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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).

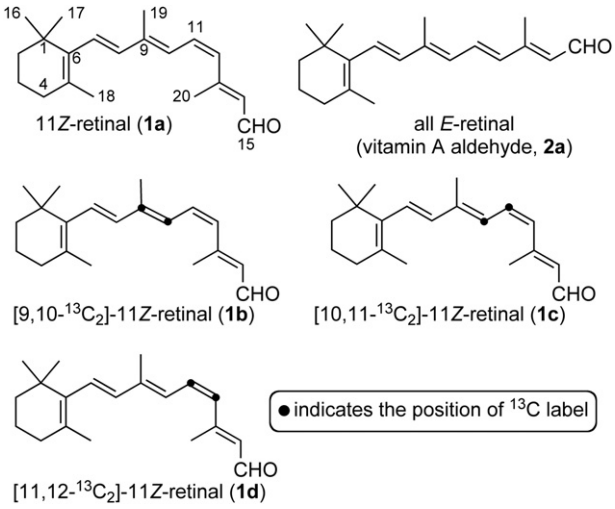

Fig. 1. Structures of 11*Z*-retinal isotopomers **1a**–**d** and all *E*-retinal (**2a**).

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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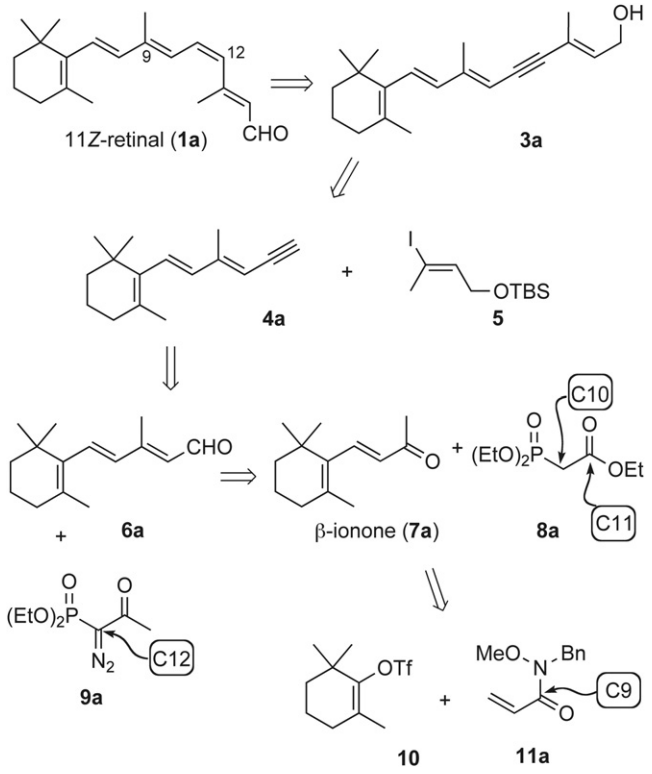
degradation products by means of preparative normal phase HPLC. Stereocontrolled syntheses of unlabelled 11*Z*-retinals have been reported,[10,11] although we are not aware of reports detailing the application of these synthetic routes to obtain ^{13}C-labelled compounds. The stereocontrolled synthesis 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 $^{13}CH_3I$.



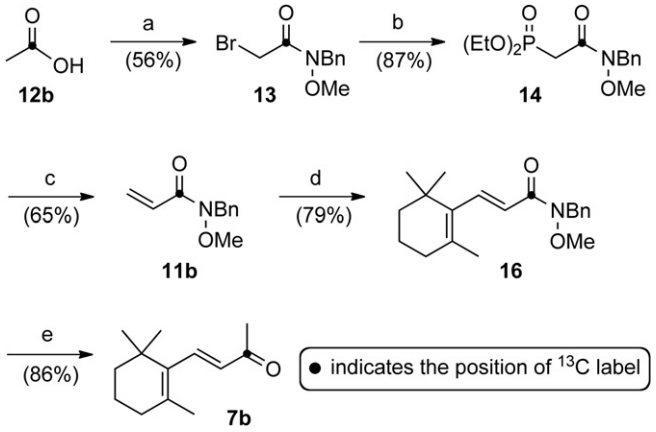
Scheme 1. Overview of the synthesis of 11*Z*-retinals suitable for introducing pairs of ^{13}C labels at the C9/C10, C10/C11 or C11/C12 positions.

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]