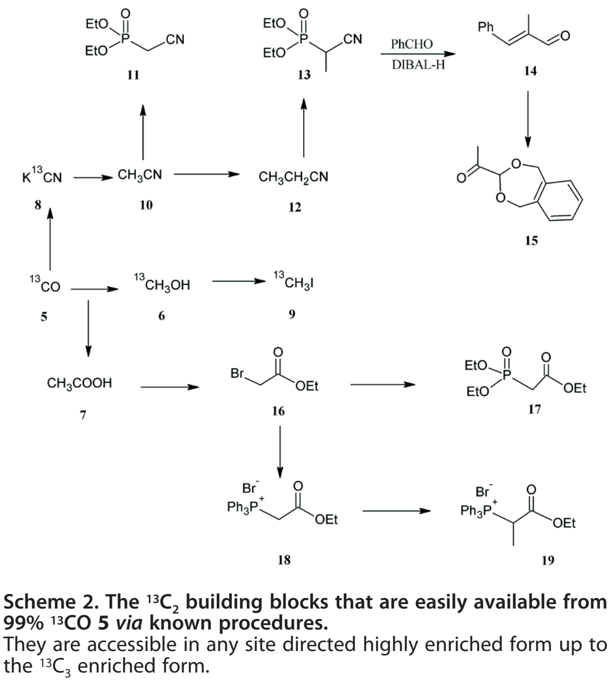
{(10,19,19,19-^{2}H)-retinyl acetate} have become a favorite (Olsen, 1997).

The separation properties may even change upon introduction of a number of deuterium isotopes; octadeutero-β-carotene can be separated from natural abundance β-carotene with HPLC techniques (Dueker *et al.*, 1994). Deuterium is situated at the periphery of the molecule, the deuterium-protium mass ratio is 2/1 and the C-D bond is shorter than the C-H bond length. In the case of multiple ^{13}C enrichment the isotope is situated in the carbon skeleton of the molecule and the mass ratio is 13/12. For these reasons ^{13}C labels systems were used recently as reference in nutritional studies (van Lieshout *et al.*, 2001; Wang *et al.*, 2008). Besides the difference in mass the ^{13}C isotope has I=1/2 which allows the ^{13}C NMR studies. Isotope sensitive non-invasive physical techniques such as NMR and resonance Raman studies allow obtaining information at atomic resolution without any perturbation of the carotene-proteins involved in light harvestation and energy conversion during photosynthesis. In this paper we discuss the site-direct ^{13}C incorporation in carotenoids at any position and combination of positions for nutritional and spectroscopic studies.

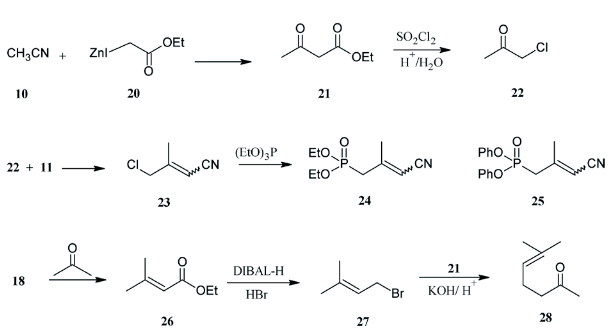
ACCESS TO HIGHLY $^{13}C_2$, $^{13}C_3$ ENRICHED BUILDING BLOCKS

In natural abundance carbon contains 1.1% ^{13}C and 98.9% ^{12}C. Commercially 99% ^{13}CO is obtained by cryogenic distillation of carbon monoxide (Lockhart, 1979). The enrichment factor is 90. As indicated from ^{13}CO **5**, $^{13}CH_3OH$ **6**, K^{13}CN **8** and $^{13}CH_3I$ **9** are easily prepared (Lockhart, 1979). Simple reactions lead to [1-^{13}C]-, [2-^{13}C]-and [1,2-$^{13}C_2$]-acetonitrile **10** and [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetic acid **7** depending if the synthesis of the two carbon building blocks is prepared with only one of the both highly ^{13}C enriched building blocks. Ethyl bromoacetate **16** is prepared *via* a bromination and subsequent esterification.

The $^{13}C_2$ building blocks have been used to make Wittig reagents (Creemers & Lugtenburg, 2002). The anion of acetonitrile **10** reacts with methyl iodide **9** in high



Scheme 2. The $^{13}C_2$ building blocks that are easily available from 99% ^{13}CO 5 *via* known procedures.
They are accessible in any site directed highly enriched form up to the $^{13}C_3$ enriched form.



Scheme 3. The preparation of the highly ^{13}C enriched building blocks for the synthesis of β-carotene.
They are available in any ^{13}C enrichment in any possible position.

yield to give propionitrile **12**. Diethyl chlorophosphate reacts with the anion of acetonitrile **10** and the anion of propionitrile **12** to give diethyl phosphonoacetonitrile **11** and diethyl phosphonopropionitrile **13**, respectively. An Arbuzov reaction of ethyl bromoacetate **16** with triethylphosphite gives ethyl diethyl phosphonoacetate **17**. The reaction of ethyl bromoacetate **16** with triphenylphosphine gives triphenyl phosphonium acetate **18**. Anion of **18** can easily be converted into the corresponding propionate system **19** by treatment with methyl iodide **9**.

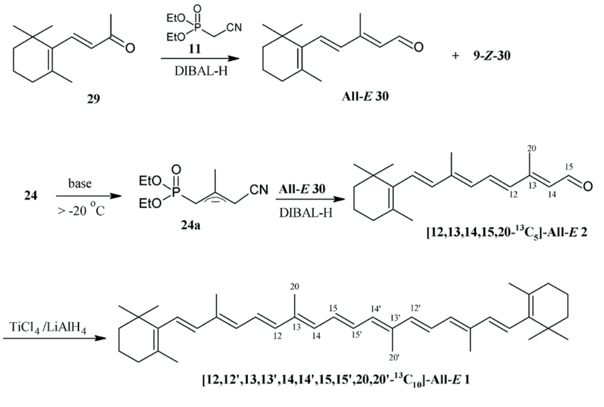
Depending on the reagents each $^{13}C_3$ building block is available in high ^{13}C enrichment at any carbon position and any combination of carbon positions. The Wittig reaction of diethyl phosphonopropionitrile **13** with benzaldehyde and subsequent DIBAL-H reduction gives 3-phenyl-2-methyl-3-propenal **14**. To obtain the acetal of prop-2-one-1-al **15**, product **14** is treated first with 1,2-dihydroxymethylene benzene to protect the aldehyde, then followed by oxidative cleavage with potassium permanganate (Jansen, 1996).

In Scheme 3 it is indicated that reagents from Scheme 2 are used to prepare ethyl-3-oxo-butyrate **21** *via* a Blaise reaction of acetonitrile and the zinc derivative of iodoacetate **20**. Monochlorination of **21** and subsequent acid hydrolysis gives 1-chloroacetone **22** (Creemers & Lugtenburg, 2002). The Wittig reaction of product **22** with diethyl phosphonoacetonitrile **11** gives (*E*/*Z*)-4-chloro-3-methylbut-2-enenitrile **23**. The Arbuzov reaction of triethylphosphite with product **23** gives the reagent (*Z*/*E*)-4-(diethylphosphono)-3-methylbut-2-enenitrile **24** and similarly, diphenyl methylphosphite gives the corresponding (*Z*/*E*)-4-(diphenylphosphono)-3-methylbut-2-enenitrile **25**.

Ethyl 3-methylbutenoate **26** is obtained by the reaction of acetone and the Wittig reagent **18** (Scheme 2), followed by the reduction with DIBAL-H and subsequent treatment with aqueous HBr to obtain 1-bromo-3-methylbut-3-ene **27**. The latter is coupled with ethyl acetoacetate **21**. Subsequently, the alkylated product is hydrolyzed and decarboxylated to obtain 6-methylhept-5-ene-2-one **28**.

^{13}C ENRICHED β-CAROTENE

In Scheme 4 it is indicated that starting from β-ionone **29** with the reagents described in Schemes 2 and 3 [12,12',13,13',14,14',15,15',20,20'-$^{13}C_{10}$]-all-*E* β-carotene **1** is prepared (Lugtenburg *et al.*, 1999). β-Ionone **29** gives a chain extension with diethyl phosphonoacetonitrile **11**, subsequent DIBAL-H reduction gives all-*E* and 9-*Z*-β-ionylidene acetaldehyde **30** which can easily be separated by column chromatography.



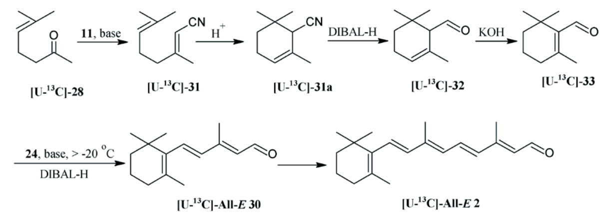
Scheme 4. The synthesis of [12,12′,13,13′,14,14′,15,15′,20,20′-$^{13}C_{10}$]-all-*E* β-carotene 1.

The product **all-*E* 30** is treated with anion of (*Z*/*E*)-4-(diethylphosphono)-3-methylbut-2-enenitrile **24a**. When the reaction is carried out at above –20°C in the absence of excess base, the Wittig reagent occurs only in the extended form without a trace of geometric isomers. The resulting retinonitrile is formed in the all-*E* form only, careful DIBAL-H reduction gives all-*E* retinal **2**.

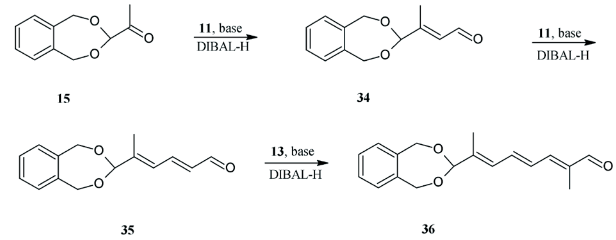
A subsequent McMurry reductive coupling of all-*E* retinal **2** gives all-*E* β-carotene **1**. The reactions in Scheme 4 have been carried out with $^{13}C_5$-phosphonate **24** resulting in [12,12',13,13',14,14'15,15',20,20'-$^{13}C_{10}$]-all-*E* β-carotene **1** with ^{13}C incorporation of 99% at each of the mentioned positions (Lugtenburg *et al.*, 1999).

It is clear that *via* the reactions in Scheme 4 all-*E* β-carotene **1** can be prepared in any possible ^{13}C enriched form that is symmetrically incorporated at the position 12 up to 12' and 20 up to 20'. Curiously carrying out the reactions with the corresponding $^{13}C_5$-diphenyl building block gives an about 1:1 mixture of for vision research important 11-*Z*-retinal **2** and all-*E* retinal **2** which can easily be separated by column chromatography (Wang *et al.*, 2004). The difference in electronegativity between the ethoxy and phenoxy group on the phosphorous is presumably the cause of this result.

The introduction of better electron withdrawing groups *via* Arbuzov reactions with 1-chloroacetone **22** was unsuccessful. Lately, the conversion of (*Z*/*E*)-4-(diethylphosphono)-3-methylbut-2-enenitrile **24** into the corresponding dichloro derivative has been reported (Monbaliu *et al.*, 2010). It is to be expected that *via* this phosphonyl chloride many better electron withdrawing groups can be introduced leading to the hope that using these reagents 11-*Z*-retinal can be obtained in pure 11-*cis* form only, which will be an important step for vision research.



Scheme 5. The synthetic Scheme to prepare [U-^{13}C]-all-*E* retinal [U-^{13}C]-all-*E* 2.



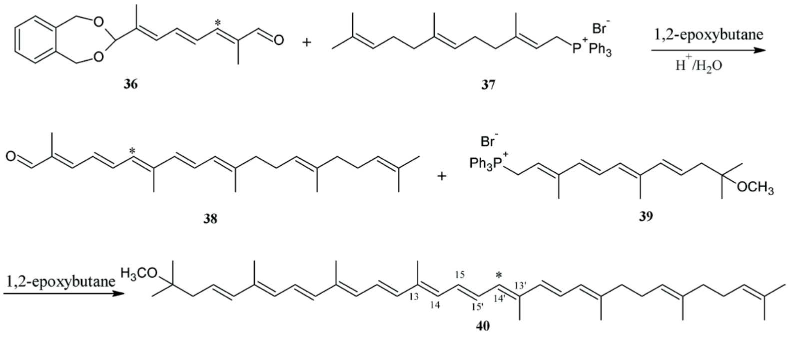
Scheme 6. The preparation of the site directed mono-acetal of the $^{13}C_{10}$-dialdehyde in all possible ^{13}C isotopomomers.

U-^{13}C ENRICHED β-CAROTENE

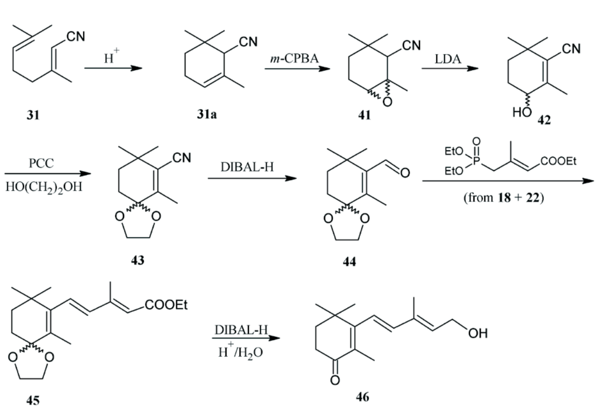
[U-^{13}C]-All-*E* retinal **2** can be converted easily into [U-^{13}C]-β- carotene **1** *via* the McMurry coupling shown in Scheme 4. The reactions to obtain [U-^{13}C]-all-*E* retinal **2** are shown in Scheme 5 (Creemers & Lugtenburg, 2002). [U-^{13}C]-6-Methylhept-5-ene-2-one **[U-^{13}C]-28** prepared *via* Scheme 3 is coupled with $^{13}C_2$-diethyl phosphonoacetonitrile **11** giving [U-^{13}C]-3,7-dimethylocta-2,6-dienenitrile **[U-^{13}C]-31** which upon treatment with concentrated sulphuric acid gives the α-cyclocitronitrile **31a** and subsequent DIBAL-H treatment gives [U-^{13}C]-α-cyclocitral **[U-^{13}C]-32**. The α-form is fully converted into the conjugated [U-^{13}C]-β-cyclocitral **[U-^{13}C]-33**.

Reaction of **[U-^{13}C]-33** with (*Z*/*E*)-4-(diethylphosphono)-3-methylbut-2-enenitrile **24** above –20°C and subsequent DIBAL-H reduction gives pure [U-^{13}C]-all-*E* β-ionylidene acetaldehyde **[U-^{13}C]-all-*E* 30**. Repeating this sequence results in pure [U-^{13}C]-all-*E* retinal **[U-^{13}C]-all-*E* 2**. [U-^{13}C]-All-*E* retinal **2** is expected to give [U-^{13}C]-all-*E* β-carotene **1** without any problem. It is clear that besides [U-^{13}C]-all-*E* β-carotene **1** all β-carotenes that have symmetrical ^{13}C isotope enrichment are accessible *via* the reactions described in Scheme 5 and the building blocks that are required in ^{13}C enriched forms can be prepared according to the literature (Creemers & Lugtenburg, 2002).

The reactions in Scheme 5 are not applicable for carotenoids with different end groups or for asymmetric introduction of ^{13}C in symmetric carotenoids. A general approach based on 2,7-dimethylocta-2,4,6-triene-1,8-dial is used in the commercial synthesis of β-carotene **1** *via* Wittig chemistry (Creemers & Lugtenburg, 2002).



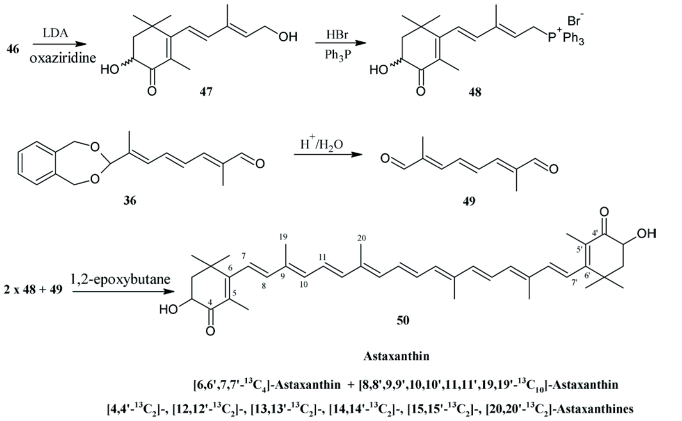
Scheme 7. Synthetic Scheme to prepare both [14-^{13}C]-all-*E* spheroidene and [14′-^{13}C]-all-*E* spheroidene.



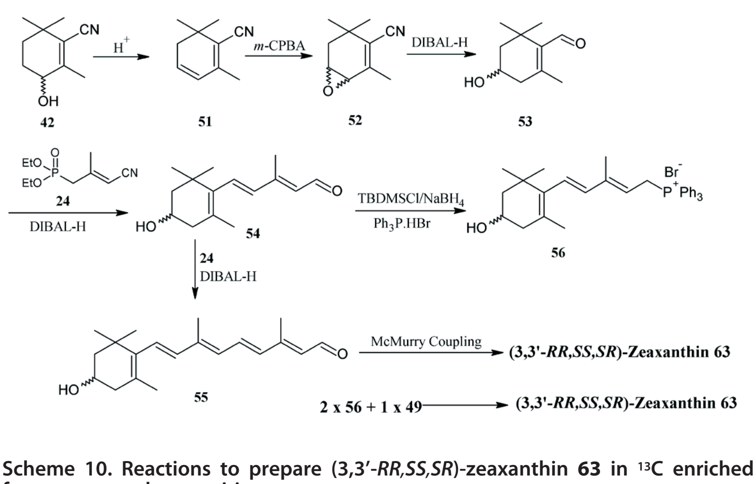
Scheme 8. Preparation of the end group of canthaxanthin in any possible ^{13}C isotopologues.

In Scheme 6 it is indicated that the acetal of prop-2-en-1-al **15** (Scheme 2) can be easily converted into the mono site protected aldehyde of 2,7-dimethylocta-2,4,6-triene-1,8-dial **36**. Product **15** is chain extended twice with diethyl phosphonoacetonitrile **11** and DIBAL-H reduction into the acetal conjugated aldehyde **35**. A final chain extension with diethyl phosphonopropionitrile **13** and subsequent reduction gives the required monoacetal of 2,7-dimethylocta-2,4,6-triene-1,8-dial **36**.

In literature the synthesis of building blocks **37** and **39** have been described (Jansen & Lugtenburg, 2009).



Scheme 9. Preparation of the end groups of astaxanthin and the final conversion into ^{13}C enriched astaxanthines.



Scheme 10. Reactions to prepare (3,3'-*RR,SS,SR*)-zeaxanthin 63 in ^{13}C enriched form at any carbon position.

The Wittig coupling of the labeled aldehyde **36** with product **37** in refluxing 1,2-epoxybutane and subsequent deprotection gives the conjugated aldehyde **38**.

Repeating this coupling with building block **39**, results in [14'-^{13}C]-spheroidene **40** where as first coupling of **36** with **39**, subsequent deprotection and final coupling with building block **37** gives [14-^{13}C]-spheroidene **40**. These reactions have actually been carried out with the all-*E* [$^{13}C_{10}$]-7-cyano-2-methylocta-2,4,6-trienal (Jansen, 1996). The reactions in Scheme 7 lead to any carotenoids ^{13}C enriched in any possible way in the central part of the carbon skeleton.

In Scheme 8 the acid catalyzed conversion of 3,7-dimethylocta-2,6-dienenitrile **31** into the cycloderivative **31a** has been discussed. Epoxidation of the double bond with *meta*-chloroperbenzoic acid (*m*-CPBA) gives the epoxide derivative **41**.

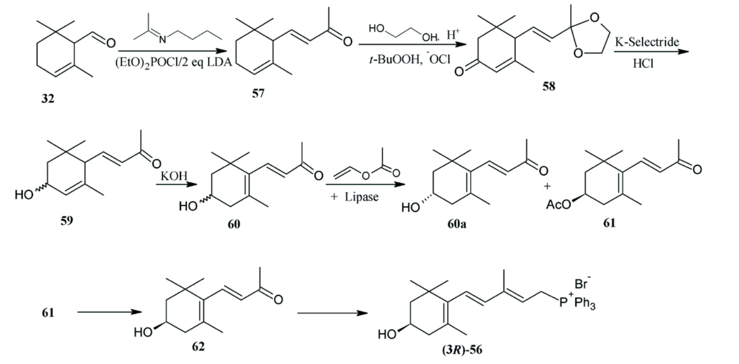
Treatment of product **41** with a base gives (4*RS*)-4-hydroxy-β-cyclocitronitrile **42** (Lugtenburg *et al.*, 1999; Jansen, 2000). Pyridinium chlorochromate oxidation and subsequent ketalisation with ethylene glycol gives compound **43** which upon DIBAL-H reduction gives the 4,4'-protected β-cyclocitral **44**. Wittig-Horner reaction with the $^{13}C_5$-phosphonato derivative results in the β-ionylidene acetoester **45**, which upon treatment with DIBAL-H and subsequent deprotection gives 4-oxo-β-ionylidene ethanol **46**.

In Scheme 9 it is shown that upon treatment with two equivalents LDA, the alcoholate anion of **46** is treated with oxaziridine to give (3*RS*)-3-hydroxy-4-oxo-β-ionylidene ethanol **47**. Treatment with triphenyl phosphine and HBr results in the Wittig salt **48** (Becker *et al.*, 1981). The Wittig coupling of the phosphonium salt **48** with the $^{13}C_{10}$-dialdehyde **49** in 1,2-epoxybutane gives (3,3'-*RR,SR,SS*)-astaxanthin **50**. The [4,4'-$^{13}C_2$]- and the other isotopologues of astaxanthin indicated in Scheme 9 have been prepared.

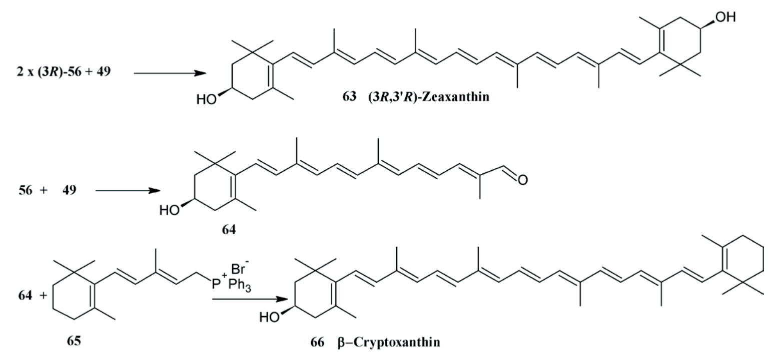
In Scheme 10 it is shown that (4*RS*)-4-hydroxy-β-cyclocitronitrile **42** is converted by acid catalyzed dehydration into the cyclohexadienenitrile **51** (van Wijk *et al.*, 2003). Oxidation with *m*-CPBA acid results epoxidation of 3,4-double bond in product **52**. DIBAL-H reduction results in (3*RS*)-3-hydroxy-β-cyclocitral **53**, chain extension and subsequent DIBAL-H reduction gives the 3-hydroxy-β-ionylidene acetaldehyde **54** followed by protection with TBDMSCl and reduction with $NaBH_4$ and subsequent reaction with $Ph_3P.HBr$ gives the Wittig salt **56**. The latter could be converted into zeaxanthin **63**.

In literature (*3R,3'R*)-all-*E* β-carotene-3,3'-diol enriched with ^{13}C at 12,12',13 and 13' has been reported (Khachik *et al.*, 1995). Product **59** can also be converted into (3*RS*)-3-hydroxy all-*E* retinal **55** which upon McMurry coupling gives the corresponding (3,3',-*RR, SR, SS*)-zeaxanthin **63** (Lugtenburg *et al.*, 1999).

In Scheme 11 it is indicated that α-cyclocitral **32** is prepared in any ^{13}C isotopologue *via* Scheme 5 that can be converted into α-ionone **57** by treating it with the imine of butylamine and acetone. If a ^{13}C enriched isotopologue is required α-cyclocitral **32** could be coupled with the diethyl phosphonoacetonitrile **11** first and then the obtained nitrile could be converted into α-ionone **57**.



Scheme 11. Synthesis of (3*R*)-3-hydroxy-β-ionylidene ethyl triphenyl phosphonium bromide (3*R*)-56 from α-cyclocitral 32.



Scheme 12. Preparation of (3*R*,3'*R*)-zeaxanthin 63 and β-cryptoxanthin 66.

The conversions of α-ionone **57** to **58** has been described in literature (Khachik & Chang, 2011). Protection of the keto function in **58** and subsequent oxidation with *t*-butyl hydroperoxide in aqueous hypochlorite gives the 3-oxo derivative **58**, which with K-Selectride and deprotection of the keto function results in (3*RS*)-3-hydroxy-α-ionone **59** which upon treatment with KOH gives the corresponding (3*RS*)-3-hydroxy-β-ionone **60**.

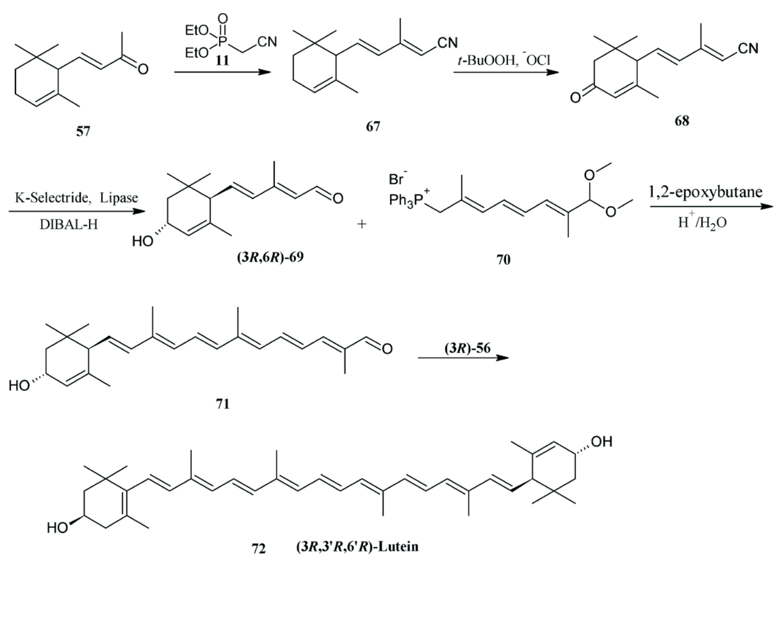
Treatment of (3*RS*)-3-hydroxy-β-ionone **60** with vinyl acetate in the presence of lipase gives (3*S*)-hydroxy-β-ionone **60a** and (3*R*)-acetoxy-β-ionone **61**. The latter has been converted into (3*R*,3'*R*)-zeaxanthin **63** *via* coupling of **(3*R*)-56** and dialdehyde **49** in Scheme 12.

The Schemes discussed so far allow access to any C_{40} carotenoid labeled with ^{13}C at any position and combination of positions. The only carotenoid that is not accessible in any ^{13}C enriched form is (3*R*,3'*R*,6'*R*)-lutein. Recently the synthesis of (3*R*,3'*R*,6'*R*)-lutein in natural form has been published (Khachik & Chang, 2009). Wittig-Horner reaction of α-ionone **57** gives α-ionylidene acetonitrile **67**, which upon treatment with *t*-butyl hydroperoxide and aqueous hypochlorite solution gives the 3-oxo-α-ionylidene acetonitrile **68**. Treatment of **68** with K-Selectride and subsequent reaction with vinyl acetate in the presence of lipase and final DIBAL-H reduction gives the (3*R*,6*R*)-3-hydroxy-α-ionylidene acetaldehyde **69**. These reactions have also been used for the conversions in Scheme 11.

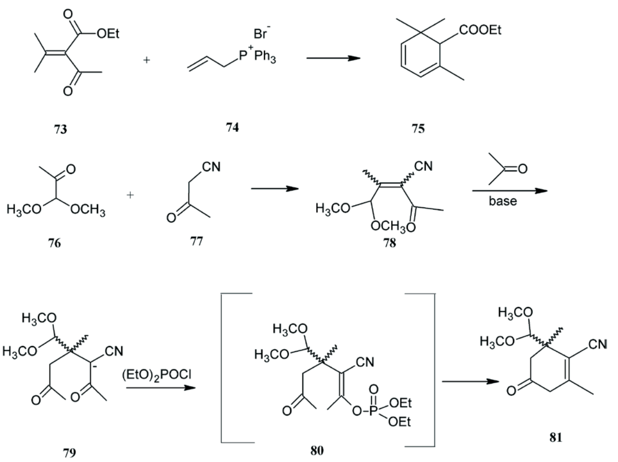
Coupling of product **(3*R*,6*R*)-69** with the protected $^{13}C_{10}$-phosphonium salt gives compound **71**. The latter is converted in a final Wittig coupling with **(3*R*)-56** (Scheme 11) into (3*R*,3'*R*,6'*R*)-lutein **72**. For the introduction of ^{13}C at any carbon position in lutein, α-ionylidene acetonitrile **67** should preferably be made via α-cyclocitral **32** and coupling with (*Z*/*E*)-4-(diethylphosphono)-3-methylbut-2-enenitrile **24** (Scheme 5).

The phosphonium salt **70** is easily available *via* the aldehyde **36**. *Via* the Schemes in this paper all important C_{40}-carotenoids are now accessible in any ^{13}C labeled form at any position and combinations of positions. The Schemes for the introduction of ^{13}C discussed so far have a few drawbacks. First, the six membered rings contain two diastereotopic methyl groups on carbon 1 and carbon 1': when the methyl groups have one ^{13}C and one ^{12}C isotope, the quaternary carbon atom 1 will be optically active, giving diastereomeric mixtures that cannot be separated. Second, the introduction of substitution in the six membered rings leads to long linear synthetic Schemes resulting in low yields of these carotenoids with ^{13}C enrichment in the six membered rings.

For the solution of both problems we have found that 4-methyl-3-carbethoxypent-3-ene-2-one **73** has been converted in one pot with allyl triphenylphosphonium bromide **74** into the cyclic ester **75** (Büchi & Wuest, 1971). We carried out a Knoevenagel condensation of 1,1-dimethoxyacetone **76** with 1-cyanoacetone **77** to give 3-cyano-5,5-dimethoxy-4-methylpent-3-ene-2-one **78** (Dawadi & Lugtenburg, 2007). Reaction of the anion of acetone at position 4 of **78** should give the stable anion **79** analogous to the reaction between **73** and **74**. The anion **79** is expected to form



Scheme 13. Preparation of (3*R*,3'*R*,6'*R*)-lutein 72 starting from α-ionone.



Scheme 14. Synthesis of well defined six membered systems for the easy access to [13]C labeled end groups of carotenoids.

the phosphate ester **80** upon treatment with diethyl phosphonyl chloride. Subsequent reaction with base should give 3-oxo-β-cyclocitronitrile **81** (Dawadi & Lugtenburg, 2010). Application of the reduction and enzymatic conversion in Scheme 14 gives the building blocks for the end groups of zeaxanthin and lutein with one of the diasteromeric methyl groups in the form of a protected aldehyde, which will allow a site-directed [13]C introduction. After deprotection and reduction of the aldehyde end groups will be available with well defined chirality at the carbon position 1.

CONCLUSION

In this paper all known[13]C enriched C_{40} carotenoids that have been published are reviewed. The Schemes described in this paper allow access to any biologically and commercially important carotenoid in well defined [13]C enriched form up to the U-[13]C enriched system. Combined with the reactions reviewed in the [13]C enriched retinal papers all apo-carotenoids are now also accessible in any [13]C enriched form (Dawadi & Lugtenburg, 2010).

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REFERENCES

Becker E, Albrecht R, Bernhard K, Leuenberger HGW, Mayer H, Müller RK, Schüp W, Wagner HP (1981) Synthese von Astaxanthin aus β-ionen. *Helv Chim Acta* **64**: 2419–2435.

Britton G (2009) Functions of intact carotenoids. In *Carotenoids* Britton G, Liaaen-Jensen S, Pfander H, eds, vol 4, pp 189–212. Birkhauser Verlag, Basel, Switzerland.

Britton G (2009) Functions of carotenoid metabolites and breakdown products. In *Carotenoids* Britton G, Liaaen-Jensen S, Pfander H, eds, vol 4, pp 309-324. Birkhauser Verlag, Basel, Switzerland.

Büchi G, Wuest H (1971) Synthetic studies on damascenones. *Helv Chim Acta* **54**: 1767–1776.

In Carotenoids (2009) *Volume 5: Nutrition and Health*; Britton G, Liaaen-Jensen S, Pfander H, eds. Birkhauser Verlag, Basel, Switzerland.

Creemers AFL, Lugtenburg J (2002) The preparation of all-*trans* uniformly [13]C-labeled retinal *via* a modular total organic synthetic strategy. Emerging central contribution of organic synthesis toward the structure and function study with atomic resolution in protein research. *J Am Chem Soc* **124**: 6324–6334.

Dawadi PBS, Lugtenburg J (2007) Efficient preparation of [2-[13]C]- and [3-[13]C]-3-cyano-4-methyl-3-pyrrolin-2-one. *Eur J Org Chem* 1294-1300.

Dawadi PBS, Lugtenburg J (2010) Synthesis and use of stable isotope enriched retinals in the field of vitamin A. *Molecules* **15**: 1825-1872.

Dueker SR, Jones AD, Smith GM, Clifford AJ (1994) Stable isotope methods for the study of β-carotene-d_8 metabolism in humans utilizing tandem mass spectrometry and high-performance liquid chromatography. *Anal Chem* **66**: 4177–4185.

Goodwin TW (1980) *The biochemistry of the carotenoids*. Vol. 1 Plants, 2nd edn. Chapman and Hall London, New York.

Jansen FLHM (1996) *Synthesis of [13]C labeled astaxanthin, spheroidene and β-carotenene*. Dissertation, Leiden.

Jansen FLHM (2000) Synthesis of all-*E* 4,4'-[13]C_2-astaxanthin strategies for labeling the C_{15}-end groups in carotenoids. *Eur J Org Chem* 829–830.

Jansen FJ, Lugtenburg J (2009) Chapter 3. Carotenoid synthesis. Part VI. Labelled carotenoids. In *Carotenoids*. Britton G, Liaaen-Jensen S, Pfander H, eds, vol 2, pp 265-308. Birkhauser Verlag, Basel, Switzerland.

Khachik F, Beecher GR, Li BW, Englert G (1995) Synthesis of [13]C-labelled (all-*E*,3R,3'R)-β,β-carotene-3,3'-diol (zeaxanthin) at C(12), C(13), C(12'), and C(13') via all-*E*-2,7-dimethylocta-2,4,6-triene-1,8-dial-[13]C_4. *J labelled Compd Radiopharm* **XXXVI**: 1157–1172.

Khachik F, Chang AN (2009) Total synthesis of (3R,3'R,6'R)-Lutein and its stereo isomers. *J Org Chem* **74**: 3875–3885.

Khachik F, Chang AN (2011) Synthesis of (3*S*)- and (3*R*)-3-hydroxy-β-ionone and their transformation into (3*S*)- and (3*R*)-β-cryptoxanthin. *Synthesis* 509–516.

Lockhart IM (1979) Stable isotopes-separation and application in *Isotopes: Essential Chemistry and Applications*; Elvidge JA, Jones JR, eds. pp 1–35. The Chemical Society: London, UK.

Lugtenburg J, Creemers AFL, Verhoeven MA, Van Wijk AAC, Verbengen PJE, Munnee MCF, Jansen FLHM (1999) Synthesis of [13]C-labeled carotenoids and retinoids. *Pure Appl Chem* **7**: 2245–2252.

Monbaliu JC, Tinant B, Marchand-Brynaert J (2010) [4 + 2] Cycloadditions of 1-phosphono-1,3-butadienes with nitroso heterodienophiles: a versatile synthetic route for polyfunctionalized aminophosphonic derivatives. *J Org Chem* **20**: 5478–5486.

Olsen JA (1997) Isotope-dilution techniques: A wave of the future in human nutrition. *Am J Clin Nutr* **66**: 186–187.

Paust J (1996) Chapter 3, Synthesis part VII. Technical synthesis. In *Carotenoids*. Vol 2 Synthesis, pp 259–292. Birkhauser Verlag, Basel, Switzerland.

Schoenheimer R (1942) *The dynamic state of body constituents*. Harvard University Press: Cambridge, USA.

Telfer A, Pascal A, Gall A (2009) Carotenoids in photosynthesis. In *Carotenoids*. Britton G, Liaaen-Jensen S, Pfander H, vol 4, eds. pp 265-308. Birkhauser Verlag, Basel, Switzerland.

Thibodeux CJ, Liu HW (2009) Unraveling the mechanisms of isoprenoid biosynthetic enzymes: Mechanistic studies of the early stage enzymes. *Chimia* **63**: 334–339.

van Lieshout M, West C, Muhilal E, Permesan D, Wang Y, Xu X, van Bremen RB, Creemers AFL, Verhoeven MA, Lugtenburg J (2001) Bioefficiency of β-carotene dissolved in oil studied in children in Indonesia. *Am J Clin Nutr* **73**: 949–958.

Van Wijk AAC, Van de Weerd MB, Lugtenburg J (2003) Synthetic Scheme for the preparation of [13]C-labeled 3,4-didehydroretinal, 3-hydroxyretinal and 4-hydroxyretinal up to uniform [13]C enrichment. *Eur J Org Chem* 863–868.

Wang J, Wang Y, Wang Z, Li L, Qin J, Lai W, Fu Y, Suter PM, Russell RM, Grusak MA, Tang G, Yin S (2008) Vitamin A equivalence of spirulina β-carotene in Chinese adults as assessed by use of a stable-isotope reference method. *Am J Clin Nutr* **87**: 1730–1737.

Wang Y, Woo WS, Van der Hoef I, Lugtenburg J (2004) 9-Demethyl-9-haloretinals by Wadsworth-Emmons Coupling — Easy preparation of pure (all-*E*), (9*Z*) and (11*Z*) isomers. *Eur J Org Chem* 2166–2175.