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Syntheses of $^{13}C_2$-labelled 11*Z*-retinals

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ABSTRACT

To enable solid-state NMR investigations of the rhodopsin chromophore and its photointermediates, a series of 11*Z*-retinal isotopomers have been synthesised containing pairs of adjacent ^{13}C labels at C9/C10, C10/C11 or C11/C12, respectively. The C9 labelled carbon atom was introduced through the Heck reaction of a ^{13}C-labelled Weinreb acrylamide derivative, and the label at the C12 position derived from a ^{13}C-containing ethoxy Bestmann–Ohira reagent. The ^{13}C labels at C10 and C11 were introduced through the reaction of β-ionone with labelled triethyl phosphonoacetate.

1. Introduction

Located in the rod cells of the retina, rhodopsin is responsible for dim light vision in vertebrates. It is a 40-kd G-protein coupled receptor (GPCR) consisting of an 11*Z*-retinylidene chromophore bound to the apoprotein opsin by a protonated Schiff base linkage to the ε-amino group of lysine-296. Upon absorption of a photon, the chromophore photoisomerises to all *E*-retinylidene in approximately 200 fs leading to conformational changes within the protein producing the active form of rhodpsin, metarhodpsin II.[1]

Members of the GPCR super-family of receptors are frequently identified as targets for the development of therapies to treat a diversity of diseases.[2] Detailed structural knowledge of GPCRs and their bound ligands is therefore of great significance, and rhodopsins have been one of the most widely studied GPCRs due to the availability of crystal structural data and adequate amounts of material.[3] Understanding in detail how the protein environment in rhodopsin affects and accelerates isomerisation of the retinylidene chromophore, and how isomerisation of the chromophore influences conformational changes in the protein, is important for GPCR research. Recently, solid-state NMR has been used as a powerful tool to study the conformation of the retylidene chromophore in rhodopsin and its photointermediates with a level of resolution unmatched using other techniques.[4,5] Double-quantum filtered ^{13}C magic angle spinning structural studies,[6] however, require access to 11*Z*-retinals containing two adjacent ^{13}C labels in defined positions (Fig. 1).

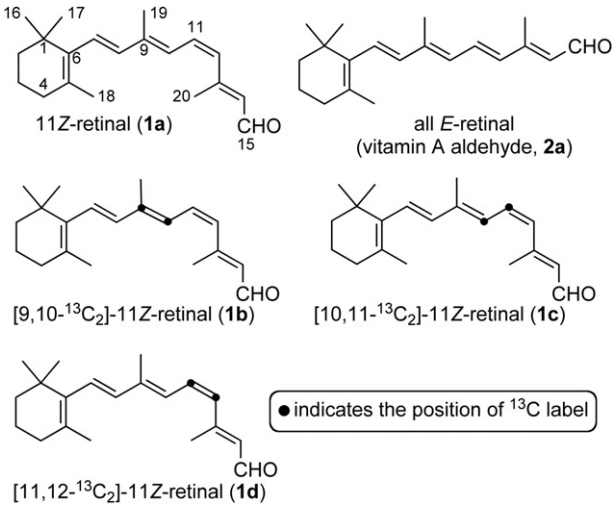

Fig. 1. Structures of 11*Z*-retinal isotopomers **1a**–**d** and all *E*-retinal (**2a**).

Previously, ^{13}C-labelled11*Z*-retinal isotopomers have been obtained by photoisomerisation of all *E*-retinals already containing appropriately positioned ^{13}C labels introduced by total synthesis.[7–9] This approach requires separation of the desired 11*Z*-retinal from a complex mixture of retinal stereoisomers and

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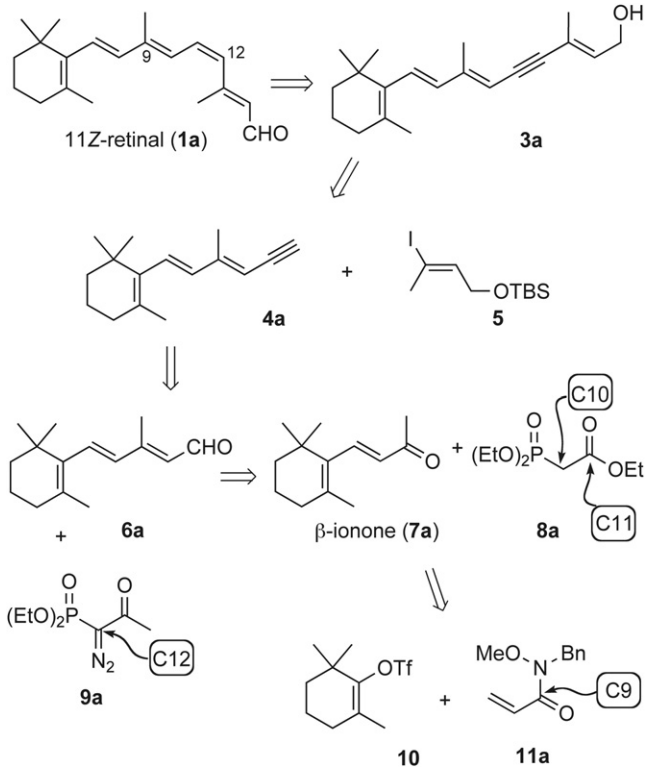
degradation products by means of preparative normal phase HPLC. Stereocontrolled syntheses of unlabelled 11*Z*-retinals have been reported,[10,11] although we are not aware of reports detailing the application of these synthetic routes to obtain ^{13}C-labelled compounds. The stereocontrolled synthesis of 11*Z*-retinal is complicated by facile chemical and photochemical isomerisation of the polyene system. In the present work, our objective was to develop stereocontrolled syntheses of 11*Z*-retinals containing adjacent ^{13}C labels at positions close to the site of isomerisation in the rhodopsin retylidene chromophore. Here we describe syntheses of three 11*Z*-retinal isotopomers **1b**–**d**.

For the solid-state NMR studies, three different doubly labelled11*Z*-retinals **1b**–**d** were required, containing pairs of adjacent ^{13}C labels at C9/C10, C10/C11 and C11/C12, respectively. Our synthetic approach was based on the previously demonstrated coupling of the alkyne and iodoalkene fragments **4a** and **5** to afford dehydroretinol (**3a**), followed by zinc-mediated semi-hydrogenation of the alkyne **3a**, and subsequent oxidation of the allylic alcohol to afford 11*Z*-retinal (Scheme 1).[10b,12] Alkyne fragment **4a** was to be derived from aldehyde **6a**, which would in turn come from *E*-selective olefination of β-ionone (**7a**).[13,14] Synthesis of β-ionone would proceed by Heck coupling of cyclohexenyl triflate **10** and the acrylamide derivative **11a**.[15] With the synthetic plan in place, appropriate commercially available ^{13}C-labelled starting materials were identified to be: [1-^{13}C]-acetic acid, [2-^{13}C]-triethyl phosphonoacetate, [1,2-$^{13}C_2$]-bromoethyl acetate and $^{13}CH_3I$.



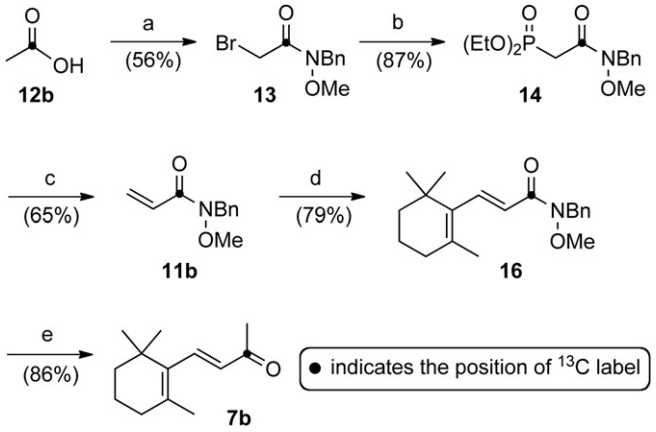
Scheme 1. Overview of the synthesis of 11*Z*-retinals suitable for introducing pairs of ^{13}C labels at the C9/C10, C10/C11 or C11/C12 positions.

2. Results and discussion

For clarity, the syntheses of the three different doubly $^{13}C_2$-labelled isotopomers **1b**–**d** are described separately. Some of the reactions discussed below were initially performed using the unlabelled material, and any significant differences in yields or stereoselectivities, where observed, are discussed.

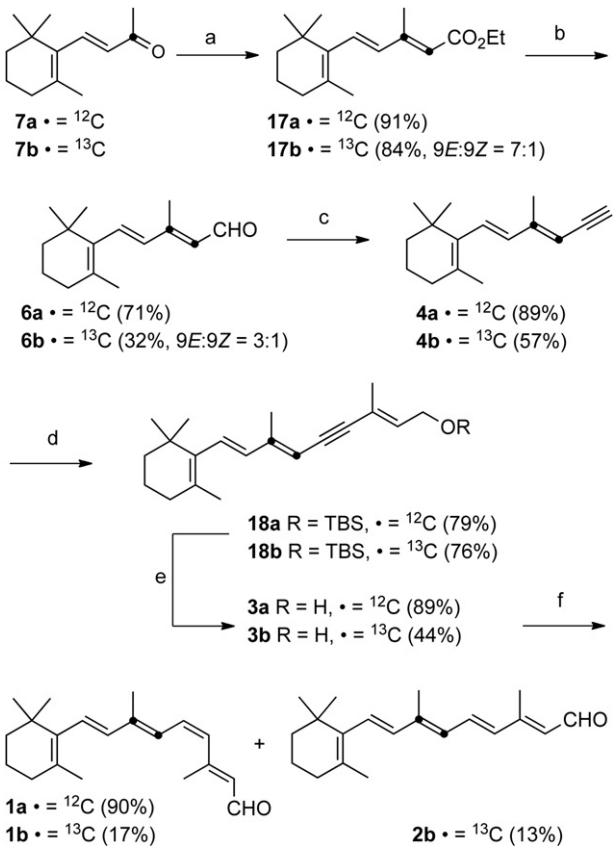
2.1. [9,10-$^{13}C_2$]-11*Z*-Retinal

Incorporation of the ^{13}C label at the C9 position in retinal required its introduction during the synthesis of ^{13}C-β-ionone **7b** (Scheme 2). The direct Heck reaction between labelled methyl vinyl ketone and the cyclohexenyl triflate **10** was considered to be unattractive due to the practical difficulties in manipulating relative small quantities of volatile and expensive labelled building blocks.[15] We therefore targeted a novel Weinreb amide derivative **11b**, which would serve as a precursor to the methyl ketone once coupled to vinyl triflate **10**. Thus, bromination of commercial [1-^{13}C]-acetic acid (**12b**) and subsequent treatment with *N*-benzylmethoxyamine afforded bromoacetamide derivative **13** in 56% yield over the two steps. Arbuzov reaction of **13** with triethyl phosphite followed by Horner–Emmons olefination with formaldehyde provided acrylamide **11b**. Triflate **10** was prepared from 2,6-dimethylcyclohexanone as described in good yield,[15] then coupled with acrylamide **11b** to afford the Weinreb amide derivative **16**. Finally, addition of methyllithium to the Weinreb amide analogue **16** provided the desired β-ionone isotopomer **7b** containing the required ^{13}C label at C9.



Scheme 2. Reagents and conditions: (a) (i) [1-^{13}C]-acetic acid, PBr_3, Br_2, reflux, (ii) BnNH(OMe), Et_3N, CH_2Cl_2, 0 °C; (b) $P(OEt)_3$, 180 °C; (c) CH_2O, K_2CO_3, H_2O, 40 °C; (d) **10**, $Pd(PPh_3)_2Cl_2$, Et_3N, DMF, 75 °C; (e) MeLi, THF, −78 °C→rt.

Olefination of the β-ionone isotopomer **7b** with commercial [2-^{13}C]-triethyl phosphonoacetate proceeded with good *E*/*Z* selectivity (*E*/*Z*=7:1) and excellent yield (Scheme 3).[13] However, the two-step ester reduction–alcohol oxidation sequence returned an unexpectedly poor yield of aldehyde **6b** due to decomposition and isomerisation at the C9 double bond during silica gel purification. The same chemistry, previously carried out on the unlabelled material, had progressed smoothly in 71% yield and without substantial isomerisation, although the sensitivity of the aldehyde **6** has been noted by others.[14] Reaction of the mixture of stereoisomers **6b** (*E*/*Z*=3:1) with $TMSCHN_2$ afforded alkyne **4b** in 57% yield, with enrichment of the *E*-isomer to 12:1 after chromatography. Palladium-catalysed cross-coupling of alkyne **4b** with the vinyl iodide fragment **5** returned the desired enyne **18b** as a mixture with unreacted vinyl iodide **5**.[10b] This mixture was subjected to silyl deprotection to secure [9,10-$^{13}C_2$]-11*Z*-dehydroretinol (**3b**) in 44% isolated yield. Hydrogenation with activated zinc provided [9,10-$^{13}C_2$]-11*Z*-retinol with *Z*/*E* ratio at C11 of 3:1.[10b] Oxidation of the crude unseparated retinols with TPAP and NMO occurred rapidly to provide pure samples of [9,10-$^{13}C_2$]-11*Z*-retinal (**1b**) and [9,10-$^{13}C_2$]-11*E*-retinal (**2b**) after preparative HPLC separation on a silica column. The isomers were identified on the basis of HPLC retention times, and through successful incorporation of [9,10-$^{13}C_2$]-11*Z*-retinal into rhodopsin.[16]

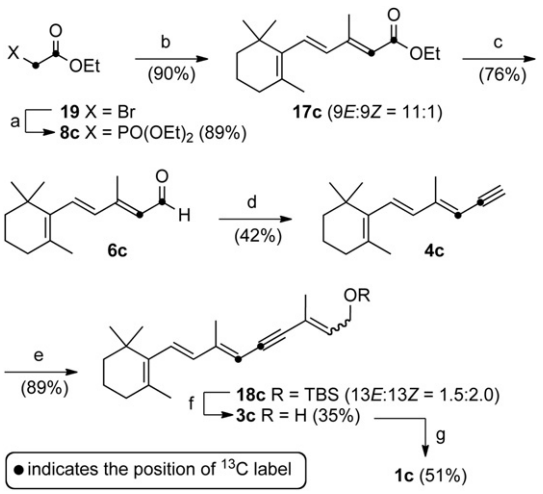


Scheme 3. Synthesis of [9,10-$^{13}C_2$]-11*Z*-retinal (**1b**). Reagents and conditions: (a) triethyl phosphonoacetate or [2-^{13}C]-triethyl phosphonoacetate, NaH, Et_2O; (b) (i) $LiAlH_4$, Et_2O, −78 °C→rt, (ii) TPAP, NMO, 4 Å sieves, CH_2Cl_2; (c) $TMSCHN_2$, LDA, THF, −78 °C; (d) **5**, $Pd(PPh_3)_4$, CuI, *i*-$PrNH_2$; (e) TBAF, THF, 0 °C→rt; (f) (i) Zn, $Cu(OAc)_2$, $AgNO_3$, H_2O/MeOH, 40 °C, (ii) TPAP, NMO, 4 Å sieves, CH_2Cl_2.

The final steps of the syntheses had been conducted in the dark, or in dim red light when necessary. Despite these precautions, some isomerisation of 11*Z*-retinal occurred during manipulation of material. It is evident that several of the steps involving the labelled intermediates were accomplished with significantly reduced yields and selectivities in comparison to the same reactions using unlabelled material. The cause was later traced to a poor quality batch of silica gel, and although similar problems were largely avoided in subsequent syntheses, these results do highlight the well-known sensitivity of the polyene intermediates towards thermal, acid catalysed and photochemical isomerisation.[8d,17] However, once purified, the samples of 11*Z*-retinals have been stored at −78 °C in the dark, and have been used for incorporation into rhodopsin without problem over a number of years.

2.2. [10,11-$^{13}C_2$]-11*Z*-Retinal

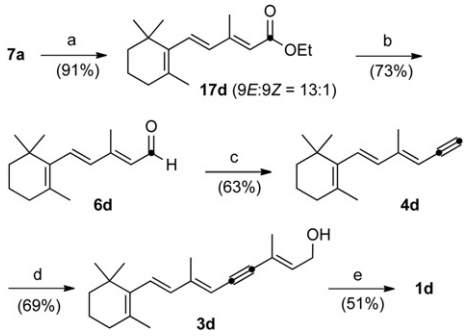
[10,11-$^{13}C_2$]-11*Z*-Retinal (**1c**) was the most conveniently accessible of the three isotopomers due to the availability of both ^{13}C labels in commercially available [1,2-$^{13}C_2$]-bromoethyl acetate, which was readily converted to the phosphonate **8c** using the Arbuzov reaction (Scheme 4).[9] Sequential Horner–Emmons reaction with β-ionone (**7a**), ester reduction then oxidation gave aldehyde **6c** in excellent yield and *E*/*Z* ratio of 11:1. Subsequent reaction with $TMSCHN_2$ provided the alkyne **4c**, which was then taken through the sequence described above to afford [10,11-$^{13}C_2$]-11*Z*-retinal (**1c**). Unfortunately, we were once again inconvenienced by an unexpected isomerisation, this time at C13 (*E*/*Z*=1.5:2.0) in the enyne **18c** during column chromatography. This isomerisation had not been observed in previous syntheses, and was attributed to direct exposure of the reaction mixture to silica gel column chromatography. As a precaution in subsequent reactions, an aqueous extraction was conducted and reaction mixtures were filtered through basic alumina prior to purification on silica gel. Separation of the desired 13*E*-isomer from the 13*Z*-isomer was possible following silyl deprotection. Pleasingly, selective hydrogenation provided the [10,11-$^{13}C_2$]-11*Z*-retinol as the only observable isomer by NMR, a considerable improvement on the 3:1 previously attained. This result maybe attributed to the exclusion of light and the use of HPLC grade solvents (including water). Final oxidation proceeded smoothly and [10,11-$^{13}C_2$]-11*Z*-retinal was isolated by preparative HPLC in good yield along with smaller amounts of the 11*E*-isomer and mixed isomers, which were formed during manipulation of the material rather than as a result of the reactions themselves.



Scheme 4. Synthesis of [10,11-$^{13}C_2$]-11*Z*-retinal (**1c**). Reagents and conditions: (a) $P(OEt)_3$, 180 °C; (b) β-ionone (**7a**), NaH, Et_2O; (c) (i) $LiAlH_4$, Et_2O, −78 °C→rt, (ii) TPAP, NMO, 4 Å sieves, CH_2Cl_2; (d) $TMSCHN_2$, LDA, THF, −78 °C; (e) **5**, $Pd(PPh_3)_4$, CuI, *i*-$PrNH_2$; (f) TBAF, THF, 0 °C→rt; (g) (i) Zn, $Cu(OAc)_2$, $AgNO_3$, H_2O/*i*-PrOH, 40 °C, (ii) TPAP, NMO, 4 Å sieves, CH_2Cl_2.

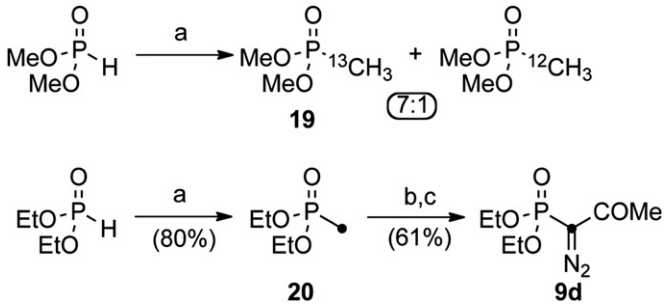
2.3. [11,12-$^{13}C_2$]-11*Z*-Retinal

Incorporation of the ^{13}C label at C11 was performed as previously described (10,11-$^{13}C_2$-11*Z*-retinal) utilising a ^{13}C-labelled triethyl phosphonate in the Horner Emmons reaction with β-ionone (**7a**) in high yield and *E*/*Z* selectivity (Scheme 5). Ester reduction and immediate oxidation yielded the labelled aldehyde **6d**. The previous isotopomer syntheses had made use of $TMSCHN_2$ to



Scheme 5. Synthesis of [11,12-$^{13}C_2$]-11*Z*-Retinal. Reagents and conditions: (a) [1-^{13}C]-triethyl phosphonoacetate, NaH, Et_2O; (b) (i) $LiAlH_4$, Et_2O, −78 °C→rt, (ii) TPAP, NMO, 4 Å sieves, CH_2Cl_2; (c) **9d**, NaOMe, MeOH (1 equiv), THF, −78 °C→rt; (d) (i) **5**, $Pd(PPh_3)_4$, CuI, *i*-$PrNH_2$, (ii) TBAF, THF, 0 °C→rt; (e) (i) Zn, $Cu(OAc)_2$, $AgNO_3$, H_2O/MeOH, 40 °C, (ii) TPAP, NMO, 4 Å sieves, CH_2Cl_2.

introduce C12 into **4a**–**c**. However, a ^{13}C-labelled Bestmann–Ohira reagent was preferred to TMS $^{13}CHN_2$on the basis of synthetic accessibility.[18] Initial investigations began with the preparation of a labelled methoxy reagent **19** using a modified Michaelis–Becker reaction,[19] however isotopic dilution was observed (Scheme 6). This problem was resolved by starting from diethyl phosphite, resulting in the formation of the desired phosphonate **20** in high yield.[20] Acetylation of phosphonate **20** followed by diazo transfer furnished the [1,2-$^{13}C_3$]-Bestmann–Ohira reagent **9d**. Deacylative activation of **9d** was carried out by pre-treatment with 1 equiv of NaOMe before adding aldehyde **6d**, which was smoothly converted to the alkyne **4d**. More conventional reaction conditions (K_2CO_3, MeOH) resulted in the formation of the desired product **4d** in 74% yield,[18] but with isomerisation (6:1) resulting from methoxide addition–elimination to the unsaturated aldehyde **6d**. Sonogashira reaction of the alkyne **4d** with iodide **5** proceeded efficiently, and the crude coupling reaction mixture was subjected to silyl deprotection. Careful filtration of crude mixture through neutral alumina followed by silica gel chromatography helped to minimise isomerisation. Subsequent alkyne reduction, final alcohol oxidation and purification by preparative HPLC afforded [11,12-$^{13}C_2$]-11*Z*-retinal (**1d**).



Scheme 6. Synthesis of a ^{13}C-labelled Bestmann–Ohira Reagent. Reagents and conditions: (a) $^{13}CH_3I$, K_2CO_3, 35 °C then μW, 110 °C; (b) *n*-BuLi, CuI, THF, −60 °C→−30 °C, then AcCl, THF, −40 °C→rt; (c) TsN_3, NaH, benzene/THF, 0 °C→rt.

3. Conclusions

The stereoselective synthesis of three isotopomers, [9,10-$^{13}C_2$]-11*Z*-retinal (**1b**), [10,11-$^{13}C_2$]-11*Z*-retinal (**1c**), [11,12-$^{13}C_2$]-11*Z*-retinal (**1c**), has been achieved. The syntheses utilised relatively inexpensive ^{13}C enriched building blocks, lead to the development of a Weinreb acrylamide and incorporates the use of a ^{13}C-labelled Bestmann–Ohira reagent on an α,β-unsaturated aldehyde, using the pre-deacetylation of the reagents to minimise isomerisation. Samples of the doubly ^{13}C-labelled retinal isotopomers have been successful combined with the apoprotein opsin to generate rhodopsin with a labelled chromophore, and these specifically labelled proteins have been used in solid-state NMR experiments to study conformational changes in the rhodopsin chromophore and its photointermediates.[5c] Some difficulties were encountered due to undesired isomerisation of certain polyunsaturated intermediates, highlighting their sensitive nature. However, these technical challenges were largely overcome during the synthesis of the final isotopomer **1d**.

4. Experimental

4.1. General methods

All reactions were carried out under an inert atmosphere in oven dried glassware. THF and Et_2O were distilled from sodium and benzophenone prior to use. Triethylamine and dichloromethane were dried by distillation from CaH_2. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or C_6D_6 solutions using Bruker AC300, AV300 (300 and 75 MHz, respectively) or Bruker DPX400 (400 and 100 MHz, respectively) spectrometers. ^{19}F and ^{31}P NMR spectra were recorded in solution using a Bruker AV300 (282 and 121 MHz, respectively). Chemical shifts are reported in δ units using $CHCl_3$ or C_6H_6 as an internal standard (δ 7.27 ppm ^{1}H, δ 77.36 ppm ^{13}C, δ 7.15 ppm ^{1}H, δ 128.62 ppm ^{13}C, respectively). Infrared spectra were recorded on a Nicolet 380 fitted with a Diamond platform, as solids or neat liquids. Melting points were obtained using a Gallenkamp Electrothermal apparatus and are uncorrected. Electron impact and chemical ionisation mass spectra were obtained using a Fisons VG platform single quadropole mass spectrometer. Electrospray mass spectra were obtained using a Micromass platform mass analyser with an electrospray ion source. Reactions introducing and containing the 11*Z*isomers were carried out in dim red light conditions using base washed glassware. HPLC purification of retinal was performed with a Shimadzu VP series HPLC and Phenomenex silica column, eluting with Et_2O/hexane. The positions of ^{13}C labels are indicated using the retinal numbering system, except for compounds **9d** and **20**.

4.2. Preparation of compounds

*4.2.1. [9-^{13}C]-N-Benzyl-2-bromo-N-methoxyacetamide (**13**).* To [1-^{13}C]-acetic acid (1.00 g, 16.4 mmol) and PBr_3 (1.95 mL, 16.4 mmol) was added bromine (2.10 mL, 41.0 mmol) dropwise. The mixture was heated to 75 °C and stirred for 3 h. Distillation at atmospheric pressure removed excess bromine and then [1-^{13}C]-bromoacetyl bromide (155–158 °C). The resulting [1-^{13}C]-bromoacetyl bromide was dissolved in CH_2Cl_2 (45 mL), then cooled to 0 °C. A solution of *N*-benzyl-*O*-methylhydroxylamine[21] (2.25 g, 16.4 mmol) in CH_2Cl_2 (5 mL) was added dropwise. After 10 min, Et_3N (2.28 mL, 16.4 mmol) was added dropwise and the reaction stirred at 0 °C. After 1 h the reaction was quenched with H_2O and extracted into CH_2Cl_2 and the combined organics were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel column chromatography eluting with 1% Et_2O/CH_2Cl_2 gave the amide **13** as a colourless oil (2.39 g, 9.21 mmol, 56%). FT-IR (neat) ν_{max}1620 (^{13}C=O)cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.30 (5H, m), 4.83 (2H, d, J_{CH}=1.8 Hz), 4.05 (2H, d, J_{CH}=3.8 Hz), 3.74 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1 (^{13}C), 135.9 (C), 128.9 (CH), 128.7 (CH), 128.3 (CH), 62.8 (CH_3), 49.7 (CH_2), 25.8 (CH_2, d, J_{CC}=57.7 Hz) ppm; LRMS (CI, NH_3) *m/z* 259 [M (^{79}Br)+H]$^+$, 261 [M (^{81}Br)+H]$^+$;HRMS (ES^+) $C_9{}^{13}CH_{13}{}^{79}BrNO_2$, calculated 259.0159, found 259.0160.

*4.2.2. [9-^{13}C]-Diethyl(N-benzyl-N-methoxycarbamoyl)-methyl phosphonate (**14**).* A mixture of amide **13** (2.37 g, 9.16 mmol) and triethyl phosphite (1.57 mL, 9.16 mmol) was heated to 180 °C for 1 h giving a colourless oil. Purification by silica gel column chromatography eluting with EtOAc then 10% $MeOH/CH_2Cl_2$ gave the title compound**14** as a colourless oil (2.51 g, 7.95 mmol, 87%). FT-IR (neat) ν_{max} 1615 (^{13}C=O), 1250 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.20 (5H, m), 4.83 (2H, d, J_{CH}=2.2 Hz), 4.17 (4H, qd, J_{HH}=7.0 Hz, J_{HP}=8.2 Hz), 3.72 (3H, s), 3.20 (2H, dd, J_{CH}=6.8 Hz, J_{HP}=22.0 Hz), 1.33 (6H, td, J_{HH}=7.0 Hz, J_{HP}=0.6 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5 (^{13}C), 136.3 (C), 128.9 (CH), 128.8 (CH), 128.1 (CH), 62.9 (CH_2, d, J_{CP}=6.1 Hz), 62.6 (CH_3), 49.1 (CH_2), 32.0 (CH_2, dd, J_{CP}=135.5 Hz, J_{CC}=53.1 Hz), 16.6 (CH_3, d, J_{CP}=6.0 Hz) ppm; ^{31}P NMR (121 MHz, $CDCl_3$) δ 21.6 (s) ppm; LRMS (ES^+) *m/z* 339 [M+Na]$^+$; HRMS (ES^+) $C_{13}{}^{13}CH_{23}NO_5P$, calculated 317.1342, found 317.1340.

*4.2.3. [9-^{13}C]-N-Benzyl-N-methoxyacrylamide (**11b**).* Phosphonate **14** (2.45 g, 7.73 mmol) and K_2CO_3 (3.21 g, 23.2 mmol) were suspended in H_2O (5 mL) and stirred for 15 min. CH_2O(aq) (1.15 mL, 15.5 mmol) was added dropwise and the reaction was warmed to

40 °C and stirred for 30 min. To the reaction 6 aliquots of CH_2O(aq) (0.58 mL, 7.73 mmol) were added at 30 min intervals. The reaction was diluted with Et_2O and H_2O then separated and the aqueous phase extracted with Et_2O (×3). The combined organics were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Purification silica gel column chromatography eluting with 30% EtOAc/hexane gave the title compound **11b** as a colourless oil (965 mg, 5.02 mmol, 65%). FT-IR (neat) ν_{max}1634 (^{13}C=O), 1595 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45–7.22 (5H, m), 6.77 (1H, ddd, J_{HH}=17.0, 10.2 Hz, J_{CH}=4.4 Hz), 6.50 (1H, ddd, J_{HH}=17.0, 2.0 Hz, J_{CH}=6.8 Hz), 5.81 (1H, ddd, J_{HH}=10.2, 2.0 Hz, J_{CH}=12.4 Hz), 4.87 (2H, d, J_{CH}=2.0 Hz), 3.65 (3H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.8 (^{13}C), 136.7 (C), 130.0 (CH_2), 128.9 (CH) 128.8 (CH), 128.1 (CH), 126.4 (CH, J_{CC}=65.8 Hz), 63.1 (CH_3), 49.7 (CH_2) ppm; LRMS (ES^+) *m/z* 215 [M+Na^+]; HRMS (ES^+) $C_{10}{}^{13}CH_{14}NO_2$, calculated 193.1053, found 193.1050.

*4.2.4. [9-^{13}C]-(2E)-N-Benzyl-N-methoxy-3-(2,6,6-trimethylcyclohex)-1-enyl acrylamide (**16**).* To $Pd(PPh_3)_2Cl_2$ (56 mg, 0.08 mmol) suspended in DMF (5 mL) was added a solution of amide **11b** (548 mg, 2.85 mmol), triflate **10** (981 mg, 3.60 mmol) and Et_3N (1.76 mL, 12.6 mmol) in DMF (5 mL) the reaction was warmed to 75 °C and stirred for 22 h. The mixture was diluted with Et_2O and H_2O then separated. The aqueous phase was extracted with Et_2O (×4) and the combined organics washed with brine (×3), dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel column chromatography eluting with 15% EtOAc/hexane gave the title compound **16** as an amber oil (708 mg, 2.25 mmol, 79%). FT-IR (neat) ν_{max} 1631 (^{13}C=O), 1596 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52 (1H, dd, J_{HH}=16.1 Hz, J_{CH}=6.5 Hz), 7.40–7.25 (5H, m), 6.43 (1H, dd, J_{HH}=16.1 Hz, J_{CH}=4.3 Hz), 4.89 (2H, d, J_{CH}=1.8 Hz), 3.64 (3H, s), 2.07 (2H, t, J=6.3 Hz), 1.79 (3H, d, J=0.5 Hz), 1.70–1.60 (2H, m), 1.55–1.45 (2H, m), 1.09 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7 (^{13}C), 143.8 (CH), 137.1 (C), 136.9 (C, J_{CC}=5.8 Hz), 135.0 (C), 128.9 (CH), 128.8 (CH), 127.9 (CH), 119.9 (CH, J_{CC}=66.6 Hz), 63.2 (CH_3), 49.8 (CH_2), 40.1 (CH_2), 34.4 (C), 33.8 (CH_2), 29.2 (CH_3), 22.1 (CH_3), 19.3 (CH_2) ppm; LRMS (ES^+) *m/z* 315 $[M+H]^+$; HRMS (ES^+) for $C_{19}{}^{13}CH_{28}NO_2$, calculated 315.2148, found 315.2150.

*4.2.5. [9-^{13}C]-β-Ionone (**7b**).* To acrylamide **16** (702 mg, 2.23 mmol) in THF (40 mL) at −78 °C was added MeLi (2.23 mL of 1.6 M in Et_2O, 3.57 mmol) dropwise. The reaction was stirred at −78 °C for 45 min then warmed to −20 °C and stirred for 20 min. The reaction was quenched with a saturated NH_4Cl(aq), diluted with Et_2O and separated. The organics were washed with a saturated NH_4Cl(aq) and the aqueous phase extracted with Et_2O (×3). The combined organics were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel column chromatography eluting with 10% Et_2O/hexane gave the title compound **7b** as a pale yellow oil (370 mg, 1.91 mmol, 86%). Spectroscopic data were consistent with selected reported values for the labelled and unlabelledβ-ionone.[22] FT-IR (neat) ν_{max} 1665 (^{13}C=O), 1647 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28 (1H, dd, J_{HH}=16.5 Hz, J_{CH}=6.8 Hz), 6.12 (1H, dd, J_{HH}=16.5 Hz, J_{CH}=3.1 Hz), 2.30 (3H, d, J_{CH}=5.7 Hz), 2.08 (2H, t, J=6.1 Hz), 1.77 (3H, s), 1.70–1.55 (2H, m), 1.53–1.40 (2H, m), 1.08 (6H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.1 (^{13}C), 143.5 (CH, d, J_{CC}=1.7 Hz), 136.4 (2× C), 131.9 (CH, d, J_{CC}=52.7 Hz), 40.1 (CH_2), 34.4 (C), 33.9 (CH_2), 29.1 (2× CH_3), 27.5 (CH_3, d, J_{CC}=42.0 Hz), 22.1 (CH_3), 19.2 (CH_2) ppm; LRMS (CI, NH_3) *m/z* 194 $[M+H]^+$; HRMS (EI) for $C_{12}{}^{13}CH_{20}O$, calculated 193.1548, found 193.1549.

*4.2.6. [11-^{13}C]-(2E,4E)-Ethyl3-methyl-5-(2,6,6-trimethylcyclo hex-1-enyl)penta-2,4-dienoate (**17d**).* To a slurry of NaH (178 mg, 4.44 mmol) in Et_2O (5 mL) was added [1-^{13}C]-triethyl phosphonoacetate (1.00 g, 4.44 mmol) dropwise. This mixture was stirred for 2 h. To the reaction was added β-ionone (**7a**) (569 mg, 2.96 mmol) in Et_2O (2 mL) dropwise, the yellow solution was stirred for 62 h forming a white suspension. H_2O was added and the mixture was extracted with hexane (×4). The combined organics were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel column chromatography eluting with 2%→3% EtOAc/hexane afforded the title ester **17d** as a pale yellow oil (713 mg, 2.71 mmol, 91%, *E*/*Z*=13:1). Spectroscopic and physical data were consistent with reported values for the unlabelled compound.[23] FT-IR (neat) ν_{max} 1669 (^{13}C=O), 1606 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.60 (1H, d, J=16.1 Hz), 6.10 (1H, d, J=16.1 Hz), 5.75 (1H, s), 4.18 (2H, qd, J_{HH}=7.1 Hz, J_{CH}=3.0 Hz), 2.34 (3H, t, J=1.3 Hz), 2.03 (2H, t, J=6.0 Hz), 1.70 (3H, s), 1.67–1.59 (2H, m), 1.51–1.45 (2H, m), 1.30 (3H, t, J=7.1 Hz), 1.03 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6 (^{13}C), 153.1 (C), 137.6 (C), 136.6 (CH, d, J_{CC}=8.7 Hz), 134.0 (CH), 131.4 (C), 118.4 (CH, d, J_{CC}=77.8 Hz), 59.9 (CH_2, d, J_{CC}=1.9 Hz), 39.9 (CH_2), 34.6 (C), 33.4 (CH_2), 29.2 (CH_3), 22.0 (CH_3), 19.5 (CH_2), 14.7 (CH_2, d, J_{CC}=1.4 Hz), 14.0 (CH_3) ppm; LRMS (ES^+) *m/z* 264 $[M+H]^+$; HRMS (ES^+) for $C_{16}{}^{13}CH_{27}O_2$, calculated 264.2040, found 264.2039.

*4.2.7. [11-^{13}C]-(2E,4E)-3-Methyl-5-(2,6,6-trimethylcyclohex-1-enyl) penta-2,4-dienal (**6d**).* To a slurry of $LiAlH_4$ (162 mg, 4.28 mmol) in Et_2O (8 mL) at −78 °C was added ester **17d** (705 mg, 2.68 mmol, 9-*E*/*Z*=13:1) in Et_2O (27 mL) dropwise, and the reaction stirred for 1 h at −78 °C. The reaction was warmed to rt and stirred for 2.5 h. The reaction was quenched with H_2O (0.2 mL), 15% NaOH (0.2 mL) and H_2O (0.6 mL) sequentially and stirred for 20 min producing a white precipitate. The heterogeneous mixture was dried ($MgSO_4$) and the precipitate removed by filtration, the solution was concentrated in vacuo giving a colourless oil. The oil was taken up in CH_2Cl_2 (28 mL) and crushed molecular sieves (1.6 g), NMO (628 mg, 5.36 mmol) and TPAP (94 mg, 0.27 mmol) added. After stirring at rt for 30 min the black suspension was filtered through Celite and concentrated in vacuo. Purification by silica gel column chromatography eluting with 4% EtOAc/hexane afforded the title aldehyde **6d** as a pale yellow oil (431 mg, 1.97 mmol, 73%) and 9*Z*-aldehyde (44 mg, 0.20 mmol, 7%). ^1H NMR data were consistent with reported values for the unlabelled compound.[14,24] FT-IR (neat) ν_{max}1629 (^{13}C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.14 (1H, dd, J_{HH}=8.0 Hz, J_{CH}=169.8 Hz), 6.75 (1H, d, J=16.1 Hz), 6.22 (1H, d, J=16.1 Hz), 5.94 (1H, d, J=8.0 Hz), 2.32 (3H, s), 2.06 (2H, br t, J=6.3 Hz), 1.73 (3H, s), 1.68–1.59 (2H, m), 1.52–1.45 (2H, m), 1.05 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.6 (^{13}CH), 155.3 (C, d, J_{CC}=2.9 Hz), 137.4 (C), 136.0 (CH, d, J_{CC}=6.8 Hz), 135.9 (C), 133.1 (CH), 129.1 (CH, d, J_{CC}=56.4 Hz), 39.9 (CH_2), 34.6 (C), 33.6 (CH_2), 29.3 (CH_3), 22.1 (CH_3), 19.4 (CH_2), 13.3 (CH_3, d, J_{CC}=4.9 Hz) ppm; LRMS (ES^+) *m/z* 220 $[M+H]^+$; HRMS (ES^+) for $C_{14}{}^{13}CH_{23}O$, calculated 220.1777, found 220.1780.

*4.2.8. [11,12-$^{13}C_2$]-1,3,3-Trimethyl-2-((1E,3E)-3-methylhexa-1,3-dien-5-ynyl)cyclohex-1-ene (**4d**).* To ^{13}C-labelled diazophosphonate **9d** (391 mg, 1.77 mmol) in THF (20 mL) at −78 °C was added NaOMe (0.5 M in MeOH, 3.54 mL, 1.77 mmol) dropwise over 15 min. After stirring for 1 h at −78 °C aldehyde **6d** (259 mg, 1.18 mmol) in THF (8 mL) was added dropwise over 10 min. The yellow solution was stirred at −78 °C for 30 min, warmed to rt over 30 min and stirred for 7.5 h. The reaction was quenched with H_2O, dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel column chromatography eluting with 5% EtOAc/hexane gave the desired alkyne **4d** as a pale yellow oil (162 mg, 0.75 mmol, 63%) and starting aldehyde **6d** (39 mg, 0.18 mmol, 15%).Spectroscopic data were consistent with selected reported values for the unlabelled compound.[25] FT-IR (neat) ν_{max}2010 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.29 (1H, d, J=16.1 Hz), 6.10 (1H, d, J=16.1 Hz), 5.41 (1H, br s), 3.29(1H, m), 2.09 (3H, s), 2.02 (2H, t, J=6.0 Hz), 1.70 (3H, br d, J=1.0 Hz), 1.66–1.58 (2H, m), 1.50–1.45 (2H, m), 1.02 (6H, s) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ 149.6 (C), 137.7 (C), 135.4 (CH, d, J_{CC}=8.8 Hz), 130.6 (CH), 130.5 (C), 107.6 (CH, dd, J_{CC}=70.0, 30.1 Hz), 84.1 (^{13}CH, d, J_{CC}=200.2 Hz), 82.4 (^{13}C, d, J_{CC}=200.2 Hz), 39.9 (CH_2), 34.6 (C), 33.4 (CH_2), 29.2 (CH_3), 22.0 (CH_3), 19.6 (CH_2), 15.4 (CH_3) ppm; LRMS (CI, NH_3) *m/z* 217 [M+H$^+$].

*4.2.9. [11,12-$^{13}C_2$]-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)-nona-2,6,8-trien-4-yn-1-ol (**3d**).* Following the method of Borhan et al.,[10b] to a solution of iodide **5**(405 mg, 1.30 mmol) in *i*-PrNH_2 (3 mL) was added $Pd(PPh_3)_4$ (13 mg, 10.82 μmol) and the reaction was stirred for 5 min. CuI (2 mg, 10.82 μmol) was added and the reaction was stirred for a further 5 min. Alkyne **4d** (234 mg, 1.08 mmol) in *i*-PrNH_2 (1.6 mL) was added dropwise and the reaction stirred for 3.5 h. The reaction was concentrated in vacuo, redissolved in Et_2O, washed with H_2O (×3) and brine, and the organics concentrated in vacuo. The residue as a mixture of the desired alkyne **18d**[26] and iodide **5** (4:1, 465 mg, ~1.21 mmol) was dissolved in THF (6 mL) and cooled to 0 °C. TBAF (1.0 M in THF, 1.34 mL, 1.34 mmol) was added dropwise and the reaction warmed to rt and stirred for 1 h. The reaction was quenched with H_2O and the extracted with Et_2O (×3). The combined organics were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The crude material was filtered through a plug of neutral alumina eluting with 20% EtOAc/hexane and concentrated in vacuo. Purification by silica gel column chromatography eluting with 15% EtOAc/hexane gave the desired dehydroretinol **3d** (191 mg, 0.67 mmol, 69% over two steps) and the 13*Z*-isomer (23 mg, 0.08 mmol, 8%). *Data for* **3d**: FT-IR (neat) ν_{max}3313 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (1H, d, *J*=16.1 Hz), 6.12 (1H, d, *J*=16.1 Hz), 6.01 (1H, br t d, J_{HH}=7.5 Hz, J_{CH}=7.5 Hz), 5.53 (1H, d, J_{CH}=4.8 Hz), 4.27 (2H, br t, *J*=5.4 Hz), 2.07 (3H, s), 2.02 (2H, t, *J*=6.3 Hz), 1.90 (3H, m), 1.70 (3H, s), 1.66–1.58 (2H, m), 1.51–1.44 (2H, m), 1.03 (6H, s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8 (C), 137.8 (C), 135.8 (CH, d, J_{CC}=9.3 Hz), 134.7 (CH, d, J_{CC}=3.9 Hz), 130.4 (C), 129.9 (CH), 108.8 (CH, dd, J_{CC}=91.4, 11.7 Hz), 98.8 (^{13}C, d, J_{CC}=177.9 Hz), 87.2 (^{13}C, d, J_{CC}=177.9 Hz), 59.6 (CH_2, d, J_{CC}=6.8 Hz), 39.9 (CH_2), 34.6 (C), 33.4 (CH_2), 29.3 (CH_3), 22.0 (CH_3), 19.6 (CH_3), 18.0 (CH_2), 15.4 (CH_3, d, J_{CC}=3.9 Hz) ppm; LRMS (ES$^+$) *m/z* 269 [M−H_2O+H]$^+$. *Data for* 13*Z-Isomer*: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.29 (1H, d, *J*=16.1 Hz), 6.12 (1H, d, *J*=16.1 Hz), 5.86 (1H, tqd, J_{HH}=6.8, 1.5 Hz, J_{CH}=13.8 Hz,), 5.57 (1H, br s), 4.36 (2H, d, *J*=6.8 Hz), 2.08 (3H, dd, J_{HH}=0.8 Hz, J_{CC}=0.8 Hz), 2.02 (2H, t, *J*=5.9 Hz), 1.95 (3H, m), 1.70 (3H, br s), 1.67–1.55 (2H, m), 1.52–1.43 (2H, m), 1.03 (6H, s) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.9 (C), 137.8 (C), 135.7 (CH, dd, J_{CC}=5.5, 3.3 Hz), 134.8 (CH), 130.5 (C), 130.2 (CH), 121.9 (C, dd, J_{CC}=58.1, 38.7 Hz), 108.6 (CH, dd, J_{CC}=61.1, 41.2 Hz), 95.4 (^{13}C, d, J_{CC}=176.9 Hz), 93.0 (^{13}C, d, J_{CC}=176.9 Hz), 61.9 (CH_2), 39.9 (CH_2), 34.6 (C), 33.4 (CH_2), 29.2 (CH_3), 23.6 (CH_3), 22.0 (CH_3), 19.5 (CH_2), 15.5 (CH_3) ppm.

*4.2.10. [11,12-$^{13}C_2$]-11Z-Retinal (**1d**).* Following the method of Borhan et al.,[10b] argon was bubbled through a suspension of zinc dust (11.42 g, 0.175 mol) in H_2O (68 mL) for 15 min. $Cu(OAc)_2$ (1.14 g, 6.28 mmol) was then added and after 15 min stirring $AgNO_3$ (1.14 g, 6.72 mmol) added (Care!-exothermic). After stirring for 30 min the activated zinc was filtered and washed with H_2O, MeOH, acetone and Et_2O sequentially. The moist zinc catalyst was suspended in H_2O (19 mL) and *i*-PrOH (19 mL) and the labelled dehydroretinol **3d** (134 mg, 0.47 mmol) in *i*-PrOH (19 mL) added. The reaction was heated at 40 °C for 26 h. The reaction was filtered through Celite flushing with H_2O and Et_2O and the phases were separated. The aqueous was extracted with Et_2O (×4) and the combined organics washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The crude labelled retinol (136 mg, ~0.47 mmol) was dissolved in CH_2Cl_2 (5 mL) and crushed molecular sieves (280 mg), NMO (110 mg, 0.94 mmol) and TPAP (49 mg, 0.14 mmol) added sequentially. After 30 min stirring the reaction was passed through a short column of neutral alumina topped with Celite, flushing with Et_2O and concentrated in vacuo. Purification by HPLC eluting with Et_2O (2.00 mL/min) and hexane (7.99 mL/min) gave [11,12-$^{13}C_2$]-11*Z*-retinal (**1d**) as a yellow oil (69 mg, 0.24 mmol, 51% over two steps), [11,12-$^{13}C_2$]-all-*E*-retinal as a yellow oil (5 mg, 0.02 mmol, 4%) and [11,12-$^{13}C_2$]-retinal as a mixture of isomers (22 mg, 0.08 mmol, 16%). Spectroscopic data for **1d** were consistent with those reported for the unlabelled compound.[27] FT-IR (neat) ν_{max}1657 (s), 1566 (m) cm^{-1}; ^{1}H NMR (400 MHz, C_6D_6) δ 9.91 (1H, d, *J*=7.8 Hz), 6.58 (1H, br d, *J*=11.1 Hz), 6.38 (1H, dq, J_{HH}=12.1 Hz, J_{CH}=148.3 Hz), 6.34 (1H, d, *J*=16.1 Hz), 6.22 (1H, d, *J*=16.1 Hz), 6.10 (1H, br t, J_{HH}=7.8 Hz, J_{CH}=7.8 Hz), 5.58 (1H, dd, J_{HH}=11.8 Hz, J_{CH}=154.7 Hz), 1.91 (2H, t, *J*=6.4 Hz), 1.77 (3H, dd, J_{HH}=1.4 Hz, J_{CH}=4.1 Hz), 1.74 (3H, s), 1.68 (3H, s), 1.60–1.52 (2H, m), 1.46–1.42 (2H, m), 1.07 (6H, s) ppm; ^{13}C NMR (100 MHz, C_6D_6) δ 190.5 (CH), 154.6 (C, dd, J_{CC}=39.4, 13.1 Hz), 141.4 (C, d, J_{CC}=5.8 Hz), 138.8 (CH, d, J_{CC}=4.9 Hz), 138.6 (C), 131.9 (^{13}CH, d, J_{CC}=70.0 Hz), 131.1 (^{13}CH, d, J_{CC}=70.0 Hz), 131.1 (CH, dd, J_{CC}=3.9, 2.0 Hz), 130.7 (C), 130.1 (CH), 127.0 (CH, dd, J_{CC}=41.8, 13.6 Hz), 40.4 (CH_2), 35.1 (C), 33.8 (CH_2), 29.7 (CH_3), 22.4 (CH_3), 20.2 (CH_2), 18.0 (CH_3), 12.8 (CH_3, d, J_{CC}=3.4 Hz) ppm; LRMS (ES$^+$) *m/z* 287 [M+H]$^+$; HRMS (ES$^+$) for $C_{18}{}^{13}C_2H_{29}O$, calculated 287.2280, found 287.2279.

*4.2.11. [1-^{13}C]-Diethyl methyl phosphonate (**20**).* To a mixture of diethyl phosphite (0.70 mL, 5.38 mmol) and K_2CO_3 (1.49 g, 10.8 mmol) was added $^{13}CH_3I$ (1.00 g, 6.99 mmol) dropwise and the vessel was sealed. The reaction was stirred at 35 °C for24 h. After which crushed molecular sieves and K_2CO_3 (740 mg, 5.38 mmol) were added. The reaction was stirred for a further 24 h at 35 °C. After this time the mixture was transferred to a microwave tube washing with $CHCl_3$ (2 mL), before the mixture was irradiated (110 °C, 300 W), the reaction was stopped at 9 min. The suspension was filtered washing with $CHCl_3$ and CH_2Cl_2 and the solution was concentrated in vacuo giving a yellow oil. Purification by vacuum transfer (0.4 mbar, rt) yielded the desired phosphonate **20**as a colourless oil (663 mg, 4.33 mmol, 80%). Spectroscopic data were consistent with those reported for the unlabelled compound.[28] FT-IR (neat) ν_{max} 1305, 1227, 1026 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (4H, qdd, J_{HH}=7.1 Hz, J_{CH}=4.6 Hz, J_{HP}=8.2 Hz), 1.47 (3H, dd, J_{CH}=128.2 Hz, J_{HP}=17.5 Hz), 1.33 (6H, t, *J*=7.1 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$)™ 61.8 (CH_2, d, J_{CP}=6.1 Hz), 16.7(CH_3, d, J_{CP}=6.1 Hz), 11.6 ($^{13}CH_3$, d, J_{CP}=144.8 Hz) ppm; ^{31}P NMR (121 MHz, $CDCl_3$)™ 31.1 (d, J_{CP}=144.8 Hz) ppm; LRMS (ES$^+$) *m/z* 154 [M+H]$^+$.

*4.2.12. [1-^{13}C]-Diethyl (1-diazo-2-oxopropyl)phosphonate (**9d**).* Following the procedure of Mathey and Savignac,[29] to a solution of phosphonate **20** (303 mg, 1.98 mmol) in THF (2.5 mL) at −60 °C was added *n*-BuLi (2.34 M in hexane, 0.93 mL, 2.18 mmol) dropwise, after 5 min CuI (415 mg, 2.18 mmol) was added. The reaction was slowly warmed to −30 °C and stirred for 1.5 h, then cooled to −40 °C. Acetyl chloride (0.15 mL, 2.08 mmol) in Et_2O (1.5 mL) was added slowly and the reaction stirred at −35 °C for 2.5 h. The reaction was warmed to rt and stirred for 17 h. The reaction was quenched with H_2O producing a white suspension, which was filtered through Celite, flushing with THF then CH_2Cl_2. The phases were separated and the aqueous extracted with CH_2Cl_2 (×3), and the combined organics were dried ($MgSO_4$) and concentrated in vacuo. Purification by distillation under reduced pressure (0.4 mbar, 60 °C) gave [1-^{13}C]-diethyl (2-oxopropyl)phosphonate as a colourless oil (323 mg, 1.66 mmol, 84%). Spectroscopic data were consistent with reported values for the unlabelled compound.[30] FT-IR (neat) ν_{max}1714, 1246 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.16 (4H, qdd, J_{HH}=7.0 Hz, J_{CH}=1.3 Hz, J_{HP}=8.2 Hz), 3.09 (2H, dd, J_{CH}=128.8 Hz, J_{HP}=22.9 Hz), 2.33 (3H, d, J_{CH}=1.5 Hz), 1.35 (6H, td, J_{HH}=7.0 Hz, J_{HP}=0.6 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 62.9 (CH_2, d, J_{CP}=6.6 Hz), 43.8 (CH_2, d, J_{CP}=127.2 Hz), 31.7 (CH_3, d, J_{CC}=14.9 Hz), 16.6 (CH_3, d, J_{CP}=6.1 Hz) ppm; ^{31}P NMR (121 MHz,

$CDCl_3$) δ 20.3 (d, J=127.0 Hz) ppm; LRMS (ES^+) m/z 196 $[M+H]^+$. To a slurry of NaH (42 mg, 1.06 mmol) in benzene (1.5 mL) and THF (2 mL) at 0 °C was added [1-^{13}C]-diethyl (2-oxopropyl)phosphonate (188 mg, 0.96 mmol) in THF (2 mL). After stirring for 1 h at 0 °C, TsN_3 (209 mg, 1.06 mmol) in THF (1 mL) was added slowly dropwise. The reaction was warmed to rt and stirred for 16 h. The resultant red suspension was filtered through Celite flushing with benzene (×3) followed by EtOAc (×3), the filtrate was concentrated in vacuo. Purification by silica gel column chromatography eluting with EtOAc afforded the title diazo compound **9d** as an oil (156 mg, 0.71 mmol, 73%). Spectroscopic data were consistent with reported values for the unlabelled compound.[31] FT-IR (neat) ν_{max} 2113, 1655, 1260 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 (4H, m), 2.29 (3H, d, J_{CH}=2.2 Hz), 1.40 (6H, td, J_{HH}=7.0 Hz, J_{HP}=0.7 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.6 (^{13}C, d, J_{CP}=218.4 Hz), 53.7 (CH_2, d, J_{CP}=5.5 Hz), 27.6 (CH_3, d, J_{CC}=21.6 Hz), 16.5 (CH_3, d, J_{CP}=6.6 Hz) ppm (carbonyl carbon not observed); ^{31}P NMR (121 MHz, $CDCl_3$) δ 11.7 (d, J_{CP}=218.4 Hz) ppm; LRMS (ES^+) m/z 222 $[M+H]^+$; HRMS (ES^+) for $C_6{}^{13}CH_{14}N_2O_4P$, calculated 222.0719, found 222.0718.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.07.092.

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