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DISSERTATION

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„Organic Chemistry as a Useful Tool in Structural Biology“

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Oh, ich hoffe es verirrt sich nicht,
so verwirrt wie es ist
auf dieser endlosen Suche
nach ein bisschen mehr Licht

(*KRÄNE*, Gisbert Zu Knyphausen)

Und jedem Anfang wohnt ein Zauber inne,
der uns beschützt und der uns hilft zu leben.

(*STUFEN*, Hermann Hesse)

für Julika

Danksagung

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Zusammenfassung

Die vorliegende Dissertation beschreibt fünf miteinander in direktem Zusammenhang stehende Projekte. Das Ziel dieser Arbeit war sowohl die Synthese von fluorierten Kohlenhydraten als auch die Herstellung fluorierter oder isotopenmarkierter α-Ketocarbonsäuren. Die herausragenden Eigenschaften des chemischen Elements Fluor für die Erforschung biologischer Systeme, führten im letzten Jahrzehnt zu enormen Fortschritten in der Synthese fluorierter Verbindungen, im speziellen in der Kohlenhydrat Chemie. Als eine unverzichtbare Methode zur Analyse enzymkatalysierter Reaktionen erwies sich die ^{19}F-NMR Spektroskopie.

Im ersten Projekt dieser Arbeit wurde eine effiziente und kurze Synthese von 3-Fluor-1-hydroxyacetonphosphat (FHAP) ausgearbeitet (PART A). FHAP, ein Analogon zu Dihydroxyacetonphosphat, diente als neuartiger Donor in Aldolase (aus Kaninchenmuskel (RAMA)) katalysierten Aldol Reaktionen zur Synthese fluorierter Kohlenhydrate. Die Stereochemie des Aldolprodukts (D-*threo*) konnte mit Hilfe einer unabhängigen Synthese bewiesen werden (PART A und PART C). Darüber hinaus wurde ein sehr schneller RAMA-katalysierter Proton-Deuterium Austausch von FHAP in D_2O und die unterschiedlichen Bindungsaffinitäten der *gem*-Diol- und Keto-Form von FHAP mittels ^{19}F-NMR analysiert.

In einem Nebenprojekt wurde eine kurze Synthese für Fluorhydroxyaceton (FHA) zur Untersuchung organokatalysierter Aldoladditionen entwickelt (PART B). Die Struktur von FHA konnte mittels ^{1}H-NMR zweifelsfrei bestimmt werden. Die chemische Instabilität von FHA erlaubte keine Isolation und keine Durchführung der geplanten organokatalysierten Aldolreaktionen.

Für die stereochemische Konfigurationsbestimmung des RAMA katalysierten Aldolprodukts wurde eine neuartiges α-Hydroxyacetylanion Äquivalent zur C-C Kettenverlängerung entwickelt (PART C). Zur Synthese substituierter Dihydroxyacetone wurde eine zweistufige Sequenz, bestehend aus einer Indium vermittelten Allenylierung von Aldehyden mit 4-Brom-2-butin-1-ol gefolgt von einer Ozonolyse der resultierenden Allene, verwendet. Die Steuerung der Regioselektivität der Indium vermittelten C-C Kettenverlängerung konnte über die Hydroxylschutzgruppe erreicht werden und folglich wurden entweder Allene oder

Alkine bevorzugt gebildet. Die Vielseitigkeit dieser Synthesestrategie konnte durch eine effiziente stereoselektive Synthese von D-*erythro*-2-Pentulose (D-Ribulose) und 1-Deoxy-D-ribulose gezeigt werden.

Aufgrund der erworbenen Expertise während der Dissertation im Bereich nucleophiler Fluorierungen wurde eine Synthese für die Herstellung von 4-Fluoro-2-oxobutansäure entwickelt. Als Schlüsselschritt wurde eine homoallylische Fluorierung gewählt, welche in moderaten bis guten Ausbeuten durchgeführt werden konnte (PART D). Homoallylische Fluorierungen gehören zu den am schwersten erzielbaren Fluorierungsmethoden und werden daher nur äußerst selten in der organischen Synthese eingesetzt. Die bereits literaturbekannte α-Ketocarbonsäure galt aufgrund eines Berichts von O-Leary *et al*. als äußerst instabil mit eine Zerfallshalbwertszeit zu 2-Oxo-3-butensäure von 162 Sekunden bei pH = 7.0 in D_2O. Im Gegensatz zu O'Leary beobachten wir einen langsamen Zerfall von 4-Fluoro-2-oxobutansäure zu 3-Fluoropropansäure durch den Verlust eines Kohlenstoffatoms über zehn Tage.

Das Ziel des letzten Projekts war die Entwicklung einer isotopenmarkierten Vorstufe für die Herstellung selektiv Histidin markierter Proteine. Das häufige Vorliegen der Aminosäure Histidine in aktiven Zentren von Enzymen, macht selektiv isotopenmarkiertes Histidin zum Studium enzymkatalysierter Reaktionen hochinteressant. Die α-Ketocarbonsäure Imidazol-5-ylpyruvat, normalerweise ein Produkt eines Histidin Abbauweges, wurde als möglicher Precursor für die *in vivo E. coli* vermittelten Synthese von Histidin ausgewählt. Eine konvergente Synthesestrategie für Imidazol-5-ylpyruvat, die eine Isotopenmarkierung des Imidazolringes und der resultierenden Peptidkette ermöglicht, wurde ausgehend von billigen isotopenangereicherten Kohlenstoff (C-13) Quellen untersucht.

Abstract

The present PhD thesis assembles five closely associated projects for the synthesis of fluorinated carbohydrates and fluorinated or isotope labeled α-keto acids for protein labeling. In the last decade an enormous research effort was directed towards the synthesis of fluorinated compounds, especially carbohydrates, due to the favorable properties of fluorine for studying biological systems. The ^{19}F-nucleus is an ideally suited reporter for visualizing enzyme catalyzed reactions by NMR spectroscopy.

In the first project, an efficient gram scale synthesis of 3-fluoro-1-hydroxyacetone phosphate (FHAP) has been developed (PART A). As a close analog to dihydroxyacetone phosphate (DHAP), FHAP was further used as a novel donor substrate for rabbit muscle aldolase (RAMA) catalyzed reactions in the synthesis of fluorinated carbohydrates. The stereochemistry of the aldol condensation product (D-*threo*) was confirmed by an independent chemical synthesis (PART A and C). In addition, a fast RAMA-catalyzed proton-deuterium exchange of FHAP with D_2O and the different binding affinities of the *gem*-diol and keto form of FHAP were studied by ^{19}F-NMR.

In a side project a short synthesis for fluorohydroxyacetone (FHA) was developed (PART B). The structure of FHA was characterized by NMR spectroscopy in solution, since pure FHA is highly instable and cannot be isolated. The initial idea behind this project was to explore the organocatalyzed aldol addition of various aldehydes to FHA for the synthesis of fluorinated carbohydrates and carbohydrate derived compounds.

For the determination of the stereochemical configuration of the product from the RAMA catalyzed aldol addition, we developed a facile preparation and application of α-hydroxyacetyl anion equivalents for carbon chain elongations (PART C). A two step sequence of an indium mediated allenylation of aldehydes with 4-bromo-2-butyn-1-ols followed by ozonolysis of the resulting allene was used to generate the desired substituted dihydroxyacetone fragments. The regioselectivity of the indium-promoted C-C bond forming reaction can be controlled by the hydroxyl-protecting group on 4-bromo-2-butyn-1-ol, preferentially yielding either allenes or alkynes. The versatility of this strategy was demonstrated by the stereoselective and efficient synthesis of D-*erythro*-2-pentulose (D-ribulose), 1-deoxy-D-ribulose.

An efficient synthesis of 4-fluoro-2-oxo-butanoic acid has been developed (PART D). In the key step of this synthesis the fluorine atom was introduced by a homoallylic fluorination in moderate yield. These types of fluorinations are known to be extremely difficult and seldom used in organic synthesis. In an earlier report by O'Leary *et al.*, 4-fluoro-2-oxo-butanoic acid was claimed to be unstable in D_2O solution with a half-life of decomposition to 2-oxo-3-butenoic acid of 162 s at pH = 7.0. These findings are in contrast to our results since 4-fluoro-2-oxo-butanoic acid was stable in D_2O solutions at pH = 7.0 for several hours. In our hands, 4-fluoro-2-oxo-butanoic acid decomposed to 3-fluoro-propanoic acid in ten days by loss of one carbon atom.

The aim of the final project was the development of a selectively ^{13}C and deuterium labeled precursor compound for the synthesis of histidine labeled proteins (PART E). Histidine is frequently found in the active site of enzymes and therefore selectively isotope labeled histidine is of high interest for studying enzyme catalyzed reactions. The α-keto acid imidazol-5-yl pyruvic acid, normally involved in the degradation of histidine, was chosen as a possible precursor for the in vivo *E. coli* mediated synthesis of histidine. A convergent synthesis of imidazol-5-yl pyruvic acid was attempted to label the imidazole ring and the resulting peptide backbone from cheap carbon-13 enriched precursors.

List of Abbreviations

5’-ProFAR	pro-phosphoriboslyl formino-5-amino-imidazole-4-carboxamide ribonucleotide
Ac	acetyl
AIBN	azobisisobutyronitrile
AICAR	5-aminoimidazole-4-carboxamide ribonucleotide
ATP	adenosine triphosphate
Bn	benzyl
Bu	butyl
Bz	benzoyl
DAST	diethylaminosulfur trifluoride
DBU	1,8-diazabicycloundec-7-en
DHA	dihydroxyacetone
DIBAL-H	diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DHAP	dihydroxyacetone phosphate
DMF	*N*,*N*-dimethylformamide
DMP	2,6-dimethylpyridine
DMSO	dimethylsulfoxide
Et	ethyl
FBP	fructose-1,6-bisphosphate
FHA	3-fluoro-1-hydroxyacetone
FHAP	3-fluoro-1-hydroxyacetone phosphate
Fuc 1-P	fuculose 1-phosphate
G3P	D-glyceraldehyde-3-phosphate
HOL	L-histidinol
HOL-P	L-histidinol phosphate
HPA	hydroxypyruvic acid
HWE	Horner-Wadsworth-Emmons reaction

IAP	imidazolacetol phosphate
IGP	imidazoleglycerol phosphate
KDO	3-deoxy-D-*manno*-oct-2-ulosonic acid
LDA	lithium diisopropylamide
MCPBA	*meta*-chlorobenzoic acid
Me	methyl
MOM	methoxymethyl
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced from of NAD^+
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of $NADP^+$
NBS	*N*-bromosuccinimide
NFSI	*N*-fluorobenzenesulfonimide
NMO	*N*-methylmorpholin-*N*-oxid
NOE	Nuclear Overhauser effect
Pase	acid phosphatase
PBSF	n-perfluorobutanesulfonyl fluoride
PEP	phosphenol pyruvate
Ph	phenyl
PP_i	pyrophosphate
PR-AMP	*N*'-5'-phosphoribosyl-adenosin monophosphate
PR-ATP	*N*'-5'-phosphoribosyl-adenosin triphosphate
PRFAR	phosphoriboslyl formino-5-amino-imidazole-4-caroxamide ribonucleotide
PRPP	phosphor-ribosylpyrophosphate
Pyr	pyridine
RAMA	rabbit muscle aldolase
Rha 1-P	rhamnulose 1-phosphate
TBAF	tetrabutylammonium fluoride

TBAI	tetrabutylammonium iodide
TBAT	tetrabutylammonium triphenyl-difluorosilicate
TBDPS	*tert*-butyldiphenylsilyl
TBP	tagatose-1,6-bisphosphate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIM	triosephosphate isomerase
TIPS	triisopropylsilyl
TMS	trimethylsilyl
TPAP	tetrapropylammoniumperruthenate
Ts	tosyl

PART A: Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate and its Application in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions

A.1. Abstract

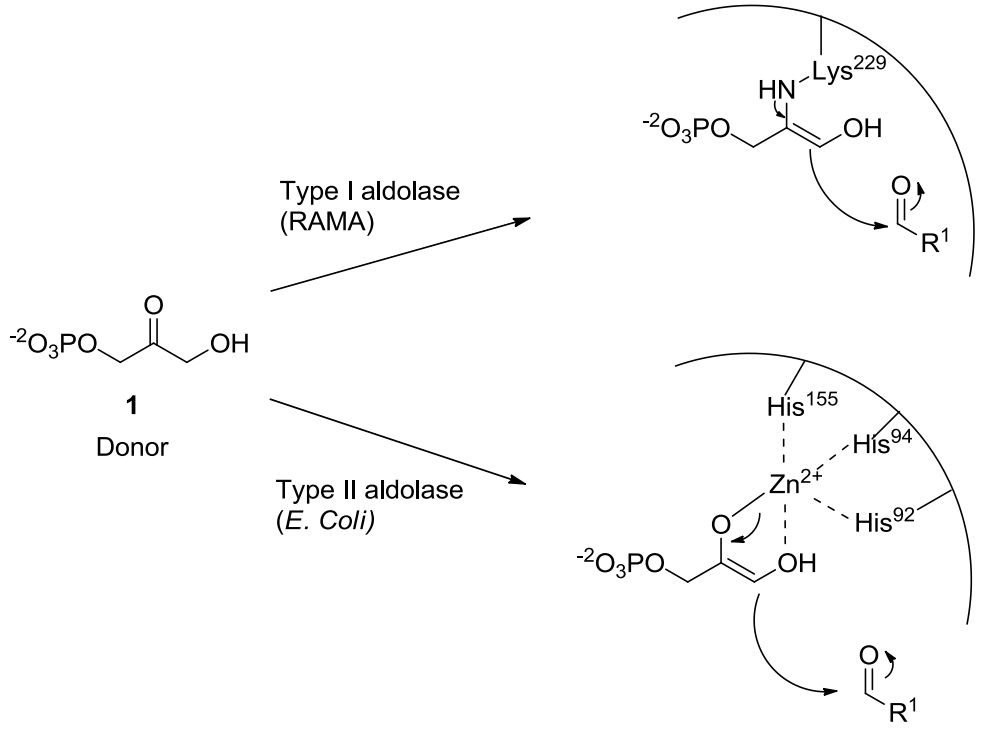
An efficient gram scale synthesis of 3-fluoro-1-hydroxyacetone phosphate (FHAP) has been developed. As a close analog to dihydroxyacetone phosphate (DHAP), FHAP was further used as a novel donor substrate for rabbit muscle aldolase (RAMA) catalyzed reactions in the synthesis of fluorinated carbohydrates. The stereochemistry of the aldol condensation product (D-*threo*) was confirmed by an independent chemical synthesis. In addition, a fast RAMA-catalyzed proton-deuterium exchange of FHAP with D_2O and the different binding affinities of the *gem*-diol and keto form of FHAP were studied by ^{19}F-NMR. Furthermore a fast RAMA-catalyzed deuterium incorporation into fructose-1,6-bisphosphate (FBP) was examined and the stereochemical configuration of the novel stereogenic center was elucidated by NOESY experiments.

A.2. Introduction and Background

The construction of carbon-carbon bonds under control of stereochemistry is of outstanding importance in organic synthesis. The aldol reaction is one of the most striking methods for carbon-carbon-bond formation and the relative and absolute stereochemistry of the formed stereogenic centers can be controlled by using stoichiometric chiral starting materials (aldehydes or auxiliaries) or chiral catalysts (biochemical catalysts or small chiral molecules and metal complexes).[1] The most prominent biochemical catalysts for this purpose are aldolases, which catalyze reversible asymmetric aldol condensations between an aldehyde acceptor and a donor substrate in nature.[2] According to their mechanism, aldolases have been classified into two categories (Scheme 1).[3]

Type I aldolases, which are predominantly found in animals and higher plants, activate the donor substrate by forming a Schiff base intermediate in the active site. The Schiff base intermediate is generated between the keto functionality of the donor substrate and the ε–amino group of a lysine residue in the active site. This activated donor adds stereoselectively to the acceptor aldehyde.

Type II aldolases use a metal cofactor for the activation of the donor substrate. The cofactor Zn^{2+} acts as a Lewis acid and leads to the activation of the donor as a zinc enolate. These types of aldolases are mainly found in bacteria and fungi.[4]



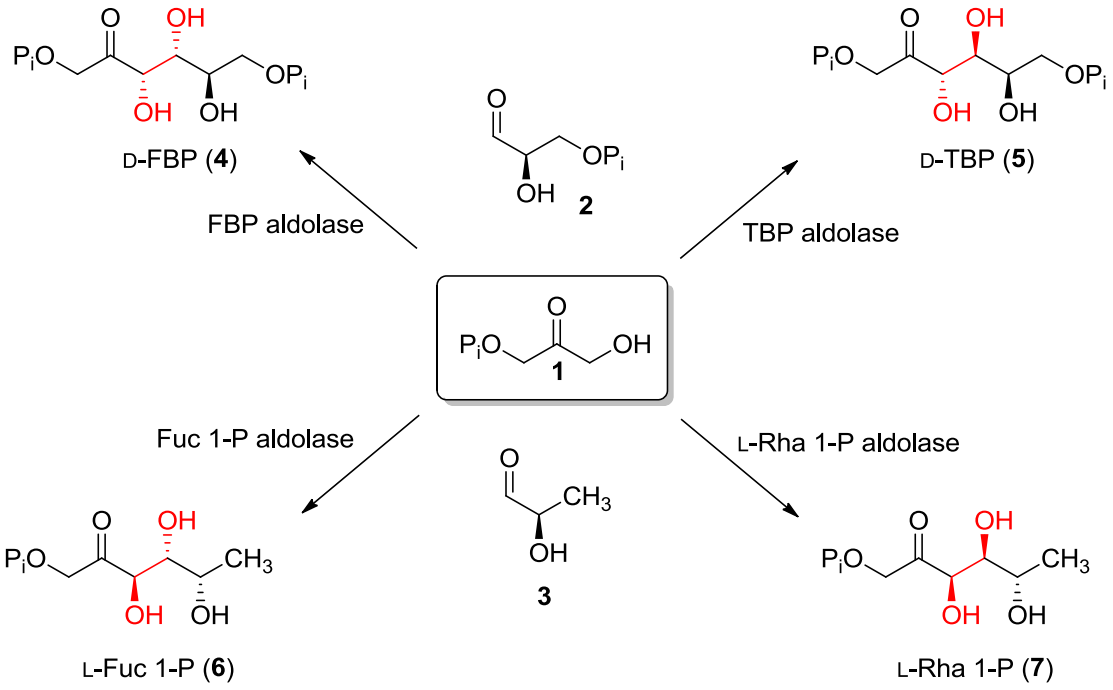
Scheme 1. Two types of aldolase mechanisms[1]

Aldolases tolerate a broad range of acceptor aldehydes, but on the same time are very restrictive towards their donor substrate. Therefore they have been further subdivided according to their donor substrate, i.e. DHAP (**1**), pyruvate/phosphenol pyruvate (PEP), glycine and acetaldehyde.[5]

DHAP-Utilizing Aldolases

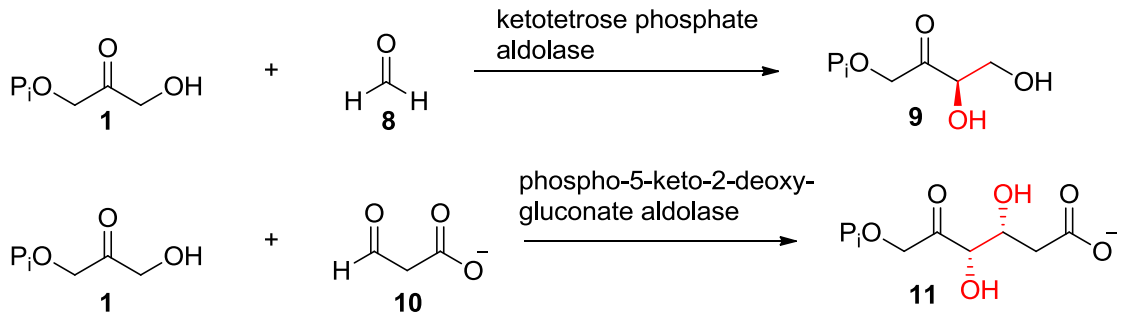
In nature, DHAP-dependent aldolases catalyze the reversible enantioselective aldol addition of DHAP (**1**) to an acceptor aldehyde. Fructose-1,6-bisphosphate (FBP)- and Tagatose-1,6-

bisphosphate (TBP)-dependent aldolases utilize D-glyceraldehyde-3-phosphate (D-G3P) (**2**) and Fuculose-1-phosphate-(Fuc 1-P), Rhamnose-1-phosphate (Rha 1-P) aldolases utilize L-lactaldehyde (**3**) as their acceptor substrate *in vivo* (Scheme 2). These four enzymes allow the stereoselective formation of the four theoretically possible diastereomeric aldol products at C3 and C4 (3*S*, 4*R*; 3*S*, 4*S*; 3*R*, 4*R*; 3*R*, 4*S* in order as mentioned above).



Scheme 2. DHAP dependent aldolases with their natural substrates[1,5]

In vivo, two further enzymes catalyze the enantioselective aldol addition of DHAP (**1**) to formaldehyde (**8**) (D-ketotetrose phosphate aldolase) and to 3-oxopropanoic acid (**10**) (phospho-5-keto-2-deoxygluconate aldolase).



Scheme 3. DHAP dependent aldolases with their natural substrates[1,5]

Fructose-1,6-bisphosphate (FBP) Aldolase (EC.4.1.2.13)

To date, FBP aldolases and especially the commercial available RAMA are the most widely used aldolases in organic synthesis. In vivo, FBP aldolases catalyze the reversible aldol addition of DHAP (**1**) to D-G3P (**2**) under the formation of D-FBP (**4**) (Scheme 4). The equilibrium constant has a value of approximately ~10^4 M^{-1} and is in favor of FBP formation.

Scheme 4. FBP aldolase with their natural substrate[5]

The type I FBP aldolases exist as tetramers (~160 kDa) and feature a high degree of sequence homology (< 50%) with the active site residues being highly conserved. Type II FBP aldolases exist as dimers of ~80 kDa and possess neither sequence homologies among each other nor to type I FBP aldolases.

Rabbit Muscle Aldolase (RAMA)

RAMA is easily isolated from rabbit muscle, not air sensitive, inexpensive and stable for ~2 days in aqueous solution at pH 7. Therefore, most mechanistic studies have been carried out on FBP aldolases from rabbit muscle (RAMA) and X-ray structures have been determined to resolution of 1.76 Å (Figure 1).[6]

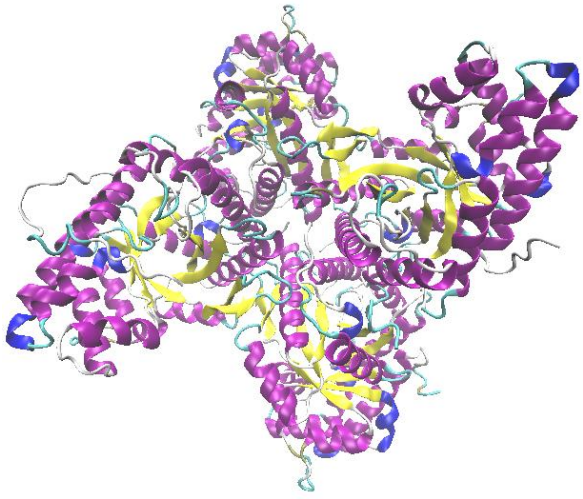
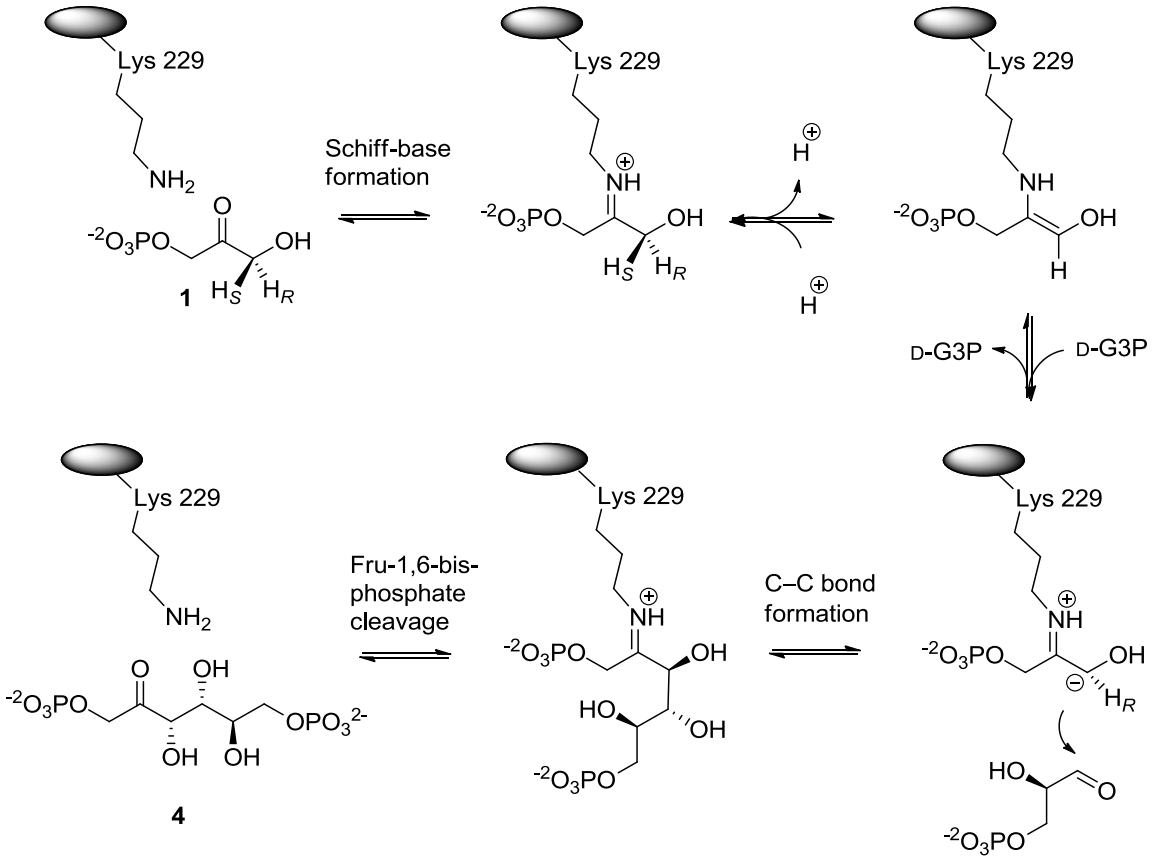


Figure 1. X-ray structure of RAMA[6,7]



Scheme 5. Simplified reaction mechanism of Type I FBP aldolases[5]

A simplified reaction mechanism for Type I FBP aldolases is shown in scheme 5. For simplicity only the Schiff-base forming amino acid Lys 229 from the active site is visualized. In the first step Lys 229 forms an iminum ion with the keto functionality of DHAP (**1**), which after abstraction of the pro-*S* hydrogen at C3, forms an enamine intermediate. Then, this enamine (or its tautomeric zwitterionic form) can add to D-G3P (**2**) under formation of D-FBP (**4**) covalently bound to the enzyme via the ε-amino group of Lys 229. In a subsequent step the imine is hydrolyzed by water and D-FBP (**4**) is liberated. The other amino acids with are involved in the active site are shown in Figure 2. For a more detailed discussion of the reaction mechanism see Sygusch *et al*.[6]

Substrate Specificity

RAMA reliably generates vicinal diols with D-*threo* configuration with a wide variety of acceptor aldehydes (> 100) using DHAP (**1**) as a donor substrate (Scheme 6).[8] In general, unhindered aliphatic and α-heteroatom substituted aldehydes, aldoses and derivatives

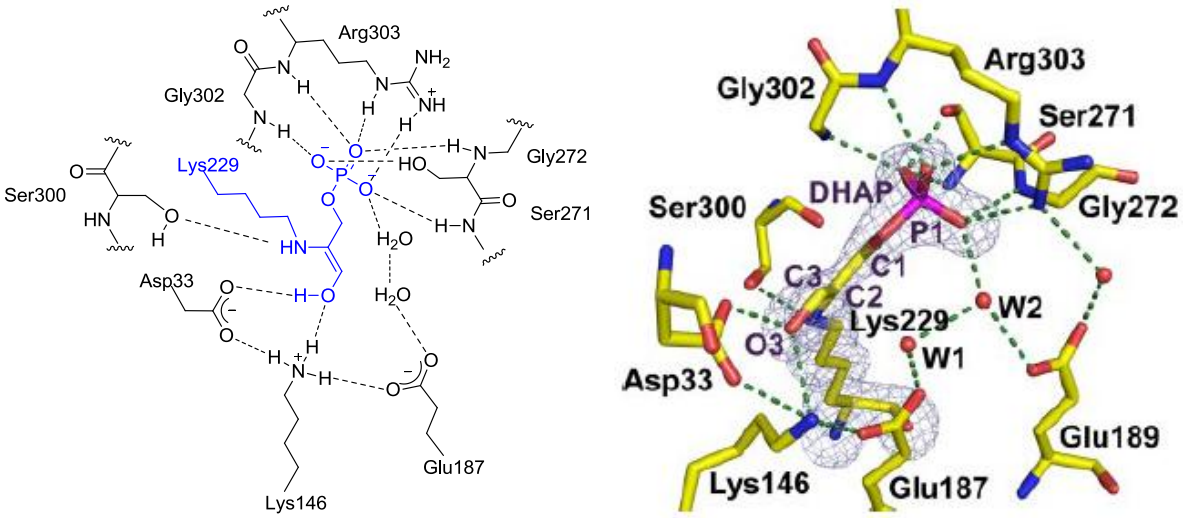
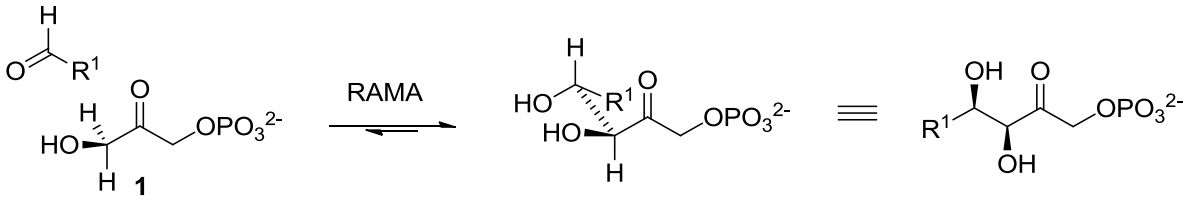


Figure 2. 2-dimensional (right side) and 3-dimensional (left side) representation of the enamine intermediate trapped in the active site of RAMA[6a]



Scheme 6. Stereospicifity of RAMA catalyzed aldol reactions[8]

thereof are suitable acceptors for RAMA-catalyzed aldol reactions. Sterically hindered, aromatic and α,β-unsaturated aldehydes are not accepted by the enzyme.

In contrast to the acceptor aldehyde, RAMA is much more restricted concerning the donor substrate. So far, RAMA tolerates only variations at position C1 of the natural donor DHAP (**1**) (Figure 3) with low catalytic activity (~10 % cf. DHAP (**1**)). For a more detailed discussion of the donor specificity of RAMA see the results section (publication).

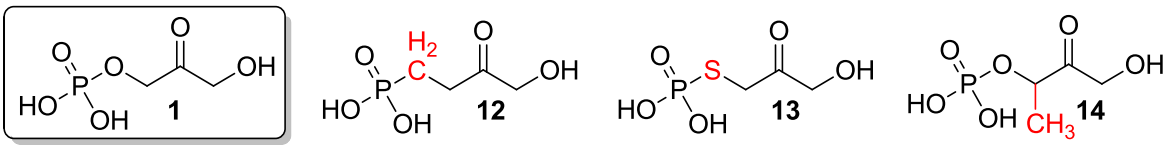


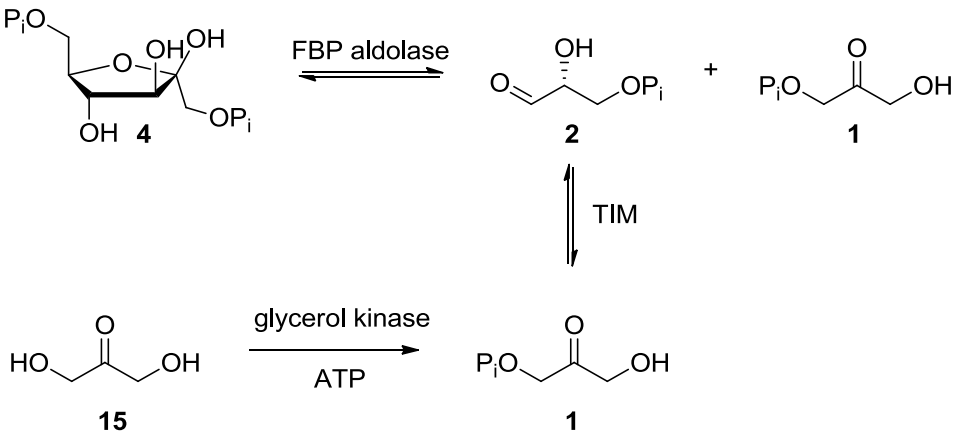
Figure 3. Donor substrate analogs[8]

Preparation of Dihydroxyacetone Phosphate (DHAP)

The donor substrate DHAP (**1**) can be synthesized by enzymatic or classical organic chemical approaches (Scheme 7-10). Enzymatic *in situ* generation of DHAP (**1**) from D-FBP (**4**) can be achieved by using FBP aldolase in combination with triosephosphate isomerase (TIM). FBP

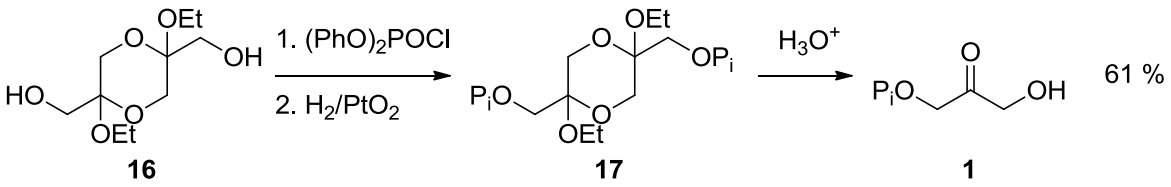
aldolase reversibly cleaves D-FBP (**4**) into DHAP (**1**) and D-G3P (**2**), whereas the latter one is further converted into DHAP (**1**) by triosephosphate isomerase (TIM). The external addition of aldehydes allows the formation of the desired products, albeit the overall reaction may not go to completion depending on the stability of the product compared to D-FBP (**4**).

An alternative enzymatic synthesis of DHAP (**1**) applies glycerol kinase catalyzed phosphorylation of dihydroxyacetone (DHA) (**8**) with ATP. The drawback of this methodology lies on the high costs of ATP in large scale synthesis (Scheme 7).[9]



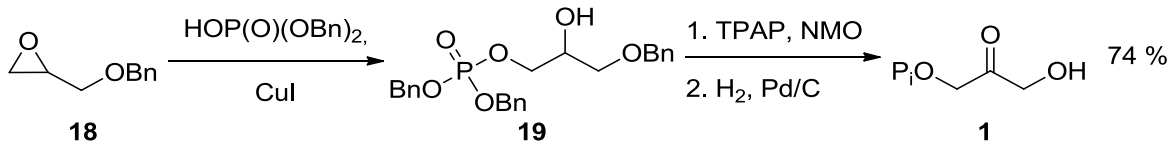
Scheme 7. Enzymatic synthesis of DHAP (1)[1,5,8]

Most chemical synthesis of DHAP (**1**) rely on the phosphorylation of the ethanol protected DHA dimer **16**. Phosphorylations have been achieved directly with $POCl_3$[10], $(PhO)_2P(O)Cl$[11], or in a two step procedure using $(BnO)_2PN(iPr)_2$ and subsequently oxidation by H_2O_2.[12] In the final step the phosphate dimer **17** can be deprotected in acid solution to give DHAP (**1**) (Scheme 8).



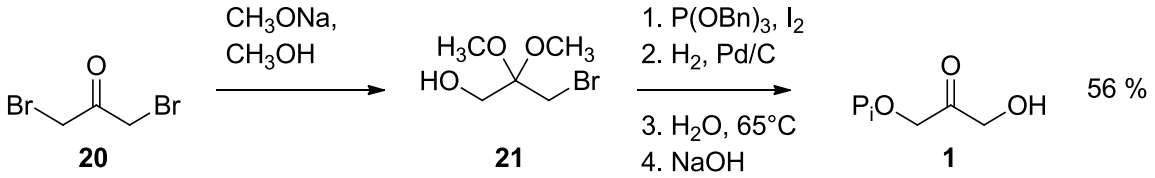
Scheme 8. A typical chemical synthesis of DHAP (1) starting from DHA dimer[1,5,8]

Alternatively DHAP (**1**) was efficiently synthesized starting with a copper catalyzed regioselective ring opening of benzylglycidol **18** with dibenzylphosphate (Scheme 8).[13] The resulting secondary alcohol **19** was oxidized with tetrapropylammoniumperruthenate/*N*-methylmorpholin-*N*-oxid (TPAP/NMO) in high yield. DHAP (**1**) was isolated after deprotection with H_2 over palladium on carbon in an overall yield of 74 %.



Scheme 9. Chemical synthesis of DHAP (1) by Meyer *et al.*[13]

Another approach used 1,3-dibromoacetone **20** as a starting material (Scheme 10).[14] Desymmetrization and simultaneous protection of the keto functionality of **20** was achieved under basic conditions. After phosphorylation of **21**, the benzyl and ketal protecting groups were cleaved and the resulting 3-bromo-1-hydroxyacetone phosphate was converted into DHAP (**1**) under basic conditions.



Scheme 10. Chemical synthesis of DHAP (1) by Gefflaut *et al.*[14]

Selected Examples of Use of RAMA in Synthesis

Due to the broad acceptor substrate tolerance, RAMA has been employed for the synthesis of nitrogen[15] or sulfur-containing, fluoro-, deoxy-, high-carbon[16] and ^{13}C-labeled carbohydrates.[17] Other molecules synthesized by FBP aldolases were various C-glycosides, cyclitols,[18] spiro compounds and (+)-exo-brevicomin (**29**) (Figure 4).

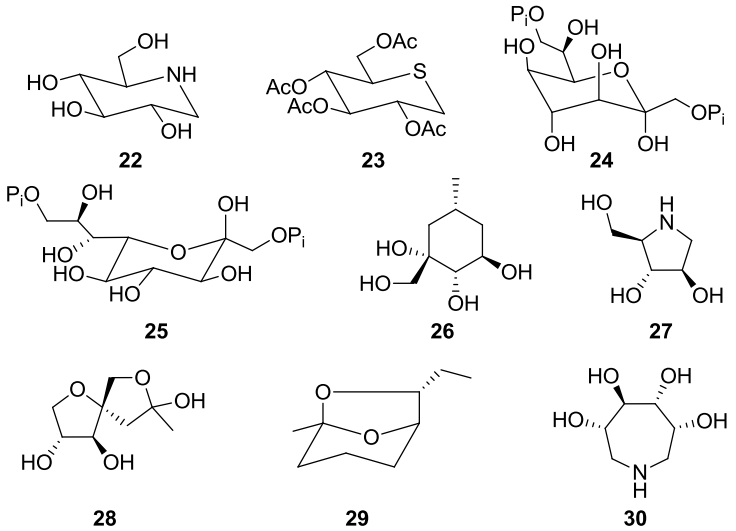
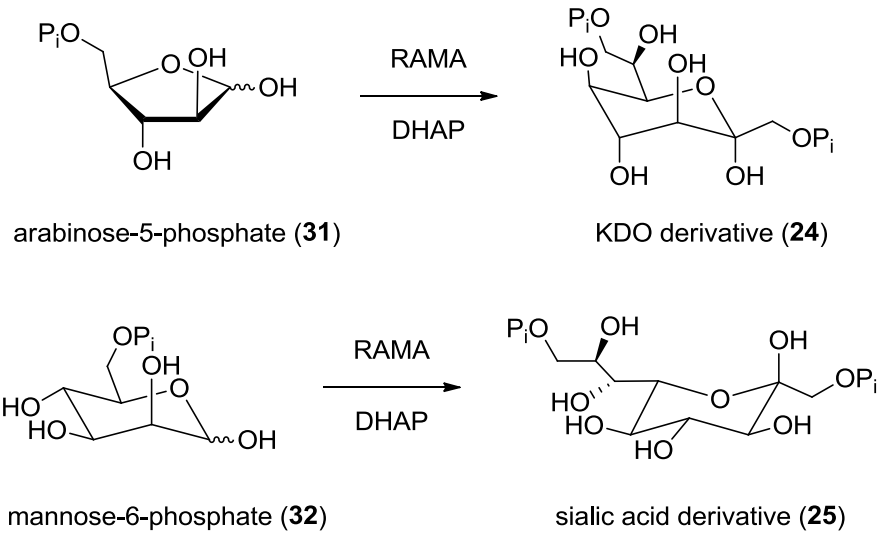


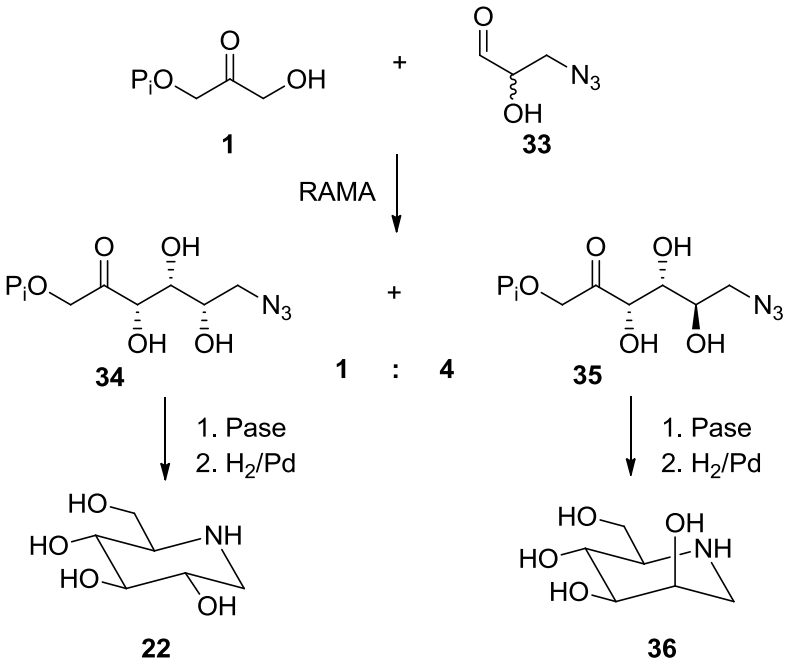
Figure 4. Synthesis of various carbohydrates and carbohydrates derived compounds with RAMA[1,5,8]

A core saccharide of the cell wall of gram-negative bacteria, 3-deoxy-D-*manno*-oct-2-ulosonic acid (KDO) and a common component of glycoproteins, the sialic acids were easily prepared by RAMA catalyzed aldol addition of DHAP (**1**) to pentose and hexose phosphates **31** and **32** (Scheme 11).



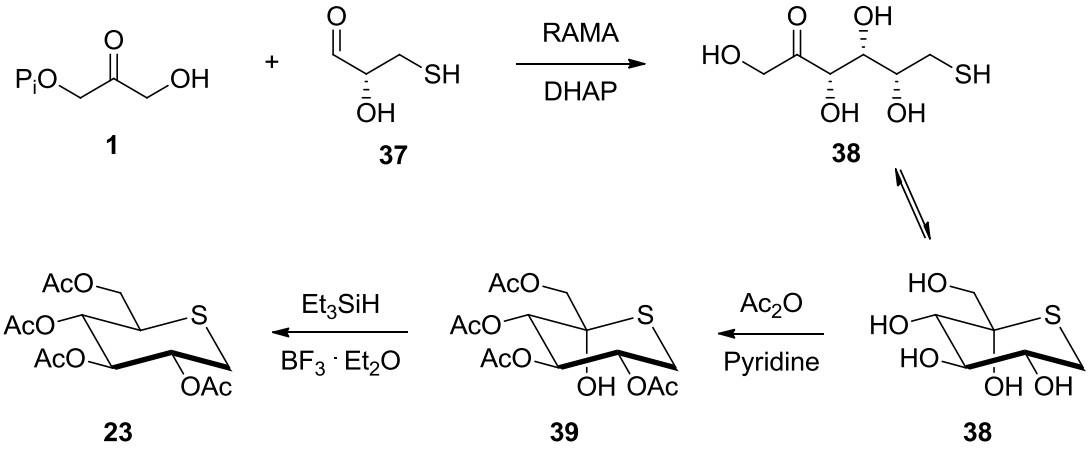
Scheme 11. RAMA-catalyzed synthesis of KDO and sialic acid derivatives[1,5,8]

RAMA was extensively exerted in the synthesis of nitrogen containing sugars, like the potent glycosidase inhibitors deoxynojirimycin (**22**) and deoxymannojirimycin (**36**) (Scheme 12).[19]

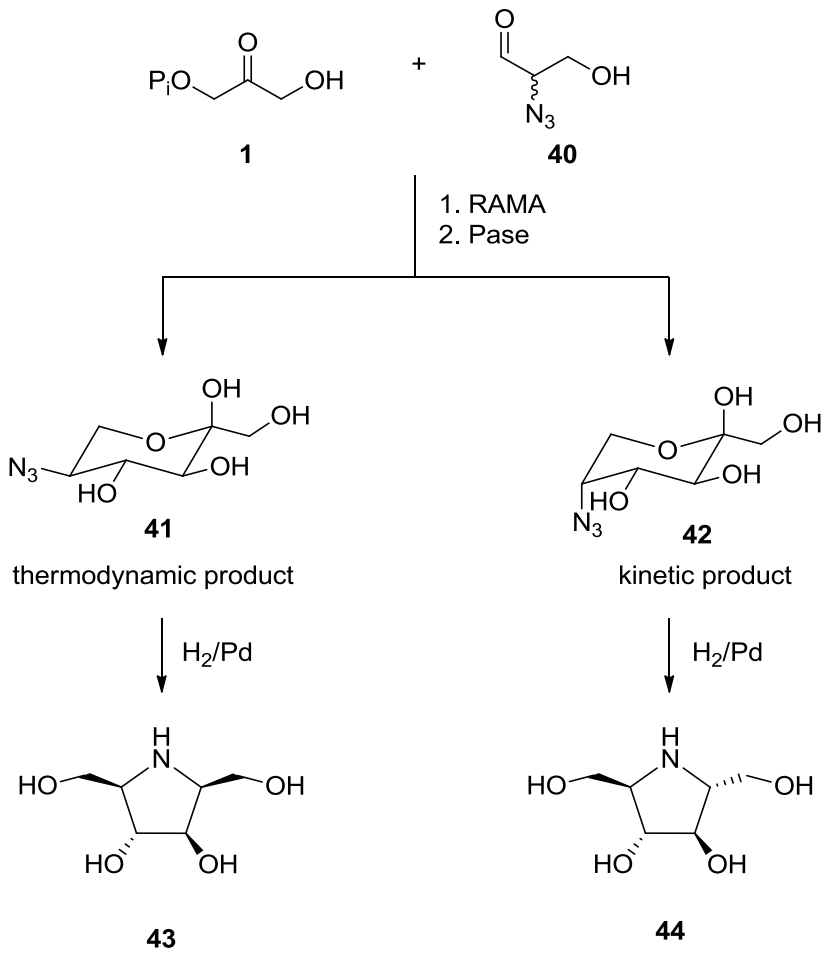


Scheme 12. Synthesis of deoxynojirimycin (22) and deoxymannojirimycin (36)[1,5,8]

Deoxy-thio sugars and bioactive five membered pyrrolidine type iminocyclitols have been synthesized via similar strategies (Scheme 13 and 14).



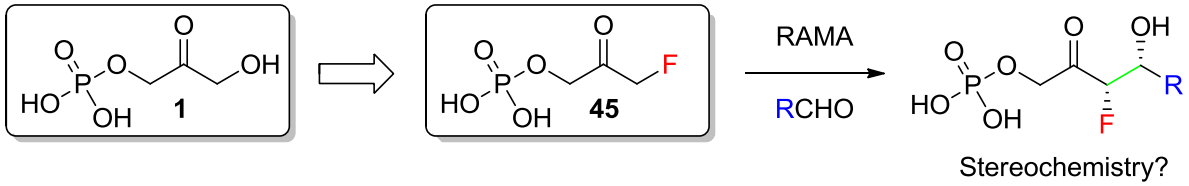
Scheme 13. Preparation of deoxy-thio-carbohydrate (23)[1,5,8]



Scheme 14. Synthesis of substituted pyrrolidines[1,5,8]

Concept and Strategy

A substitution of the free hydroxyl group of DHAP (**1**) by fluorine constitutes the smallest possible bioisosteric change (Table 1) at the C3 position (Scheme 15), while simultaneously offering a direct access to biological important fluorinated sugars and sugar-derived compounds. If FHAP (**45**) can be efficiently synthesized and is accepted by RAMA, an enormous variety of biological interesting compounds should be easily accessible in this manner. Since the substitution of the hydroxyl group at C3 by fluorine will interfere with hydrogen bonding network in the active site of the enzyme (Figure 2 and 5), the stereochemistry of the products at position C3 and C4 have to be determined by an independent methodology.



Scheme 15. Strategic concept

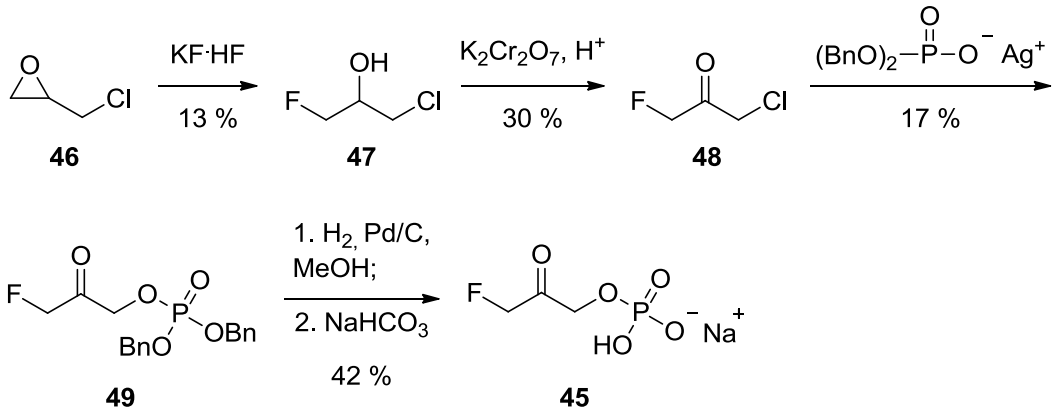
Table 1. Steric consequences of fluorine substitution[20]

Bond	Length [Å]	Van der Waals radius [Å]
C-H	1.09	1.20
C=O	1.23	1.50
C-OH	1.43	1.52
C-F	1.35	1.47
O-H	0.96	1.20

Previous Synthesis of FHAP

FHAP (**45**) was previously synthesized in a laborious sequence by Silverman *et al*. in low overall yield (~1.3 %) and purity (Scheme 16).[21] Starting from epichlorohydrin (**46**), the fluorine atom was introduced by a regioselective ring opening of the epoxide in low yield. Oxidation of the secondary hydroxyl group of **47** with potassium dichromate followed by displacement of the chloride by silver dibenzylphosphate yielded dibenzyl protected FHAP

49. The deprotection of **49** sluggishly worked with palladium on carbon in methanol under an atmosphere of hydrogen. The authors mentioned that the main problem was due to ketalisation of the carbonyl moiety of **45**. Ethyl acetate or benzene as a solvent gave purer compounds but in an even lower yields.

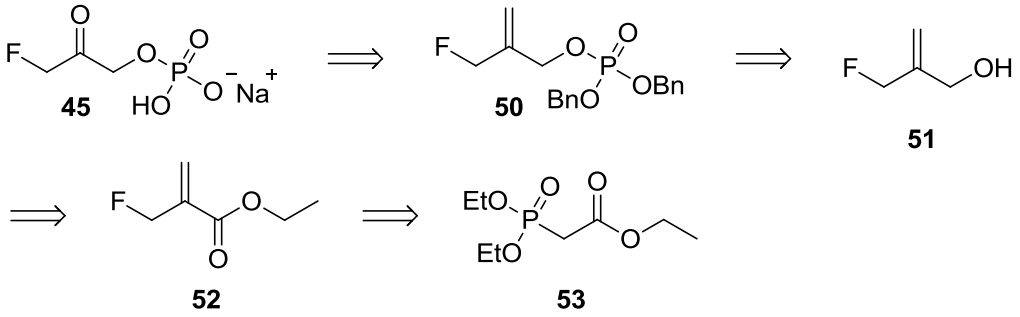


Scheme 16. Silverman's synthesis of FHAP[21]

Since FHAP is needed in large quantities for the subsequent enzymatic reactions, a novel synthesis had to be designed. Silverman's synthesis of FHAP (**45**) is afflicted by considerable disadvantages. Firstly, potassium dichromate and potassium hydrogen difluoride are toxic and the latter one is corrosive. Secondly, the silver (I) dibenzyl phosphate is commercially not available. Thirdly the overall yield is very low (< 1.3 %) and the final deprotection step is slow and incomplete.

Retrosynthetic Analysis

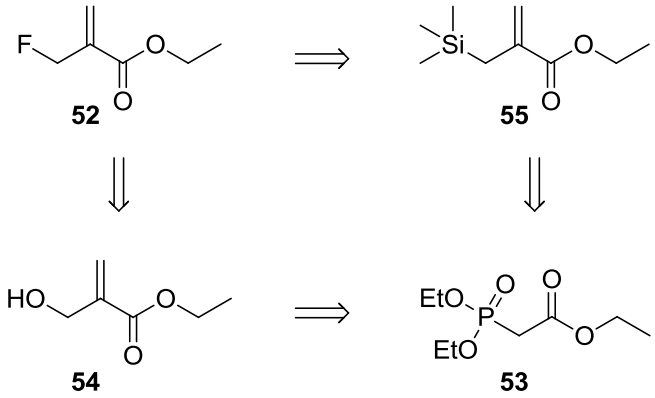
In our retrosynthetic analysis we considered following points: The fluorine atom has to be introduced via a nonhazardous method which is easily scalable. The keto moiety of FHAP (**45**) has to be generated at a late stage of synthesis, since 2-oxo phosphates tend to be unstable. Furthermore, the deprotection of the phosphate group should be accomplished by a mild methodology. Due to the fact that ketalisation can be prevented by using *tert*-butanol instead of methanol during the hydrogenolysis of benzyl phosphates, we envisaged the same final intermediate as Silverman *et al*. (Scheme 17).



Scheme 17. Retrosynthesis of FHAP (45)

The dibenzyl phosphate was planned to be introduced either by a pentavalent phosphorous reagent like $(BnO)_2P(O)Cl$ or by a much more reactive phosphor(III) species, like PCl_3 or $(BnO)_2PN(iPr)_2$[22] and subsequent oxidation.

For the introduction of the fluorine atom a halogen-halogen exchange, fluorination of allylic alcohol **54** by Ishikawa's reagent or the Gouverneur's fluorodesilylation methodology[23] could be applied (Scheme 18). In our opinion diethylaminosulfur trifluoride (DAST) has to be avoided due to its uncontrollable dangerous behavior when used in larger amounts. Considering all these facts commercially available triethyl phosphonoacetate (**50**) (~30 €/mol) was used as a starting material.



Scheme 18. Retrosynthesis of ethyl 2-(fluoromethyl)acrylate (52)

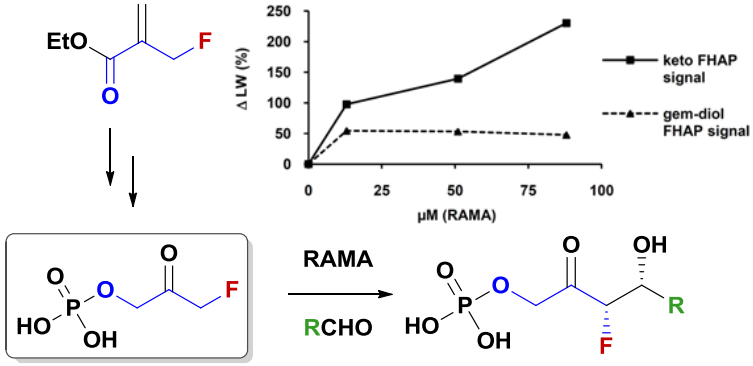
A.3. Results and Discussion

Published Work:

Gram Scale Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate: A Novel Donor Substrate in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions

Michael Fischer, Hanspeter Kählig, Walther Schmid, *Chem. Commun.* **2011**, *47*, 6647-6649.

Graphical Abstract:



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Gram scale synthesis of 3-fluoro-1-hydroxyacetone phosphate: a novel donor substrate in rabbit muscle aldolase-catalyzed aldol reactions†

Michael Fischer, Hanspeter Kählig and Walther Schmid*

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An efficient gram scale synthesis of 3-fluoro-1-hydroxyacetone phosphate (FHAP) has been developed. As a close analog to dihydroxyacetone phosphate, FHAP was used as a novel donor substrate for rabbit muscle aldolase catalyzed reactions. The different binding affinities of the *gem*-diol and keto form of FHAP were studied by ^{19}F-NMR.

Rabbit muscle aldolase (RAMA) catalyses the stereospecific aldol addition of dihydroxyacetone phosphate (DHAP) to an aldehyde, thus forming a C–C bond with D-*threo* configuration.[1] The enzyme tolerates a wide variety of functionalized aldehydes (>100) as acceptor substrates,[2] reflected by its applications in numerous syntheses of ketoses, aldoses, iminosugars and cyclitols.[1,3] In contrast to its versatile acceptor tolerance, RAMA is very restrictive concerning the nucleophilic donor DHAP, as summarized in Fig. 1. Variations at position C3 of the donor phosphate are not tolerated.[2a,4,5] The hitherto investigated 3-halo analogs of DHAP are either competitive (chloride: pH = 7) or irreversible (iodide; bromide and chloride: pH = 10) inhibitors of RAMA, acting by oxidizing sulfhydryl groups to disulfides or by forming stable covalent derivatives with sulfhydryl groups.[5] At present, only three analogs of DHAP are established

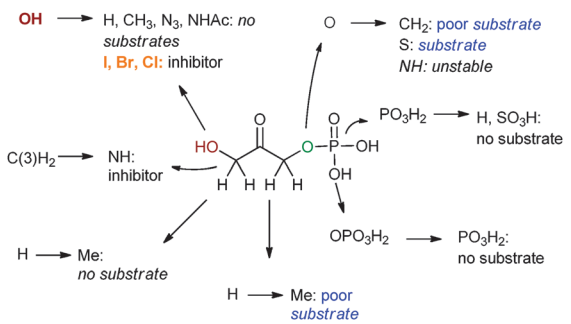
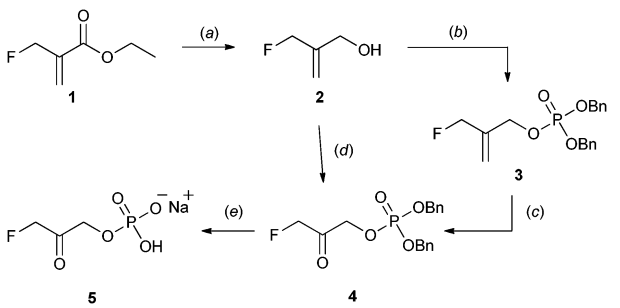


Fig. 1 Donor substrate variations of DHAP.[2a,4–8]

substrates for RAMA-catalyzed reactions (Fig. 1).[2a,6–8] A substitution of the free hydroxyl group of DHAP by fluorine constitutes the smallest possible bioisosteric change at the C3 position,[9] while simultaneously offering a direct access to biologically important fluorinated sugars and sugar-derived compounds.[10] 3-Fluoro-1-hydroxyacetone phosphate (FHAP) was previously synthesized in a laborious sequence by Silverman *et al.* starting from epichlorohydrin in low overall yield (~1.3%) and purity.[11] Previous studies on the substrate specificity of fructose-1,6-bisphosphate aldolase suggested that FHAP may not act as a substrate for the enzyme,[12] which is in contrast to our recent observations. Additionally, neither experimental details of the preparation of FHAP and inhibitor studies nor spectroscopical proof of identity were given.[12]

Herein, we report on a short and efficient gram scale synthesis of 1-fluoro-3-hydroxyacetone phosphate and its application in RAMA-catalyzed aldol reactions. The stereochemistry of the aldol condensation product **8** was proven by an independent chemical synthesis. Furthermore, a fast RAMA-catalyzed proton–deuterium exchange of FHAP with D_2O and the different binding affinities of the *gem*-diol and keto form of FHAP were studied by ^{19}F-NMR.

The synthetic sequence leading to FHAP (**5**) is outlined in Scheme 1. Starting from easily accessible ethyl α-fluoromethylacrylate (**1**),[13] allylic fluoride **2** was synthesized by reduction of the ester moiety with diisobutylaluminium hydride (DIBAL-H).[14] The phosphate functionality of FHAP was introduced *via* dibenzyl *N*,*N*-diisopropylphosphoramidite, followed by a



Scheme 1 Synthesis of FHAP (**5**): (a) DIBAL-H, CH_2Cl_2, −78 °C → 0 °C, 63%; (b) iPr$_2$NP(OBn)$_2$, 1H-tetrazole, CH_3CN; then 30% H_2O_2, 74%; (c) O_3, CH_2Cl_2, −78 °C; $(CH_3)_2S$, 95%; (d) iPr$_2$NP(OBn)$_2$, 1H-tetrazole, CH_3CN; then O_3, CH_2Cl_2, −78 °C; $(CH_3)_2S$, 52%; (e) H_2, 10% Pd/C, tBuOH; 1 N NaOH, lyophilized, 95%.

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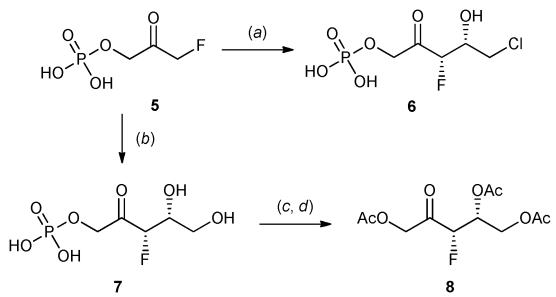
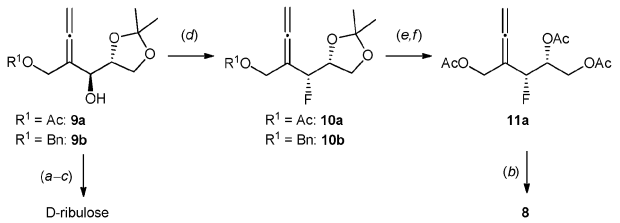
stepwise oxidation of the intermediately formed phosphite with 30% H_2O_2 to allylic phosphate **3** and ozonolysis of the double bond in a subsequent step to yield 2-oxo phosphate **4** in excellent yield. Alternatively a direct oxidation of the double bond and the phosphite with ozone gave the corresponding β-keto phosphate **4**. The dibenzyl protecting groups were cleaved by hydrogenolysis over 10% Pd/C in *t*BuOH,[15] thereby preventing a hemiketal formation which was observed when MeOH was used.[11] After conversion into the monosodium salt, lyophilized FHAP (**5**) was isolated in an overall yield of 42% over 4 steps (or alternatively in 31% yield over 3 steps) starting from ethyl α-fluoromethylacrylate (**1**). FHAP (**5**) is stable for several months at −20 °C. Suitable crystals for X-ray diffraction were obtained from the biscyclohexylammonium salt of FHAP, featuring the keto functionality in its *gem*-diol (hydrated) form (see ESI†). At neutral pD at 25 °C in D_2O,[16] FHAP (**5**) exists 91% in the *gem*-diol form (determined *via* ^{19}F-NMR), compared to 45% for DHAP.[17]

In the subsequent enzymatic reactions the ^{19}F nucleus was used to monitor the reaction progress by ^{19}F-NMR. For all conversions, spectroscopically pure FHAP (**5**) (^{1}H- and ^{19}F-NMR) was essential, since no product formation was detected with slightly impure substrates. Additionally, the acceptor substrates investigated showed the highly preferred formation of one product diastereomer, as judged by ^{19}F-NMR. Our initial studies focused on the RAMA-catalyzed condensation of FHAP (**5**) and chloroacetaldehyde in BIS–TRIS (2-[bis-(2-hydroxyethyl)amino]-2-(hydroxymethyl)-propane-1,3-diol) buffer (Scheme 2). A slow conversion of FHAP (**5**) to deoxysugar **6** was observed by NMR (see ESI†). However, all attempts towards product isolations either as the barium salt of phosphate **6** or as the dephosphorylated sugar failed due to the instability of the products. Therefore we used glycolaldehyde as the acceptor substrate. Good conversion rates forming derivative **7** were obtained by repeated addition of RAMA over 3 days. Dephosphorylation with acid phosphatase and acetylation with acetic anhydride to simplify isolation and characterization yielded 1,4,5-tri-*O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (**8**) (Scheme 2). The stereochemistry of compound **8** was assigned by an independent chemical synthesis (Scheme 3). As a prerequisite, the absolute configuration of allene **9a**[18] was confirmed by transformation into D-ribulose (see ESI†). Subsequently, the hydroxyl



Scheme 2 RAMA-catalyzed aldol condensation with chloroacetaldehyde or glycolaldehyde: (a) RAMA, $ClCH_2CHO$ solution (~50 wt. % in H_2O), BIS–TRIS buffer, pH = 6.8; (b) RAMA, $HOCH_2CHO$, BIS–TRIS buffer, pH = 6.8; (c) acid phosphatase, pH = 5.1; (d) Ac_2O, DMAP, pyridine, rt (7% over 3 steps).

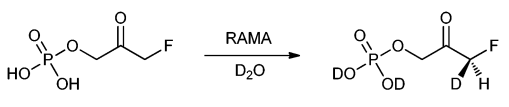


Scheme 3 Chemical synthesis of 1,4,5-tri-*O*-acetyl-3-deoxy-3-fluoro-xylulose (**8**): (a) NaOMe, MeOH, rt; (b) O_3, CH_2Cl_2, −78 °C; PPh_3; (c) Amberlyst 15 H^+, THF/H_2O (77% over 3 steps); (d) DAST, Collidine, CH_2Cl_2, −78 °C, 35%; (e) Dowex 50 H^+, THF/H_2O, rt, 80%; (f) Ac_2O, Pyridine, DMAP, rt, 95%.

functionality of **9a** was transformed to the allenylic fluoro derivative **10a** with (diethylamino)sulfur trifluoride (DAST) at −78 °C under inversion of the configuration at C3.[19] A potential 1,3-neighbouring group participation by the acetate moiety was ruled out, since using the corresponding benzyl ether **9b**[18] instead led to the formation of the same diastereomer **10b** (see ESI†). The *syn*-relationship was reflected in analogy to literature data[20] by the vicinal coupling constants (**10a**: $^3J_{H2-H3}$ = 7.8 Hz; **10b**: $^3J_{H2-H3}$ = 8.0 Hz). Cleavage of the isopropylidene protecting group and subsequent acetylation was performed under standard conditions to yield allene **11a**. After ozonolysis of the allene moiety of **11a** at −78 °C in CH_2Cl_2, pentulose **8** was isolated. All spectroscopical data were identical with the product of the enzymatic synthesis. As a consequence, the RAMA-catalyzed condensation of FHAP (**5**) with glycolaldehyde led to the identical stereochemical configuration (D-*threo*) as the one using the natural donor substrate DHAP.

In concordance with NMR studies of DHAP and RAMA in D_2O,[21] we observed a rapid proton–deuterium exchange for one of the two enantiotopic protons at the C3 position of FHAP (**5**), yielding deuterated FHAP **12** (Scheme 4 and ESI†). The rate constant determined for the deuteration of **5** (0.03 μmol min^{-1} U^{-1}) was significantly smaller than the one reported for the phosphonate analog of DHAP (0.18 μmol min^{-1} U^{-1}) and DHAP (1.2 μmol min^{-1} U^{-1}).[22] The second C3 proton of FHAP (**5**) displayed a much slower exchange rate and a further hydrogen–deuterium exchange at C1 could only be observed after several hours.[23] This finding is in contrast to the natural substrate DHAP, where the second exchange takes place at C1 at a slow rate, followed by an even slower exchange of the second C3 proton.[21] Without the enzyme FHAP does not exchange its protons for several months in D_2O at pD = 6.9.

Since line width and/or relaxation rates of ^{19}F-NMR resonances are sensitive to changes in molecular weight (molecular tumbling), reversible ligand binding processes can easily be analyzed by changes in signal broadening (ESI†). Such a significant change was only observed for the keto form of FHAP, thus indicating a specific binding to RAMA. In contrast, the signal for the *gem*-diol



Scheme 4 Deuterium incorporation into FHAP (**5**).

form of FHAP remained nearly unchanged after the first addition of enzyme.[24] These observations are in agreement with the proposed enzymatic reaction mechanism for the natural substrate (DHAP) with RAMA,[25] since only the keto form can lead to a covalent linked Schiff base intermediate with the enzyme. In analogy to the stereospecificity of RAMA with DHAP as a substrate,[26] the fast proton–deuterium exchange of the *pro-S* hydrogen of FHAP can be assumed.

The low overall yield of the enzymatic aldol addition requires some comment. Since product formation correlates with the accessibility of the carbonyl moiety,[17a] the low isolated yield may mainly be attributed to the high degree of hydration (91%) of the keto functionality and to the general difficulties in acetylation of pentuloses.[27] Furthermore, the enamine intermediates of the natural substrates DHAP and fructose-1,6-bisphosphate are stabilized by a hydrogen bond between the carboxyl functionality of Asp-33 of the enzyme and the hydroxyl group at C3 of the substrates.[28] The importance of this crucial interaction was demonstrated by mutations of Asp-33 into alanine, serine, glutamate and asparagines, which lowered V_{max} of the enzyme dramatically.[29] Our entire findings point to a similar positioning of FHAP in the active site of RAMA to that of DHAP. In contrast to DHAP, FHAP cannot form this seminal interaction with Asp-33, due to fluorine's property of lacking hydrogen bond donor capacities and functioning solely as a hydrogen bond acceptor.

In summary, we have developed an efficient gram scale synthesis of FHAP (**5**). In contrast to earlier reports[12] FHAP could be identified as a novel donor substrate for RAMA-catalyzed aldol condensations. This fact was demonstrated in the synthesis of 1,4,5-tri-O-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose **8**. FHAP is the first analog of DHAP with a modification at C3 accepted as a donor substrate by RAMA. The stereochemical configuration at C3 and C4 (D-*threo*) of the aldol condensation product has proven to be identical as for the natural substrate DHAP, which was confirmed by an independent chemical synthesis of pentulose **8**. Furthermore, a fast proton–deuterium exchange and the different binding preferences of the *gem*-diol and keto form of FHAP were analyzed by ^{19}F-NMR. A direct evolution of aldolases[30] might result in highly efficient catalysts for the aldol condensation of FHAP with aldehydes, giving access towards a great variety of fluoro containing carbohydrates with biological and medicinal relevance.[10]

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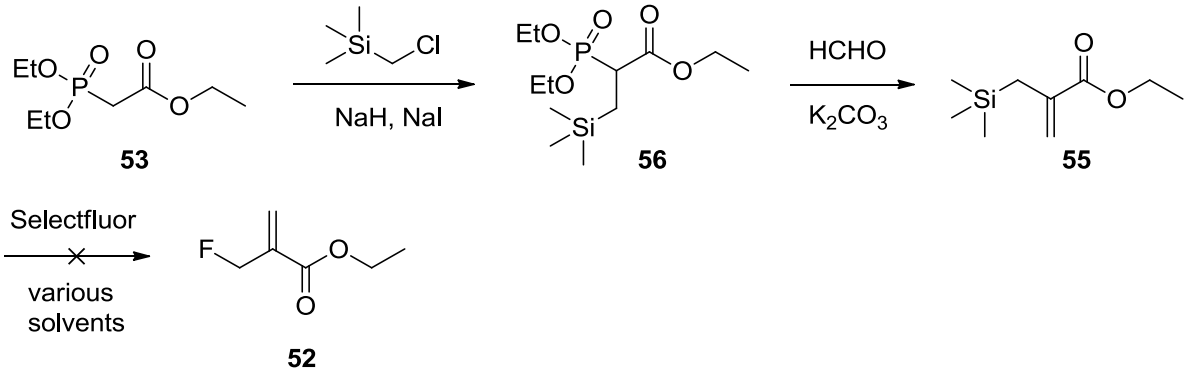
Unpublished Work:

Synthesis of Ethyl α-fluoromethylacrylate:

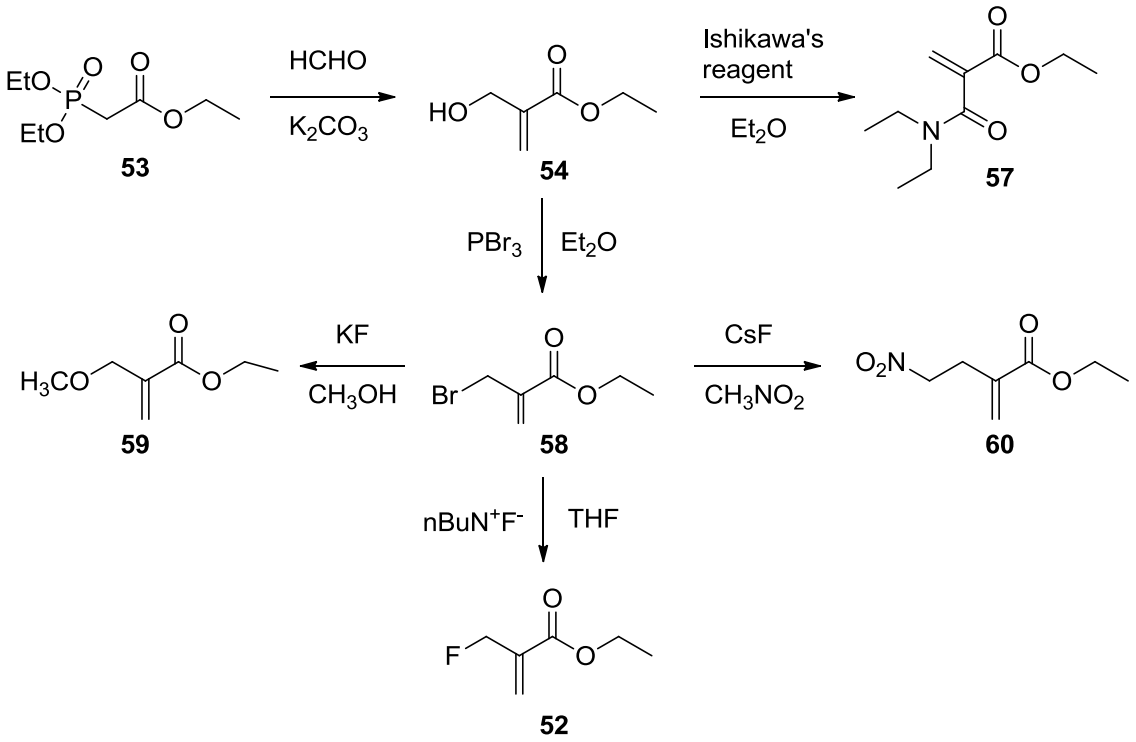
Several approaches towards the synthesis of ethyl α-fluoromethylacrylate (**52**) have been studied. All of them started from commercially available triethyl phosphonoacetate (**53**). The first approach attempted to introduce the fluorine atom by the electrophilic fluorodesilylation methodology of Gouverneur *et al*. (Scheme 19).[23] The silylated precursor acrylate **55** was prepared from **53** in two steps in high yield.[24] Although we screened several reactions conditions, the synthesis of **52** from **55** was unsuccessful using Selectfluor[25] or NFSI. This observation may be explained by electron withdrawing properties of the ester moiety, making the carbon-carbon double bond slightly electron deficient. Therefore a nucleophilic attack of the π-system towards an electrophilic fluorine source is not likely anymore. Furthermore, to the best of my knowledge Gouverneur *et al*. never published an electrophilic fluorodesilylation of ester substituted carbon-carbon double bonds.



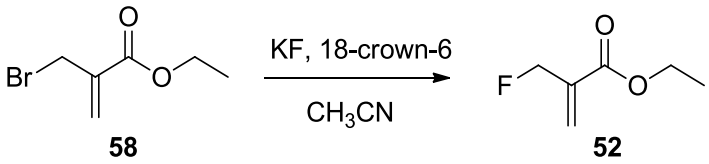
Scheme 19. Attempted synthesis of compound 52 via electrophilic flurodesilylation

In the second approach the fluorine atom was planned to be introduced by a nucleophilic substitution of the hydroxyl functionality of compound **54** (Scheme 20). Unfortunately applying Ishikawa's reagent[26] the product **57** could be isolated in moderate yield instead. Therefore a halogen-halogen exchange was used for the synthesis of **52** form **58**.[27] Bromination of **54** with phosphortribromide followed by treatment of **58** with KF in methanol yielded ether **59** due to nucleophilic attack of the solvent. For the same reason compound **60** was formed applying CsF in nitromethane. The desired product **52** was finally obtained by halogen-halogen exchange using tetrabutylammoium fluoride in THF[27] or

potassium fluoride in acetonitrile with crown ether in good yield (Scheme 21). Due to identical R_f-values of **52** and **58**, the reaction progress could only be monitored by ^{1}H-NMR. The loss in yield can be explained by co-evaporation of **52** with various solvents used. The potassium fluoride system could be up-scaled to 50 gram.



Scheme 20. Synthesis of ethyl α-fluoromethylacrylate (52)[27]

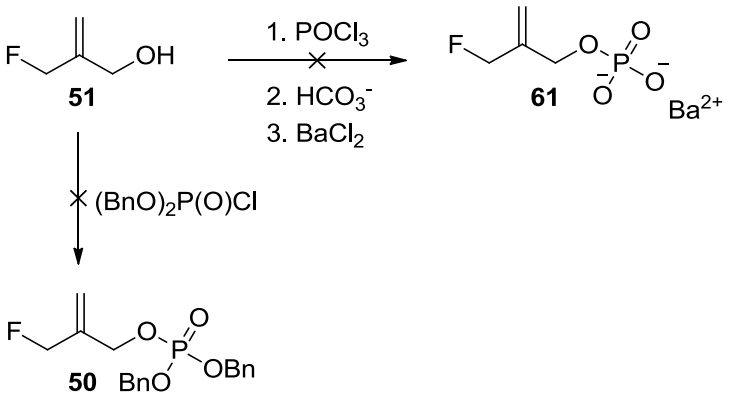


Scheme 21. Synthesis of ethyl α-fluoromethylacrylate (52)

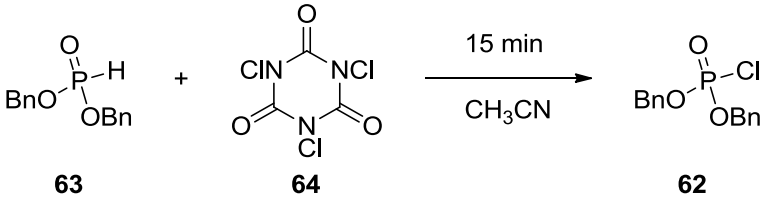
Phosphorylation of Allylic Alcohol 51:

The phosphorylation of allylic alcohol **51** proved to be complicated (Scheme 22). In analogy to the synthesis of DHAP (**1**), various methods for phosphorylation of **51** were tested. The pentavalent phosphorous reagent $(BnO)_2P(O)Cl$ (**62**) seemed to be too unreactive. Dibenzyl phosphoryl chloride (**62**) was synthesized by conversion of dibenyzlphosphite **63** with

trichlorocyanuric acid **64** in excellent yield (Scheme 23).[28] $POCl_3$, which was successfully used by Effenberger[10] for the synthesis of DHAP (1), did not give any product.



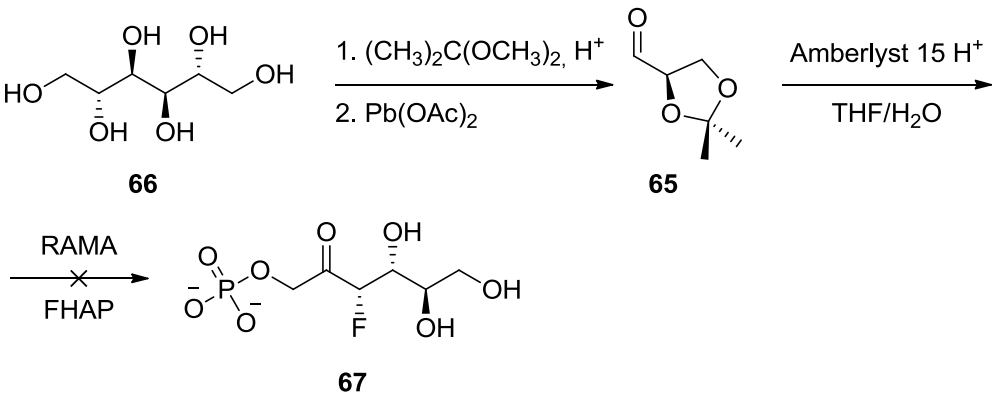
Scheme 22. Phosphorylation of allylic alcohol 51



Scheme 23. Synthesis of dibenzylphosphoryl chloride (62)[28]

Enzymatic Reactions of FHAP with D-Glyceraldehyde:

Whitesides *et al*. demonstrated that glyceraldehyde is a good acceptor substrate for RAMA-catalyzed aldol reactions.[8] To facilitate the final purification it was planned to perform the enzymatic aldol condensation with D-glyceraldehyde since theoretically only one product was expected. 1,2-*O*-Isopropylidene-D-glyceraldehyde (**65**) was synthesized starting from D-mannitol (**66**) by standard procedures.[29] The cleavage of the acetonide protecting group was performed by hydrolysis under acidic conditions in a THF/water mixture and monitored by TLC. After removal of the THF by evaporation, the crude product was used for the RAMA catalyzed aldol reaction with FHAP (**45**). Unfortunately the desired 3-deoxy-3-fluoro-D-fructose **67** could not be isolated (Scheme 24).



Scheme 24. Attempted synthesis of 3-deoxy-3-fluoro-D-fructose-1-phosphate (67)

Catalytic Role of Asp-33 of RAMA:

Tolan *et al*. have demonstrated the critical role of Asp33 in catalytic mechanism of RAMA by site-directed mutagenesis.[30] Aspartic acid 33 was changed to alanine, serine and glutamic acid, resulting in the mutant proteins, D33A, D33S and D33E, respectively. By analyzing the kinetic properties of the recombinant rabbit aldolases A using FBP (**4**), they showed that mutating the aspartate residue to alanine clearly had the most severe effect on V_{max} of the enzyme. The V_{max} of D33A, where the mutant residue is most dissimilar to the original enzyme, was 5600 times slower than wild-type. The D33S had a V_{max} 3600 and the D33E a V_{max} 960 times slower than wild type.

In 2007, Sygusch *et al*. showed that numerous interactions with active side residues stabilize the covalent intermediate, which was trapped by freezing a WT aldolase crystal in the presence of DHAP (**1**) (Fig 2).[6] As visible in Figure 5, the above mentioned Asp33 stabilizes the enamine intermediate by hydrogen bonding with DHAP C3 hydroxyl.

Assuming that FHAP (**45**) forms a similar placed covalent intermediate with the enzyme, at least this interaction is disturbed. Since the fluorine atom in FHAP (**45**) can only act as a hydrogen bond acceptor, a productive interaction with Asp33 is not possible anymore. Of course, this hypothesis can only be proved without a doubt by comparison of the X-ray structure of covalently linked FHAP (**45**) and DHAP (**1**) to RAMA. Maybe this disturbed interaction has an influence on the catalytic turnover of FHAP (**45**) by RAMA, which is reflected in the low yield of the enzymatic reactions.

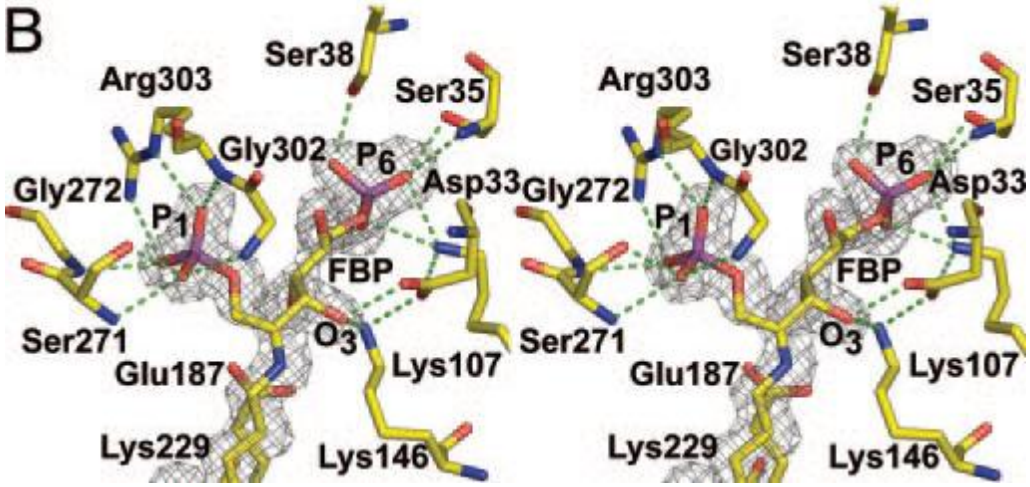
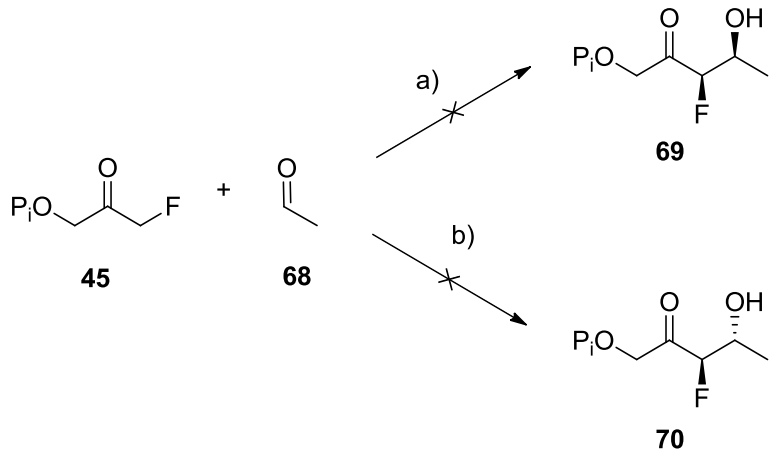


Figure 5. Catalytic role of Asp-33 with FBP (4)[6c]

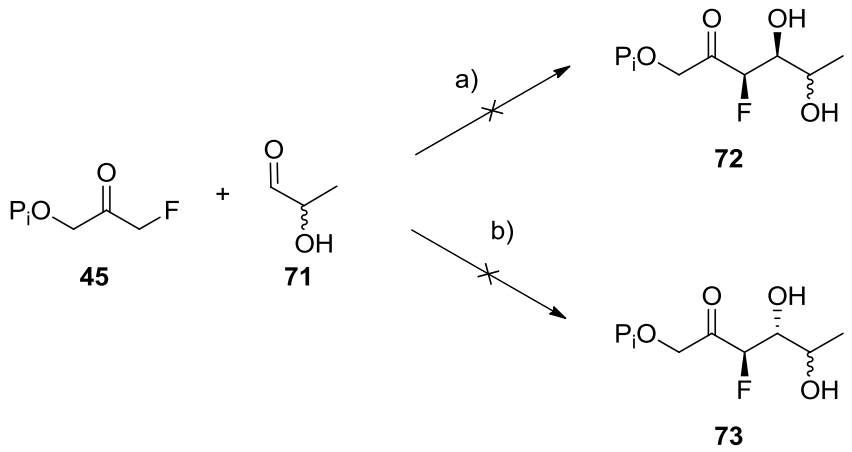
Miscellaneous Experiments:

Type I FBP Aldolase from Spinach and Type II FBP Aldolases from *E. Coli, Thermotoga Maritime and Thermoanaerobacter Ethanolicus*:

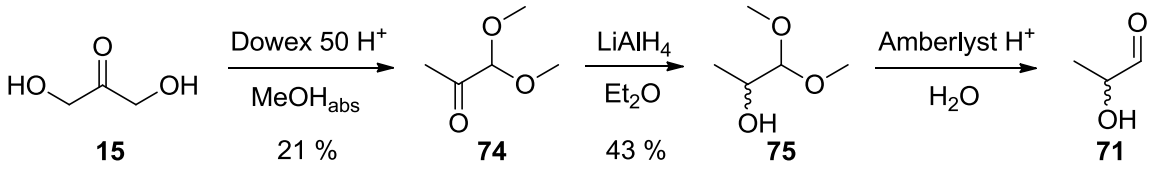
In addition to RAMA another commercial Type I FBP dependent aldolase from spinach[31] was tested in the aldol reaction between glycolaldehyde and FHAP (**45**). Similar to the reaction with RAMA, FHAP (**45**) was a bad substrate. The ^{19}F-NMR spectra showed that the reaction was even slower compared to RAMA catalyzed ones and therefore more side products were produced. Furthermore, we obtained four Type II aldolases from the company DSM, which were overexpressed in various bacteria. Two rhamnulose 1-phosphate aldolases from *E. coli* and *Thermotoga maritime* and two fuculose 1-phosphate aldolases from *E. coli* and *Thermoanaerobacter ethanolicus* were tested as catalysts for the aldol addition. Freshly distilled acetaldehyde (**68**) and 2-hydroxypropanal (**71**) were used as acceptor substrates (Scheme 25 and 26). Unfortunately no reaction progress could be observed under ^{19}F-NMR monitoring. The racemic aldehyde **71** was synthesized in two different sequences as outlined in Scheme 27 and 28, to exclude that impurities from the synthesis of 2-hydroxypropanal (**71**) were responsible for enzyme inhibition. The published reaction yields from Wong *et al*. could not be realized.[32] The deprotection of **71** was monitored by TLC and ^{1}H NMR and the aldehyde **71** was directly subjected to the enzymatic reactions after removal of amberlyst 15 H^+.



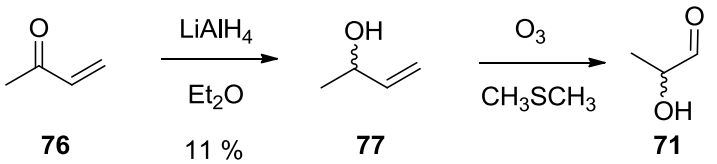
Scheme 25. Reagents and conditions: a) Rhamnulose 1-phosphate aldolases from *E. coli* (at rt) *or* Rhamnulose 1-phosphate aldolases from *Thermotoga maritime* (at 50°C) in BIS-TRIS buffer, pH = 6.8, rt; b) fuculose 1-phosphate aldolases from *E. coli* (at rt) and *Thermoanaerobacter ethanolicus* (at 50°C) in BIS-TRIS buffer, pH = 6.8, rt.



Scheme 26. Reagents and conditions: a) Rhamnulose 1-phosphate aldolases from *E. coli* (at rt) *or* Rhamnulose 1-phosphate aldolases from *Thermotoga maritime* (at 50°C) in BIS-TRIS buffer, pH = 6.8, rt; b) fuculose 1-phosphate aldolases from *E. coli* (at rt) and *Thermoanaerobacter ethanolicus* (at 50°C) in BIS-TRIS buffer, pH = 6.8, rt.



Scheme 27. Synthesis of 2-hydroxypropanal (71)[32]

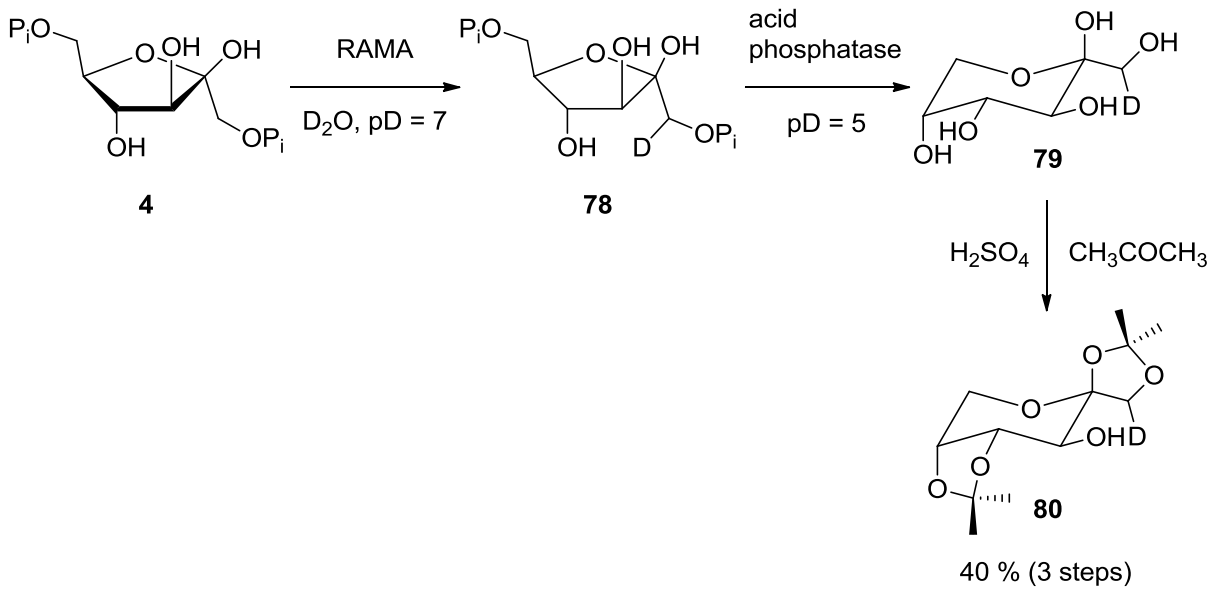


Scheme 28 Synthesis of 2-hydroxypropanal (71) via ozonolysis[32]

RAMA-Catalyzed Incorporation of Deuterium into Fructose-1,6-bisphosphate (FBP):

Since RAMA catalyzes a slow incorporation of deuterium into DHAP (**1**) at the position C1 we speculated that a hydrogen-deuterium exchange may be possible for the C1 position of D-FBP (**4**) as well. Indeed this exchange reaction was observed after addition of RAMA to FBP (**4**) in D_2O at pD = ~7 (Scheme 29). Interestingly only one deuterium was incorporated, according to the integration of the ^{1}H-NMR signals. The sight of exchange (C1) was undoubtedly determined by a combination of NMR methods (COSY, TOCSY, HSQC and HMBC) and ESI-MS^2 analysis of the fragmentation patterns. The stereochemistry of the novel stereogenic center at C1 was elucidated by comparison of NOESY crosspeaks of diisopropylidene protected, deuterium labeled fructose **80** and diisopropylidene protected fructose **81**[33] (Figure 6).

Our findings were in accordance with Lowe and Pratt, which were found after excessive literature researches.[34] Only the pro-S hydrogen was selectively exchanged by RAMA. A degradation of deuterium labeled fructose **79** to *S*-[2-D]glycolic acid, according to the procedure Rose *et al*. described for a identical tritrium labeled fructose, was unsuccessfully.[35]



Scheme 29 Synthesis of selectively deuterium labeled 1,2,4,5-*O*-diisopropylidene-D-fructose (80)

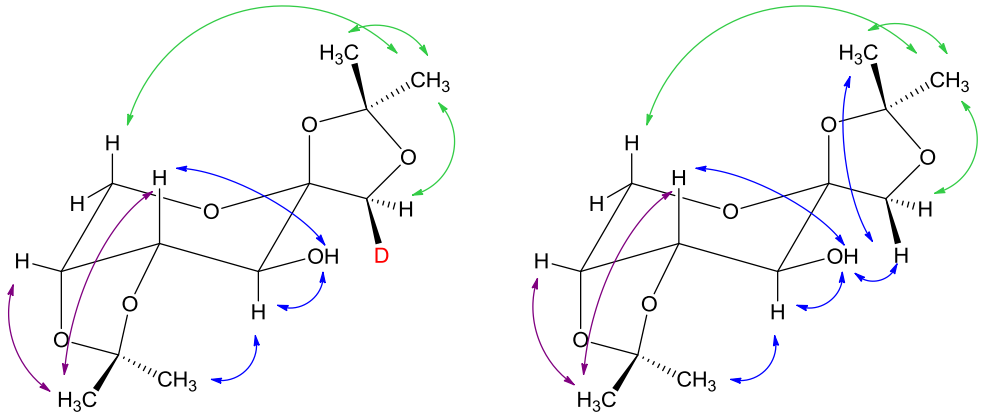


Figure 6. Determination of stereochemistry at C1 – the most important NOESY crosspeaks are visualized

A.4. Experimental Procedures

Published work:

Gram Scale Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate: A Novel Donor Substrate in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions

Michael Fischer, Hanspeter Kählig, Walther Schmid, *Chem. Commun.* **2011**, *47*, 6647-6649.

Supporting Information:

http://www.rsc.org/suppdata/cc/c1/c1cc11579k/c1cc11579k.pdf

Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Gram Scale Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate: A Novel Donor Substrate in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions

Michael Fischer, Hanspeter Kählig, and Walther Schmid*

Department of Organic Chemistry, University of Vienna, Währingerstrasse 38, 1090 Vienna, Austria

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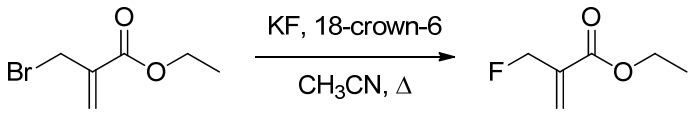
General Considerations. Anhydrous THF was distilled from potassium under argon, anhydrous CH_3CN was distilled of CaH_2. ^1H and ^{13}C NMR spectra were recorded on either 400 MHZ or 600 MHz spectrometer. Unless otherwise stated, all NMR spectra were measured either in $CDCl_3$ solutions and referenced to the residual $CHCl_3$ signal (^1H, δ=7.26 ppm; ^{13}C, δ=77.16 ppm) or in D_2O solutions (HDO, ^1H, δ=4.79 ppm). All ^1H and ^{13}C shifts are given in ppm. Flash chromatography was performed using silica gel 60 (0.004-0.063 mm). TLC monitoring was done on silica plates (silica gel 60 F_{254}), compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (48g) and $Ce(SO_4)_2$ (2g) in 10% H_2SO_4 (1L), followed by heating, or with an aqueous $KMnO_4$-solution. Ethyl α-bromomethylacrylate was synthesized according to a literature procedure.[1] pH and pD measurements were accomplished using a glass electrode (pD = pH + 0.4).[2] RAMA (lyophilized powder) and acid phosphatase (Type XA: from Sweet Potato) were purchased by Sigma Aldrich.

Ethyl α-fluoromethylacrylate (1):

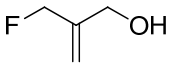


1

To a solution of ethyl α-bromomethylacrylate (48.3 g, 0.25 mol) in dry CH_3CN (200 mL), KF (43.6 g, 0.75 mol) and 18-crown-6 (1 g, 3.75 mmol) were added under argon. The mixture was refluxed until NMR monitoring (small aliquots were taken and measured in $CDCl_3$) showed complete consumption of the starting material (up to 3 days). The reaction was cooled to 0°C and quenched by addition of ice-cold water (75 mL). The aqueous phase was separated and the organic phase was extracted with water (2 x 50 mL). The combined aqueous phases were re-extracted with ether (150 mL) and the combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure (22°C, 50 Torr). The product was purified by vacuum distillation over a vigreux column to yield ethyl α-fluoromethylacrylate **(1)** (19.8 g, 60 %).

The moderate yield was due to co-evaporation of ethyl α-fluoromethylacrylate **(1)** with CH_3CN and CH_2Cl_2. Spectroscopical data were in accordance with Villieras et al.[2]

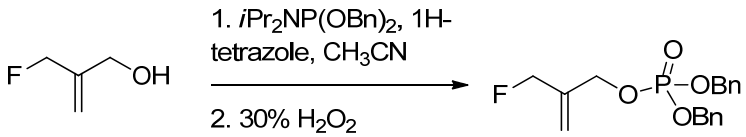
2-(Fluoromethyl)prop-2-en-1-ol (2):



2

To a solution of ethyl α-fluoromethylacrylate (**1**) (5.28 g, 0.04 mol) in dry CH_2Cl_2 (200 mL) at −78°C, DIBAL-H (1M in Hexan, 83.2 mL) was slowly added over 45 min under argon. After stirring the reaction mixture for 2 ½ hours at −65°C and for 45 min at room temperature the mixture was again cooled to −20°C. A saturated solution of Na_2SO_4 (2 mL) was slowly added and the reaction mixture was warmed up to 0°C. After addition of an ice cold saturated solution of NH_4Cl (50 mL) at 0°C, a white precipitate formed at room temperature, which was diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was carefully washed with CH_2Cl_2, the phases were separated and the combined organic phases were dried over $MgSO_4$, filtered and concentrated at reduced pressure (40°C, 750 Torr). The crude product was purified by flash chromatography (gradient: pentan/ether : 15:1→1:1) to yield 2-(fluoromethyl)prop-2-en-1-ol **(2)** (2.27 g, 63 %). The moderate yield was due to co-evaporation of 2-(fluoromethyl)prop-2-en-1-ol **(2)** with various solvents used. IR (film, cm^{-1}): ν = 3317, 2927, 1438, 1211, 1025; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.29 (m, 1H), 5.24 (m, 1H), 4.93 (m, J = 47.2 Hz, 2H), 4.23 (s, 2H), 1.58 (bs, 1OH). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 144.13 (d, J = 14.6 Hz), 114.5 (d, J = 9.8 Hz), 83.8 (d, J = 165.0 Hz), 63.33 (d, J = 2.9 Hz). ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –218.04 (dt, J = 47.2, 3.4 Hz). HRMS (EI) calcd for C_4H_7O (M-F): 71.0497, found 71.0495.

(**Dibenzyl (2-(fluoromethyl)allyl) phosphate (3):**

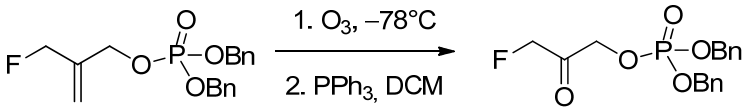


2 **3**

To a solution of 2-(fluoromethyl)prop-2-en-1-ol **(2)** (1.23 g, 13.65 mmol) in dry CH_2Cl_2 (50 mL), dibenzyl *N*,*N*-diisopropylphosphoramidite (6.60 g, 19.11 mmol) was added under argon. The

reaction mixture was cooled to -5°C and tetrazole (~0.45 M in CH_3CN, 51 mL, 22.9 mmol) was added slowly. A white solid precipitated and after the solution was stirred for 10 min at room temperature, a H_2O_2 solution (30% in H_2O, 3.1 mL, 30 mmol) was added. After TLC monitoring (hexane/EtOAc = 2/1) showed complete consumption of the intermediate phosphite (~20 min), H_2O (20 mL) and CH_2Cl_2 (20 mL) was added. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated. The crude material was purified by flash chromatography (hexane/EtOAc = 2/1) to yield dibenzyl (2-(fluoromethyl)allyl) phosphate (**3**) (3.53 g, 74 %). IR (film, cm^{-1}): $\nu = 2927$, 1456, 1272, 1012, 738, 696; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.30 (m, 10H), 5.30–5.25 (m, 2H), 5.06 (dd, J = 11.8, 8.4 Hz, 2H), 5.04 (dd, J = 11.8, 8.4 Hz, 2H), 4.82 (m, J = 46.9 Hz, 2H), 4.52 (d, J = 7.3 Hz, 2H). ^{13}C NMR (600 MHz, $CDCl_3$) δ = 139.5, 135.9 (d, J_{CF} = 6.7 Hz), 128.7, 128.1, 117.3 (d, J_{CF} = 9.5 Hz), 82.7 (d, J_{CF} = 167.0 Hz), 69.6 (d, J_{CP} = 5.5 Hz), 67.1 (dd, J_{CP} = 5.5 Hz, J_{CF} = 2.8 Hz). ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –219.26 (dt, J = 46.9, 3.3 Hz). HRMS (ESI) calcd for $C_{18}H_{21}O_4FP$ (M+H): 351.1162, found 351.1167.

Dibenzyl (3-fluoro-2-oxopropyl) phosphate (4): 3 → 4

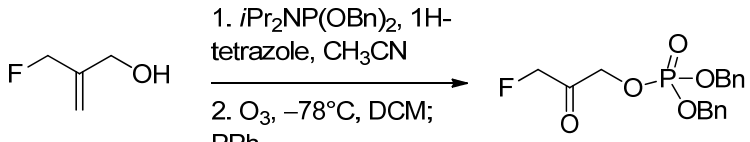


3 **4**

Ozone was bubbled through a solution of compound **3** (3.50 g, 9.99 mmol) in dry CH_2Cl_2 (25 mL) at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of $(CH_3)_2S$ (0.87 g, 14 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure to yield dibenzyl (3-fluoro-2-oxopropyl) phosphate (**4**) (3.34 g; 95 %). IR (film, cm^{-1}): ν = 2927, 1751, 1271, 1009, 737, 696. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.28 (m, 10H), 5.13 (dd, J = 11.7, 8.6 Hz, 2H) 5.09 (dd, J = 11.7, 9.1 Hz, 2H), 4.89 (d, J = 47.0 Hz, 2H), 4.70 (dd, J = 9.7, 1.7 Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 199.9 (dd, J = 19.0, 5.9 Hz, 1C), 135.6 (d, J =

6.5 Hz, 2C), 128.9 (2C), 128.8 (4C), 128.3 (4C), 84.3 (d, J = 182.5 Hz, 1C), 70.0 (d, J = 5.8 Hz, 2C), 69.3 (dd, J =5.6, 2.6 Hz, 1C). ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –237.3 (tt, J = 47.0, 1.7 Hz). HRMS (EI) calcd for $C_{17}H_{19}O_5PF$ (M+H): 353.0954, found 353.0945.

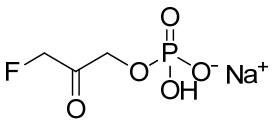
Dibenzyl (3-fluoro-2-oxopropyl) phosphate (4): 2 → 4



2 **4**

To a solution of 2-(fluoromethyl)prop-2-en-1-ol **(2)** (0.45 g, 5 mmol) in dry CH_2Cl_2 (20 mL), dibenzyl *N*,*N*-diisopropylphosphoramidite (2.42 g, 7 mmol) was added under argon. The reaction mixture was cooled to -5°C and tetrazole (~0.45 M in CH_3CN, 18.6 mL) was added slowly. A white solid precipitated and the solution was stirred for 15 min at room temperature. Ozone was bubbled through this solution at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of $(CH_3)_2S$ (0.43 g, 7 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (toluol/EtOAc = 1/1) to yield dibenzyl (3-fluoro-2-oxopropyl) phosphate (**4**) (914 mg, 52 %).

3-Fluoro-1-hydroxyacetone phosphate mono sodium salt (5):

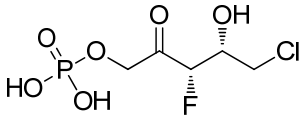


5

Compound **4** (2.04 g, 5.79 mmol) was dissolved in *t*BuOH (60 mL) and hydrogenated in the presence of 10% palladium-on-charcoal (308 mg) with a balloon of hydrogen. After TLC monitoring (tolulol/EtOAc = 1/1) showed consumption of the starting material, the catalyst was removed by filtration and the filter was rinsed with water (30 mL). After addition of NaOH (0.1 M,

5.79 mL,) the solution (pH = 6.5-6.8) was concentrated under reduced pressure (not to dryness), water added and concentrated again to remove *t*BuOH (not to dryness). The mono sodium salt of 3-fluoro-1-hydroxyacetone phosphate (**5**) (1.06 g, 95 %) was obtained after lyophilisation. ^{1}H NMR (400 MHz, D_2O): δ^{keto} = 5.30 (d, *J* = 46.5 Hz, 2H), 4.63 (d, *J* = 7.4 Hz, 2H); $\delta^{hydrate}$ = 4.40 (d, *J* = 46.6 Hz, 2H), 3.86 (dd, *J* = 5.9, 2.2 Hz, 2H) . ^{13}C NMR (400 MHz, D_2O): $\delta^{hydrate}$ = 93.5 (dd, *J* = 19.2, 7.7 Hz, 1C), 84.0 (d, *J* = 172.3 Hz, 1C), 66.4 (dd, *J* = 5.0, 1.8 Hz, 1C); δ^{keto} = 207.3 (from HMBC, 1C), 85.0 (d, *J* = 176.8 Hz, 1C), 67.9 (from HMBC, 1C); ^{19}F NMR (600 MHz, D_2O, coupled): $\delta^{hydrate}$ = –230.9 (tt, *J* = 1.9, 46.6 Hz); δ^{keto} = –237.1 (t, *J* = 46.7 Hz). HRMS (ESI-neg. mode) calcd for $C_3H_5O_5FP$ (M-H): 170.9859 found, 170.9856.

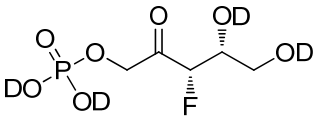
^{19}F-NMR study of RAMA with chloroacetaldehyde in H_2O: 5-Chloro-3,5-dideoxy-3-fluoro-D-*threo*-pent-2-ulose-1-phosphate (6):



6

Good resolved ^{19}F-NMR spectra for compound **6** were obtained by using a BIS-TRIS buffer (2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol). To a solution of FHAP (**5**) (42.5 mg, 0.219 mmol) in H_2O (2 mL), chloroacetaldehyde (~ 50% in H_2O; 33 µL, 0.255 mmol) and BIS-TRIS (2 mg, 0.01 mmol) was added and the pH was adjusted with small quantities of 1 M NaOH solutions to pH = 6.8. RAMA (43.6 U) was added (SI, Figure 3). After slow stirring over night, RAMA (19 U) was repeatedly added (SI, Figure 4).

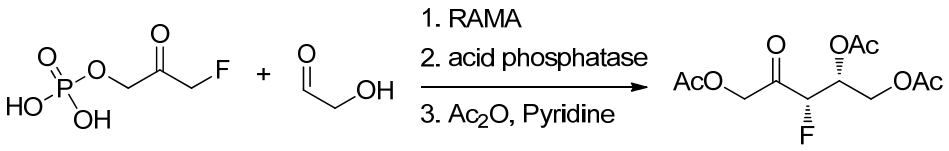
^{19}F- and ^{1}H-NMR study of RAMA with glycolacetaldehyde in D_2O: 3-deoxy-3-fluoro-D-*threo*-pent-2-ulose-1-phosphate (7):



7

To a solution of FHAP (**5**) (4 mg; 0.021 mmol) in D_2O (0.6 mL), glycolaldehyde (1.3 mg, 0.021 mmol) was added and the pD was adjusted with small quantities of 1M NaOD or DCl solutions to pD = 7.3. RAMA (2U) was added and the reaction progress was monitored by ^{1}H-NMR and ^{19}F-NMR (SI, Figure 5).

Enzymatic synthesis of 1,4,5-tri-*O*-acetyl-3-deoxo-3-fluoro-D-*threo*-pent-2-ulose (8):

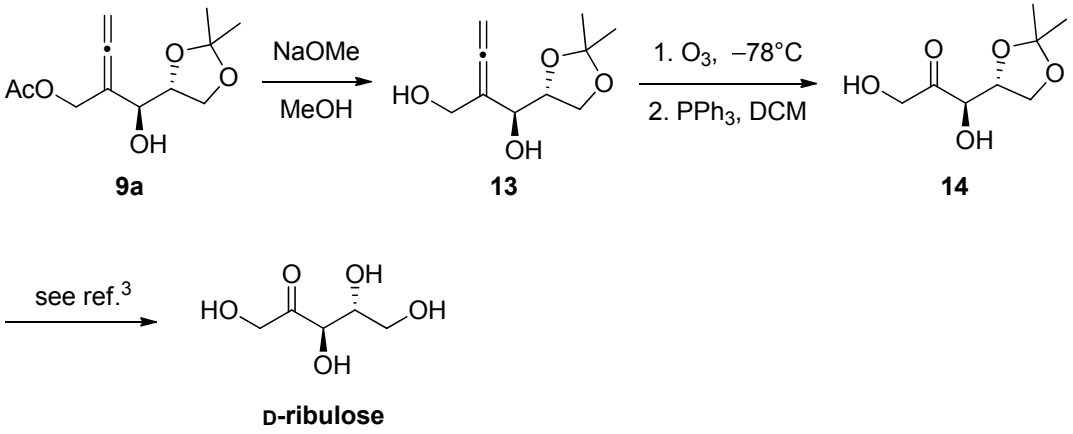


5 **8**

To a solution of FHAP mono sodium salt (100 mg, 0.52 mmol) in H_2O (30 mL), glycolaldehyde (36 mg, 0.6 mmol) and BIS-TRIS (31 mg, 0.15 mmol) was added. After the pH was adjusted with small quantities of 1 M NaOH solutions to pH = 6.8, RAMA (95 U) was added. The solution was slowly stirring over night and RAMA (47.5 U) was added again. After 2 days the pH was adjusted to pH = 5.1 with 1 N HCl solution, acid phosphatase (110 U) was added. The reaction mixture was slowly stirred over night and the pH was adjusted to 6.85 with 1N NaOH. After lyophilization the crude product was purified by flash chromatography (CHCl/MeOH = 9/1). Since a structural determination was not possible, the fractions with Rf between 0.56 and 0.30 were pooled together, evaporated and suspended in dry pyridine (2 mL). Dimethylaminopyridine (11 mg, 0.09 mmol) was added and the solution was cooled to 0°C under argon. Acetanhydride (191 mg, 1.87 mmol) was added and the solution was stirred over night. The reaction mixture was quenched by the addition

of H_2O (1 mL) at 0°C and the aqueous phase was extracted with EtOAc (3 x 5ml). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The product was purified by flash chromatography (hexane/EtOAc = 4/1) to yield 1,4,5- tri- *O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (**8**) (10 mg, 7 %). $[\alpha]_D^{20}$ = -24.5 (*c* 0.5, CH_2Cl_2); IR (film, cm^{-1}): ν = 2925, 2855, 1744, 1372, 1227, 1048; ^1H NMR (400 MHz, $CDCl_3$,): δ = 5.48 (ddt, *J* = 27.8, 6.5, 2.4 Hz, 1H), 5.14 (dd, *J* = 47.5, 2.5 Hz, 1H), 5.05 (dd, *J* = 18.2, 2.1 Hz, 1H) 4.92 (dd, *J* = 18.2, 2.1 Hz, 1H), 4.31 (dd, *J* = 11.4, 6.6 Hz, 1H), 4.24 (ddd, *J* = 11.5, 6.2, 1.0 Hz, 1H), 2.17 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 199.6 (d, *J* = 26.5 Hz), 170.3, 170.1, 170.0, 93.3 (d, *J* = 191.3 Hz), 69.7 (d, *J* = 17.8 Hz), 67.2 (d, *J* = 4.6 Hz), 60.6 (d, *J* = 6.0 Hz), 20.7, 20.6, 20.5. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –215.12 (ddt, *J* = 47.3, 27.8, 2.4 Hz). HRMS (ESI) calcd for $C_{11}H_{15}O_7FNa$ (M+Na): 301.0700, found 301.0695.

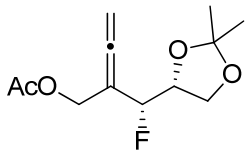
Determination of the absolute stereochemistry of compound 9a:



To a suspension of compound **9a**[3] (9 mg, 0.037 mmol) in dry MeOH under argon, NaOMe (0.5 mg, 9 µmol) was added at 0°C. The reaction mixture was stirred at room temperature until TLC monitoring (H/EtOAC = 1/1) showed conversion of the starting material. Then Dowex 50 H^+ was added and the mixture was stirred for 5 min. After neutralization the resin was removed by filtration, washed in with MeOH and the filtrate concentrated *in vacuo* to yield compound **13**.[3] The crude material was dissolved in dry CH_2Cl_2 (1 mL) and cooled to –78°C. Ozone was bubbled through this solution at -78°C until the color of the mixture turned blue. Dry air was bubbled

through the solution until the blue color disappeared. After addition of PPh_3 (12 mg, 0.044 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (hexane/EtOAc = 1/1) to yield 4,5-*O*diisopropylidine-D-*erythro*-pent-2-ulose (**14**) (6.4 mg, 91 %).[3] Compound **14** was transformed in D-ribulose as previously published.[3] The spectroscopical data for D-ribulose were identical with those reported.[4]

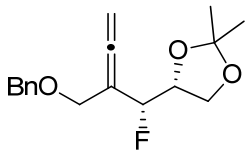
(*2R,3R*)-4-(Acetyloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10a):



10a

To a suspension of compound **9a**[3] (34 mg, 0.14 mmol) in dry CH_2Cl_2 (4 mL) under argon, collidine (34 mg, 0.281 mmol) was added and the reaction mixture cooled to −78°C. After dropwise addition of DAST (95 %) (47 mg, 0.281 mmol) the solution was stirred for 15 min at −78°C and then warmed up to −30°C. After TLC monitoring showed conversion of the starting material, the reaction was quenched by the addition of a $NaHCO_3$-solution (10%, 0.5 mL). The solution was brought to room temperature and the aqueous phase was extracted with CH_2Cl_2 (3 x 2 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo*. Flash chromatography on silica gel in hexane/EtOAc = 8/1 afforded compound **10a** (12 mg, 35 %). $[\alpha]_D^{20}$ = +35.1 (*c* = 0.2, CH_2Cl_2); IR (film, cm^{-1}): ν = 2935, 1963, 1745, 1365, 1225; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.1–5.0 (m, 2H), 4.88 (ddt, *J* = 48.7, 7.8, 1.5 Hz, 1H), 4.69–4.64 (m, 2H), 4.47–4.34 (m, 1H), 4.06 (ddd, *J* = 8.8, 6.9, 2.1 Hz, 1H), 3.69 (ddd, *J* = 8.6, 6.6, 0.9 Hz, 1H), 2.08 (s, 3H), 1.47 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.1 (d, *J* = 8.5 Hz), 170.5, 110.7, 97.2 (d, *J* = 21.3 Hz), 91.8 (d, *J* = 180.5 Hz) 79.5 (d, *J* = 2.7 Hz), 76.5 (d, *J* = 20.0 Hz), 65.7 (d, *J* = 5.9 Hz), 61.7 (d, *J* = 0.7 Hz), 26.7, 25.4, 21.0. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = −182.0 (dm, *J* = 48.8 Hz). HRMS (EI) calcd for $C_{11}H_{14}O_4F$ ($M-CH_3$): 229.0876, found 229.0875.

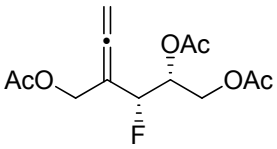
(*2R,3R*)-4-(Benzylloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10b):



10b

To a suspension of compound **9b**[3] (73 mg, 0.25 mmol) in dry CH_2Cl_2 (6 mL) under argon, collidine (61 mg, 0.5 mmol) was added and the reaction mixture cooled to −78°C. After dropwise addition of DAST (95 %) (84 mg, 0.5 mmol) the solution was stirred for 15 min at −78°C and then warmed up to −30°C. After TLC monitoring showed conversion of the starting material, the reaction was quenched by the addition of a $NaHCO_3$-solution (10%, 1 mL). The solution was brought to room temperature and the aqueous phase was extracted with CH_2Cl_2 (3 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo*. Flash chromatography on silica gel in hexane/EtOAc = 8/1 afforded compound **10b** (18 mg, 25 %). IR (film, cm^{-1}): ν = 2988, 2924, 2854, 1958, 1372, 1069, 740, 699; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.27 (m, 5H), 5.04–4.82 (m, 2H), 4.92 (ddt, *J* = 49.1, 8.0, 1.3 Hz, 1H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 4.51–4.40 (m, 1H), 4.14-4.11 (m, 2H), 4.03 (ddd, *J* = 8.6, 7.0, 2.2 Hz, 1H), 3.67 (ddd, *J* = 8.6, 6.6, 1.1 Hz, 1H), 1.46 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.3 (d, *J* = 8.5 Hz), 137.9, 128.6, 127.97, 127.96, 110.5, 98.0 (d, *J* = 21.0 Hz), 92.2 (d, *J* = 179.2 Hz), 78.3 (d, *J* = 2.9 Hz), 76.6 (d, *J* = 20.2 Hz), 72.5, 67.9, 65.8 (d, *J* = 6.4 Hz), 26.8, 25.5. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = −180.2 (dm, *J* = 49.2 Hz). HRMS (EI) calcd for $C_{16}H_{18}O_3F$ (M-CH_3): 277.1240, found 277.1233.

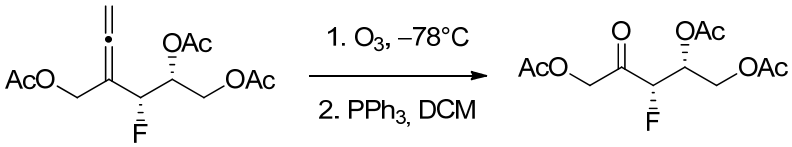
(*2R,3R*)-1,2-Di-*O*-acetyl-4-(acetyloxymethyl)-3-fluoro-hexa-4,5-dien-1,2-diol (11a):



11a

To a suspension of compound **10a** (11 mg, 0.045 mmol) in 2 mL of a mixture of water/THF (2:1), 10 mg of Amberlyst 15 (H^+-form) were added and the mixture was stirred at room temperature. After TLC monitoring showed conversion of the starting material (~4 h), the resin was removed by filtration, washed with methanol and the solvent was removed under reduced pressure. The crude product was suspended in dry pyridine (0.5 mL), dimethylaminopyridine (0.5 mg, 4 µmol) was added and the solution was cooled to 0°C under argon. Acetanhydride (18 mg, 0.176 mmol) was added and the solution was stirred over night. The reaction mixture was quenched by addition of H_2O (200 µL) at 0°C and the aqueous phase was extracted with EtOAc (3 x 1ml). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The product was purified by flash chromatography (hexane/EtOAc = 4/1) to yield (*2R,3R*)-3-fluoro-1,2,5-*O*-triacetyl-4-vinylidene-pentan-1,2,5-triol (**11a**) (10 mg, 80 %). $[\alpha]_D^{20}$ = +28.0 (*c* 0.1, CH_2Cl_2); IR (film, cm^{-1}): ν = 2922, 1973, 1744, 1374, 1222, 1047; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.39 (dddd, *J* = 17.8, 5.7, 3.9, 1.9 Hz, 1H), 5.14 (ddt, *J* = 47.2, 5.9, 1.7 Hz, 1H), 5.10–5.03 (m, 2H), 4.67 (m, 2H), 4.36 (dd, *J* = 11.9, 3.8 Hz, 1H), 4.16 (dd, *J* = 12.0, 5.9 Hz, 1H) 2.12 (s, 3H) 2.09 (s, 3H) 2.02 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.06 (d, *J* = 8.0 Hz), 170.60, 170.59, 170.2, 96.4 (d, *J* = 22.0 Hz), 88.8 (d, *J* = 182.0 Hz), 79.7 (d, *J* = 1.8 Hz), 70.8 (d, *J* = 20.9 Hz), 62.33 (d, *J* = 6.0 Hz), 61.6 (d, *J* = 1.7 Hz), 21.0, 20.9, 20.8. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –191.7 (ddt, *J* = 46.9, 17.5, 7. 8 Hz). HRMS (ESI) calcd for $C_{13}H_{17}O_6FNa$ (M+Na): 311.0907, found 311.0912.

Chemical synthesis of 1,4,5-tri-*O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (8):

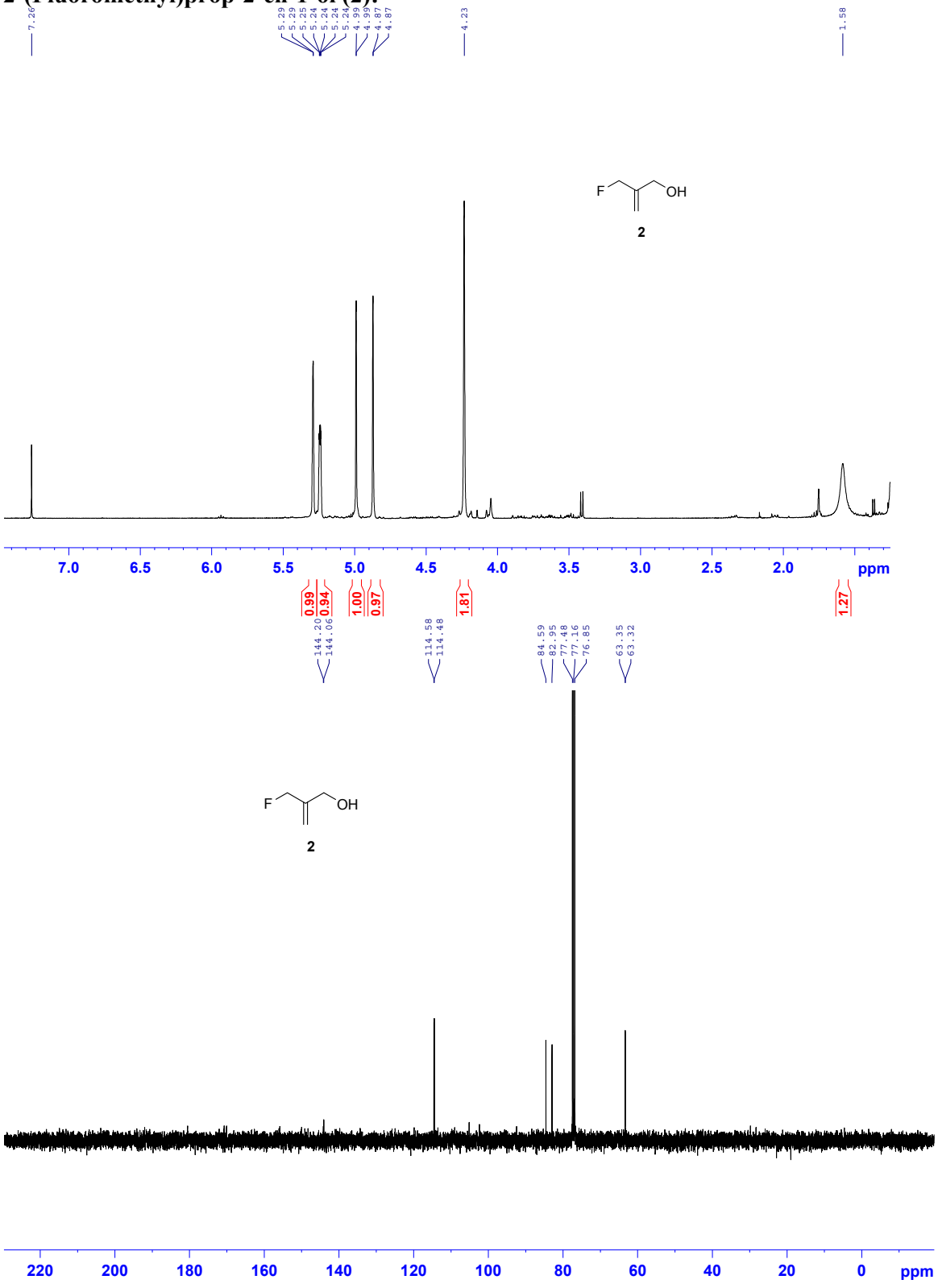


11a **8**

Ozone was bubbled through a solution of compound **11a** (3 mg, 10.4 µmol) in dry CH_2Cl_2 (1.5 mL) at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of PPh_3 (4 mg, 15 µmol), the mixture was allowed to

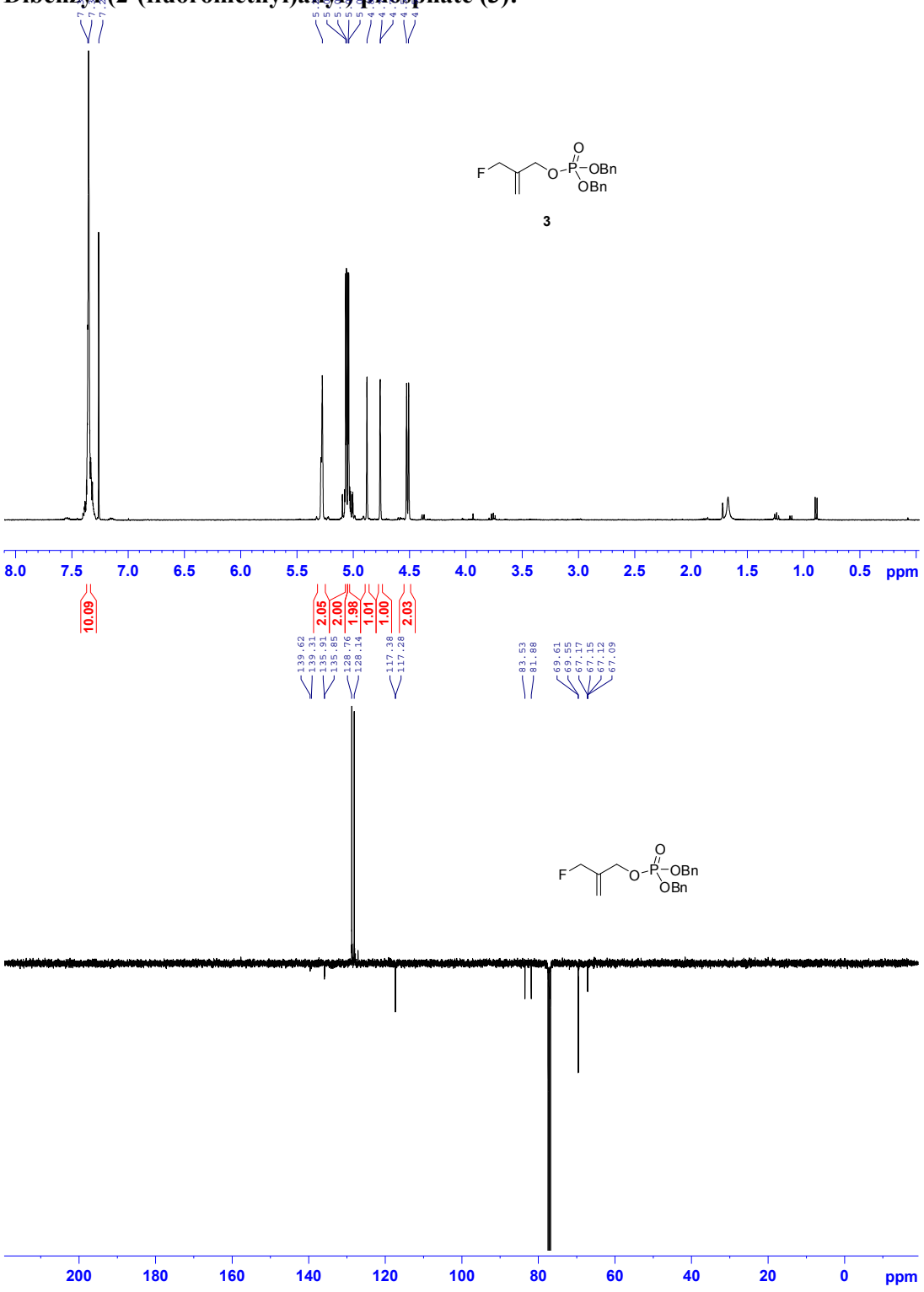
reach room temperature by stirring overnight. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (hexane/ethyl acetate = 3:1) to yield compound **8** (2.7 mg; 95 %). $[\alpha]_D^{20}$ = -26.5 (*c* 0.15, CH_2Cl_2). Spectroscopical data were identical with the data obtained from the enzymatic synthesis.

2-(Fluoromethyl)prop-2-en-1-ol (2):

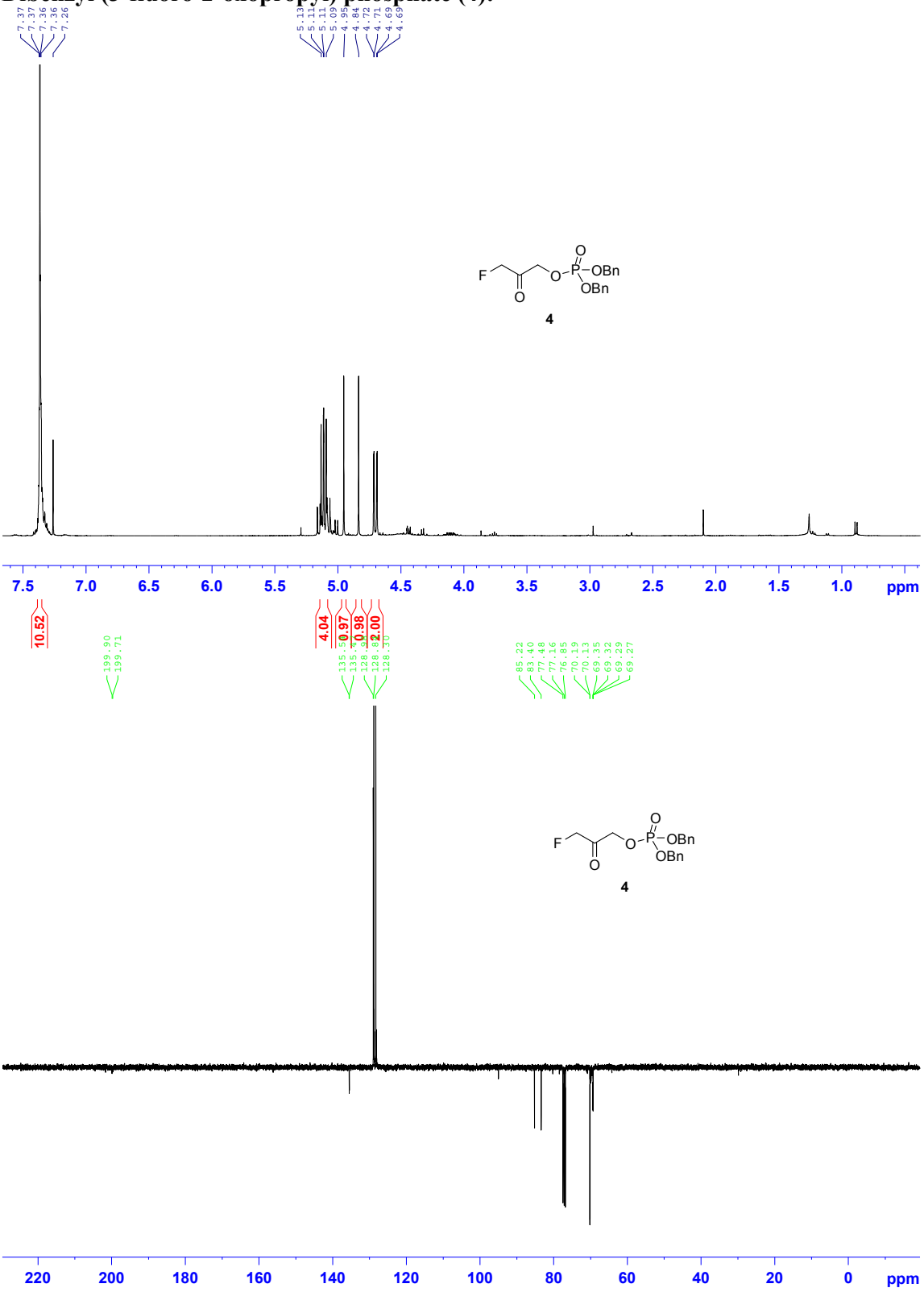


S13

Dibenzyl (2-(fluoromethyl)allyl) phosphate (3):

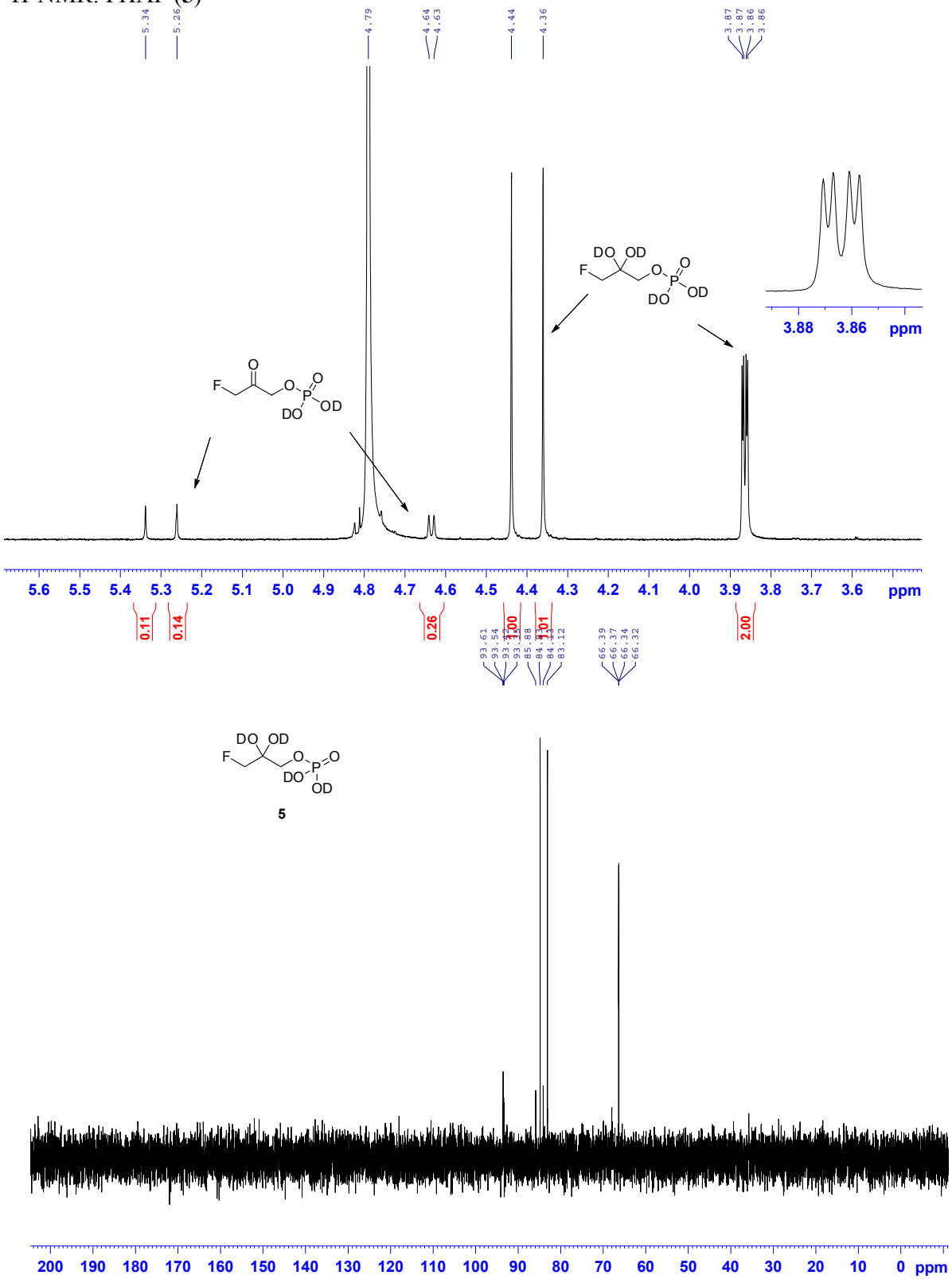


Dibenzyl (3-fluoro-2-oxopropyl) phosphate (4):



S15

^{1}H-NMR: FHAP (**5**)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3.87
3.86
3.86
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0.11
0.14
0.26
1.00
1.01
2.00
93.61
93.54
93.46
93.12
85.88
84.13
84.03
83.12
66.39
66.37
66.34
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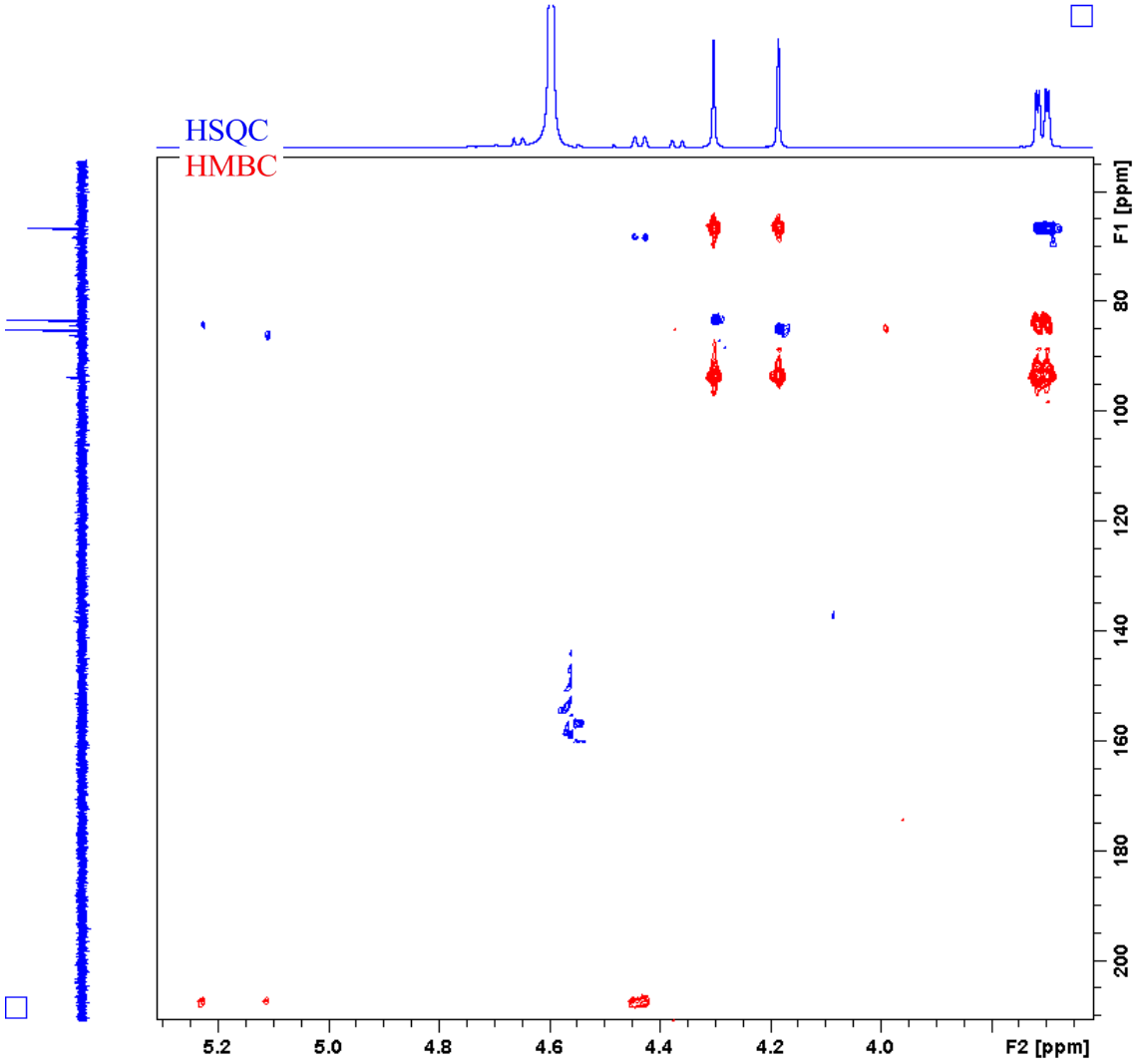
HSQC
HMBC
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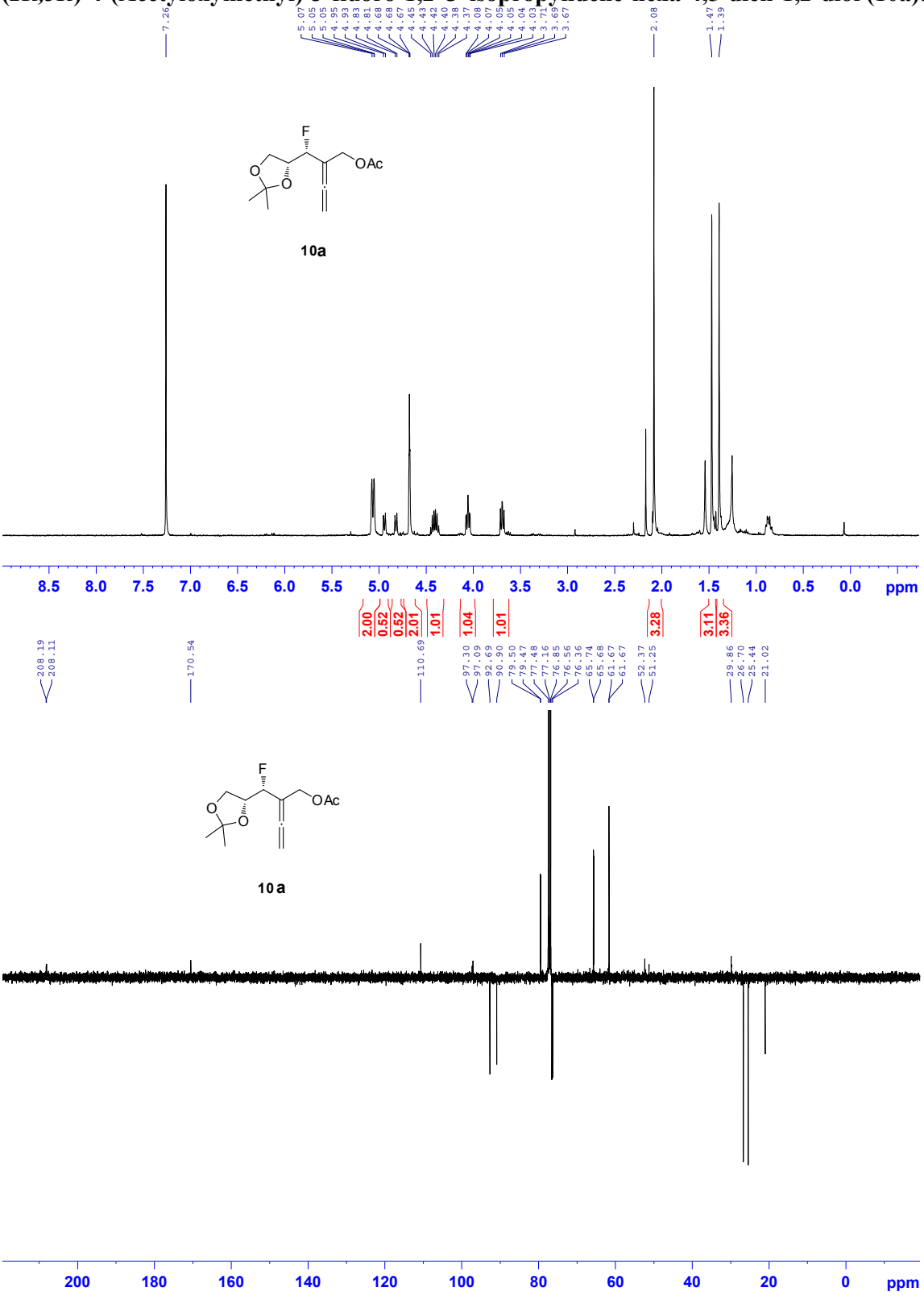
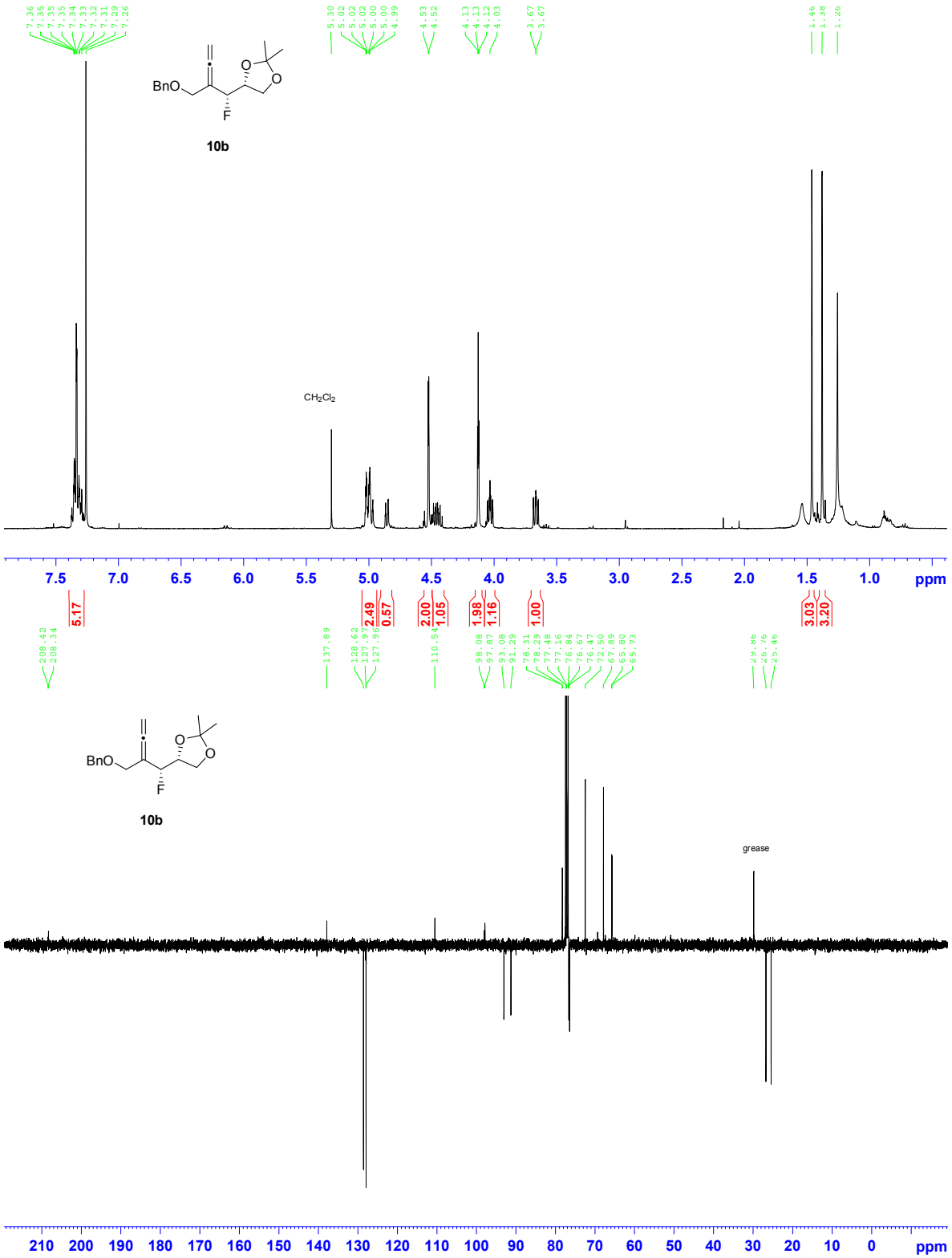
100
120
140
160
180
200
5.2
5.0
4.8
4.6
4.4
4.2
4.0
F2 [ppm]

S17

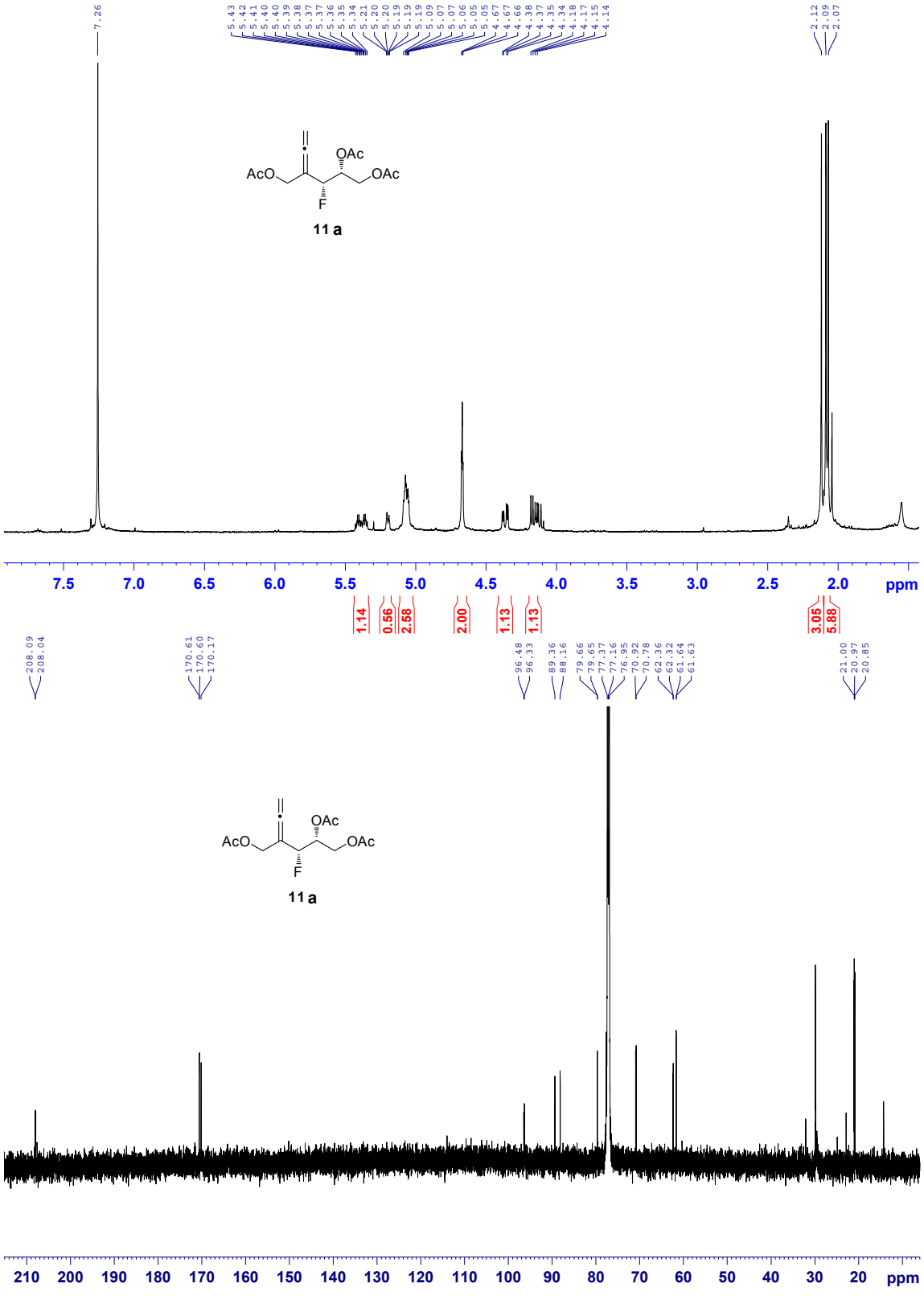
(*2R,3R*)-4-(Acetyloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10a):



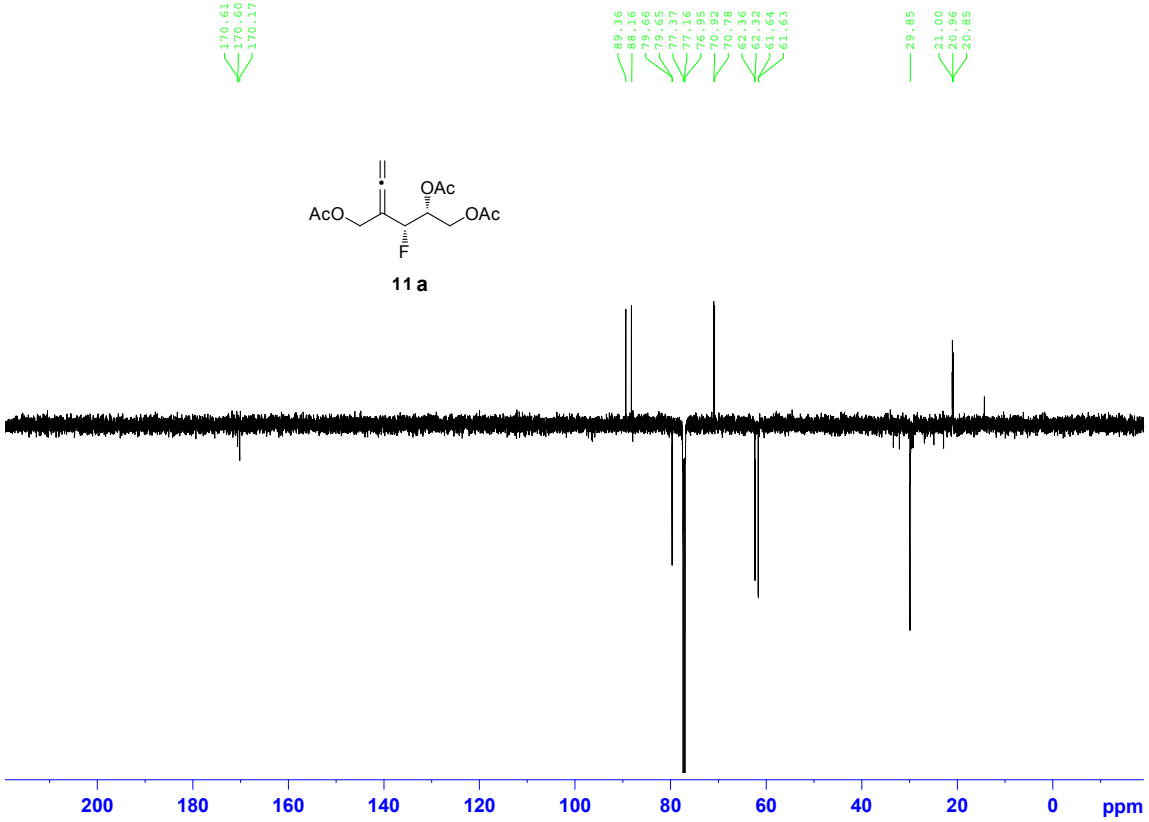
(*2R,3R*)-4-(Benzyloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10b):



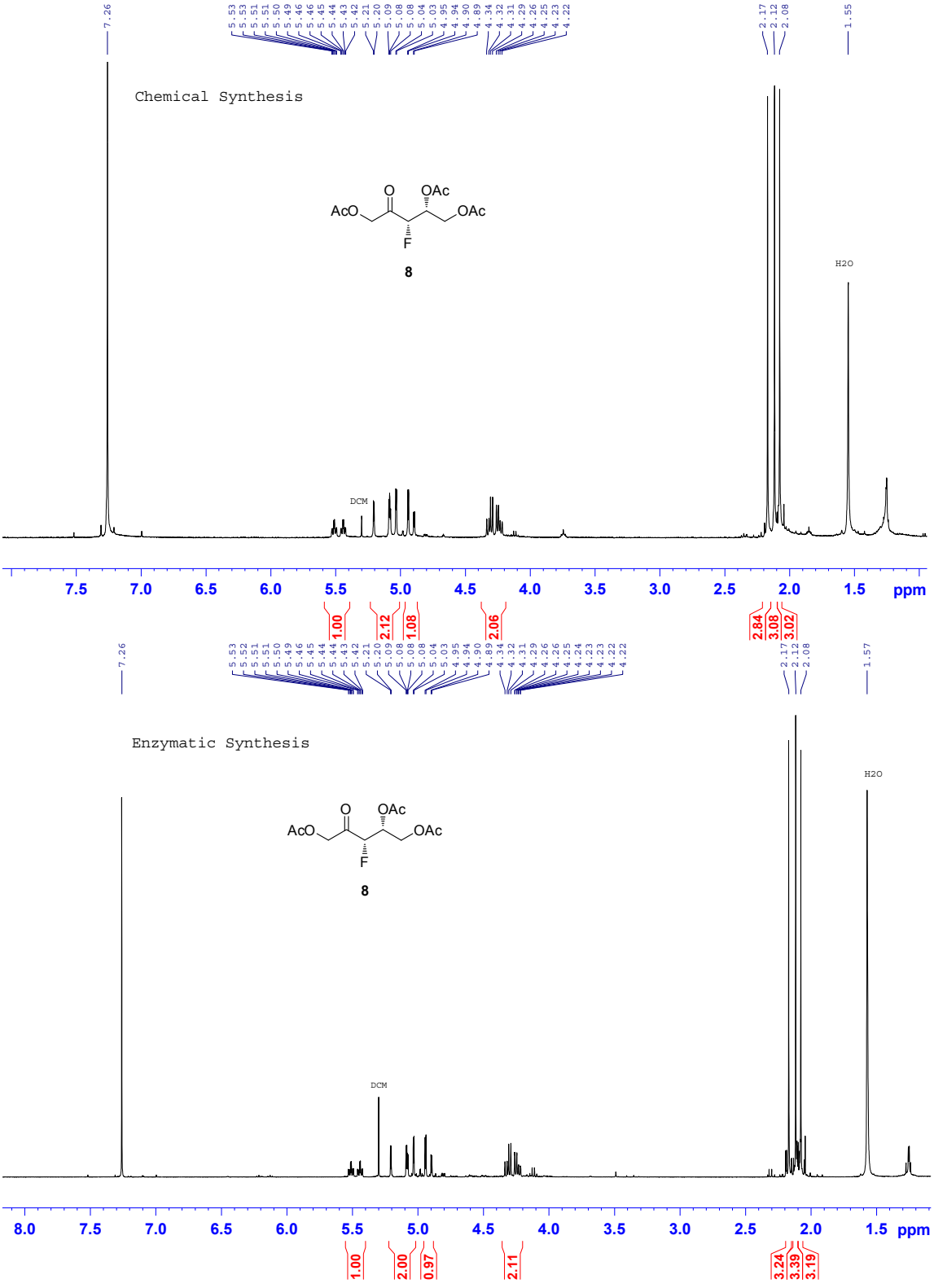
(*2R,3R*)-1,2-Di-*O*-acetyl-4-(acetyloxymethyl)-3-fluoro-hexa-4,5-dien-1,2-diol (11a):



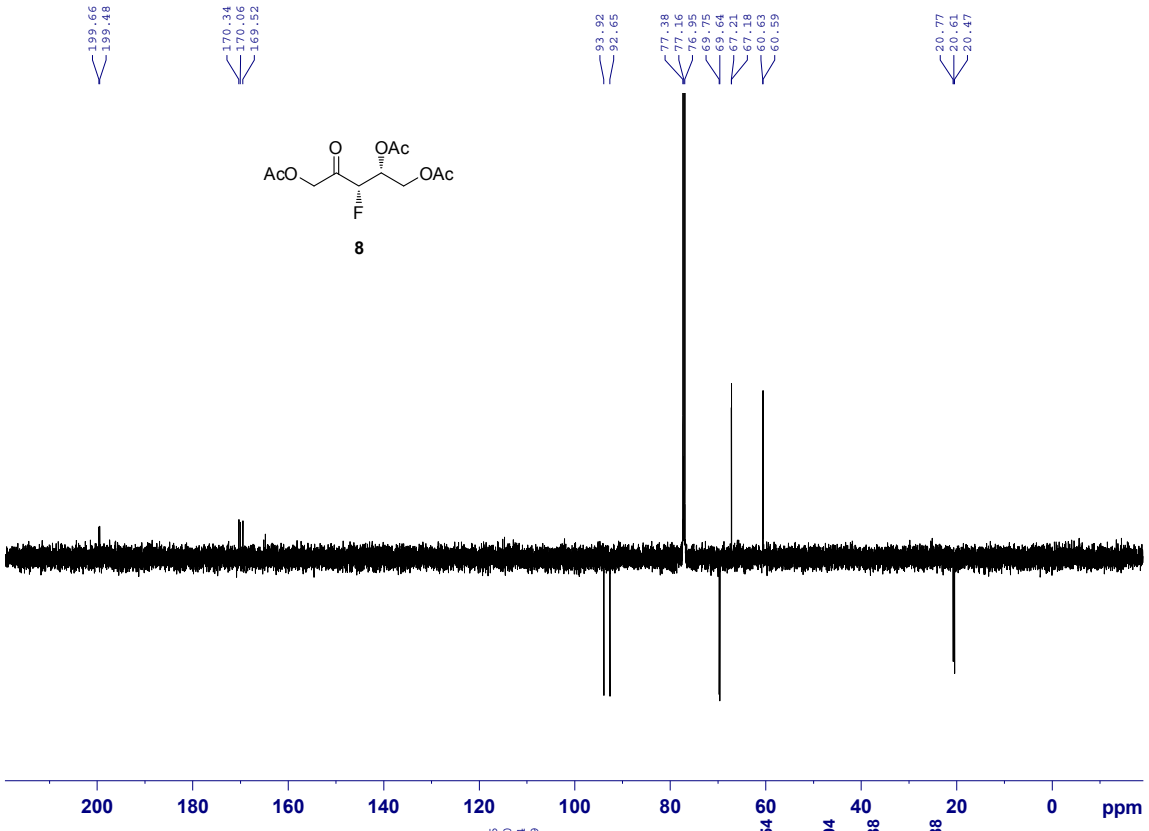
^{13}C NMR (JMOD): For the quaternary carbon of compound **11a** see above.



1,4,5-Tri-*O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (8):



^{13}C-NMR:



^{19}F NMR (coupled)

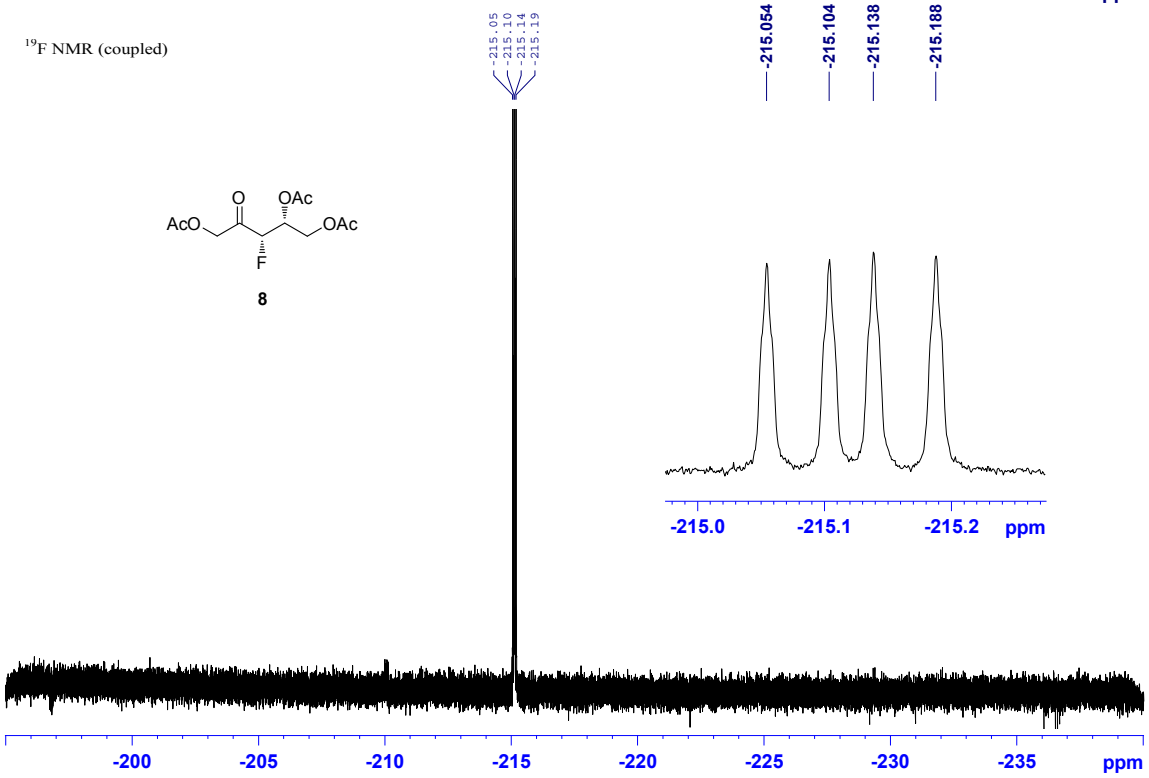
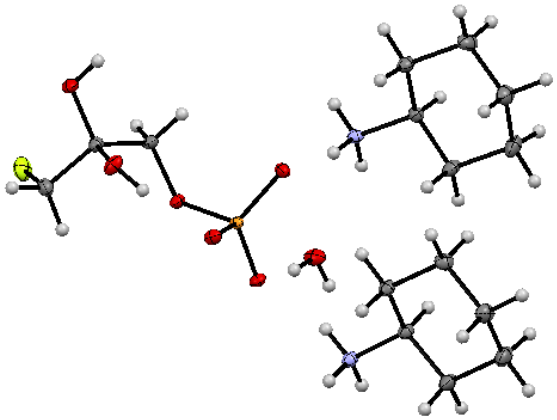


Figure 1. Crystal structure of biscyclohexylammonium salt of FHAP (**5**): The keto functionality is hydrated)[5]



Fluorine: green

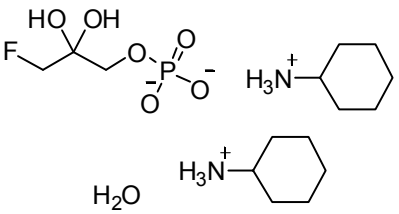
Oxygen: red

Phosphor: orange

Nitrogen: blue

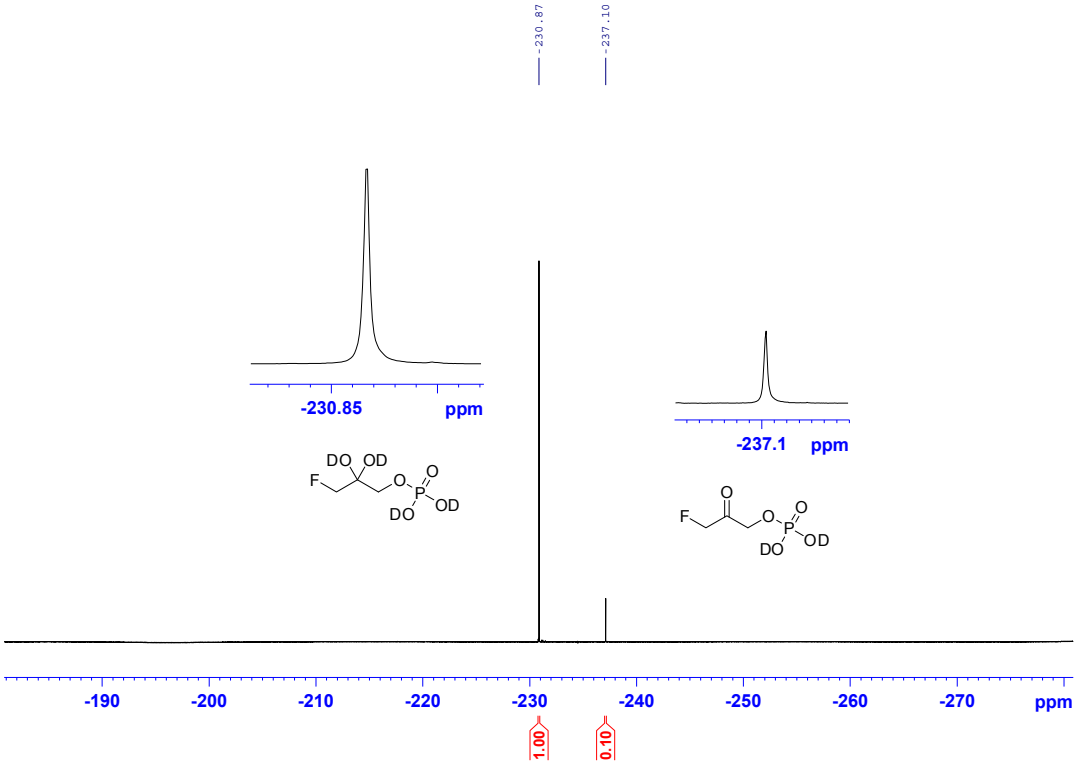
Carbon: black

Hydrogen: gray-white



CCDC-809382 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Figure 2. ^{19}F-NMR spectrum (decoupled): FHAP (**5**) in D_2O



^{19}F-NMR spectrum (coupled): FHAP (**5**) in D_2O

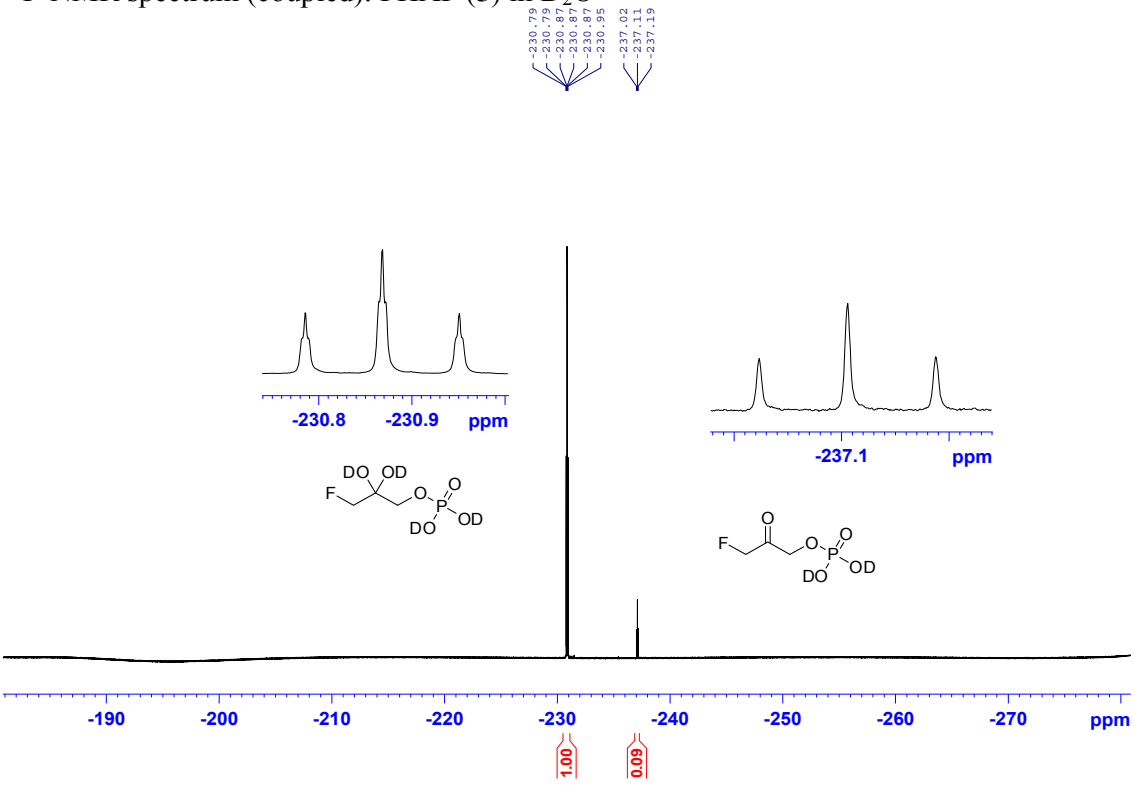


Figure 3. ^{19}F-NMR spectra: RAMA catalyzed formation of 5-chloro-3,5-dideoxy-3-fluoro-D-*threo*-pentulose 1-phosphate **(6)** in H_2O after 20 hours.

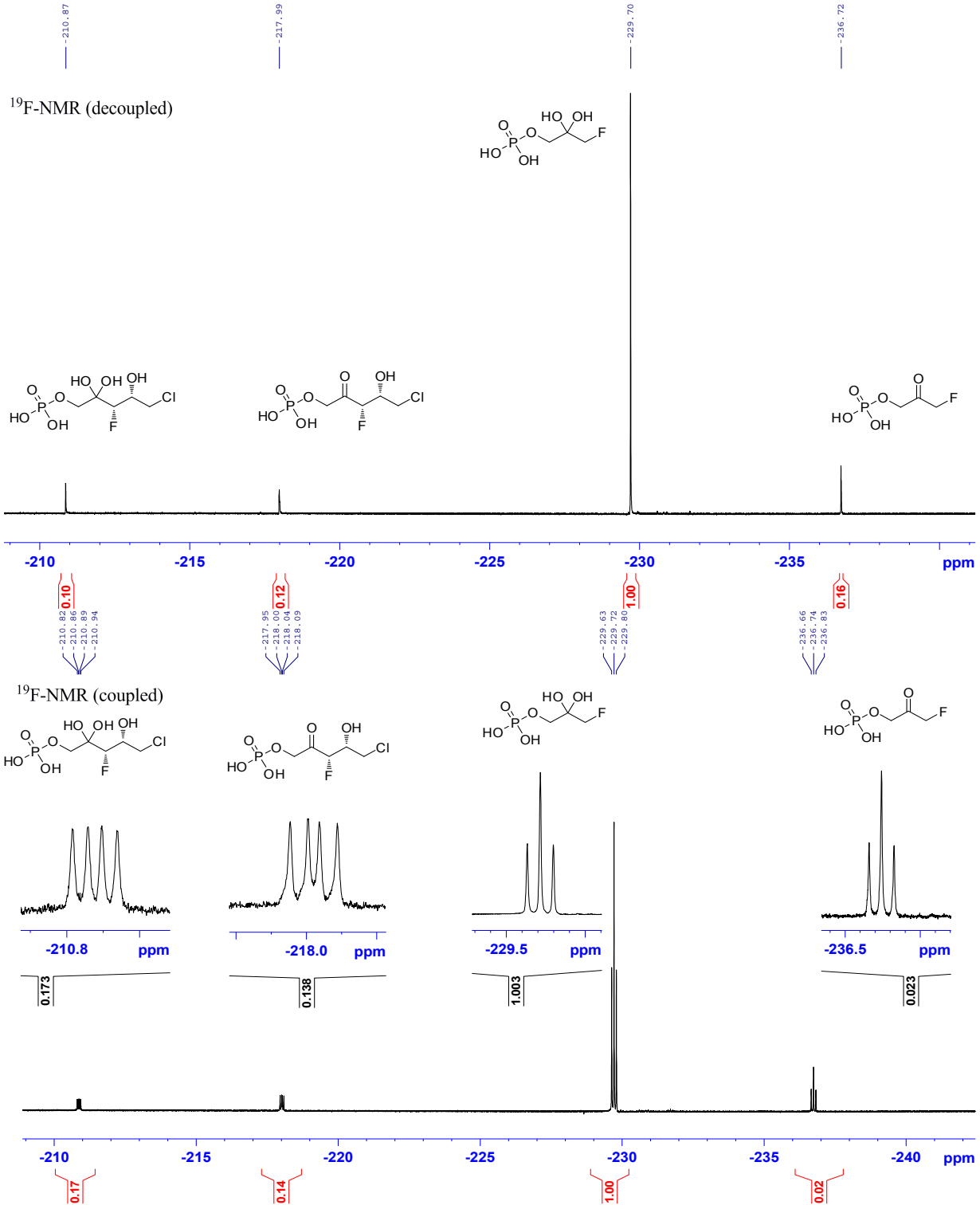


Figure 4. ^{19}F-NMR spectra (coupled): RAMA catalyzed formation of 5-chloro-3,5-dideoxy-3-fluoro-D-*threo*-pentulose 1-phosphate **(6)** in D_2O after repeated addition of RAMA over 3 days.

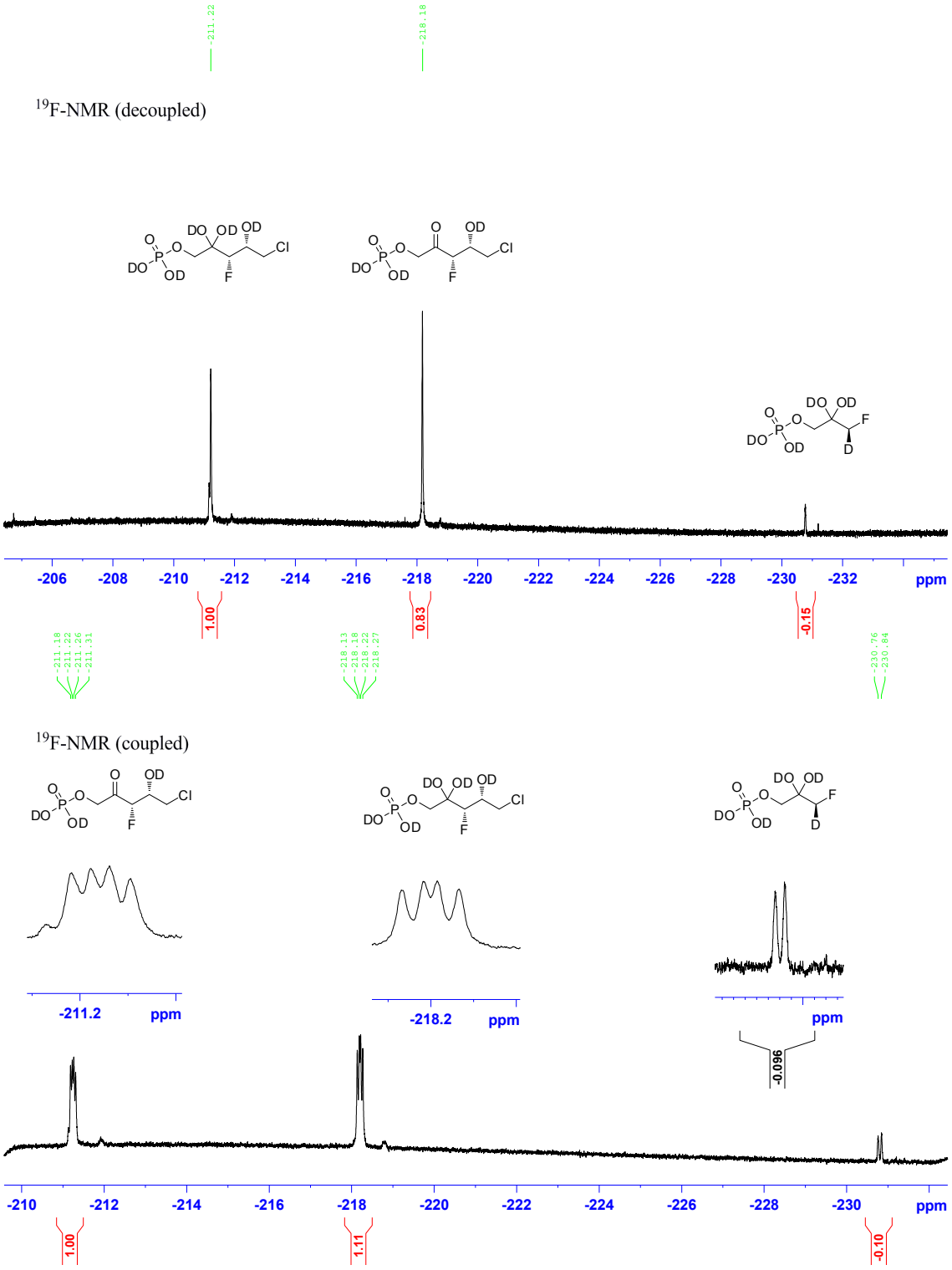
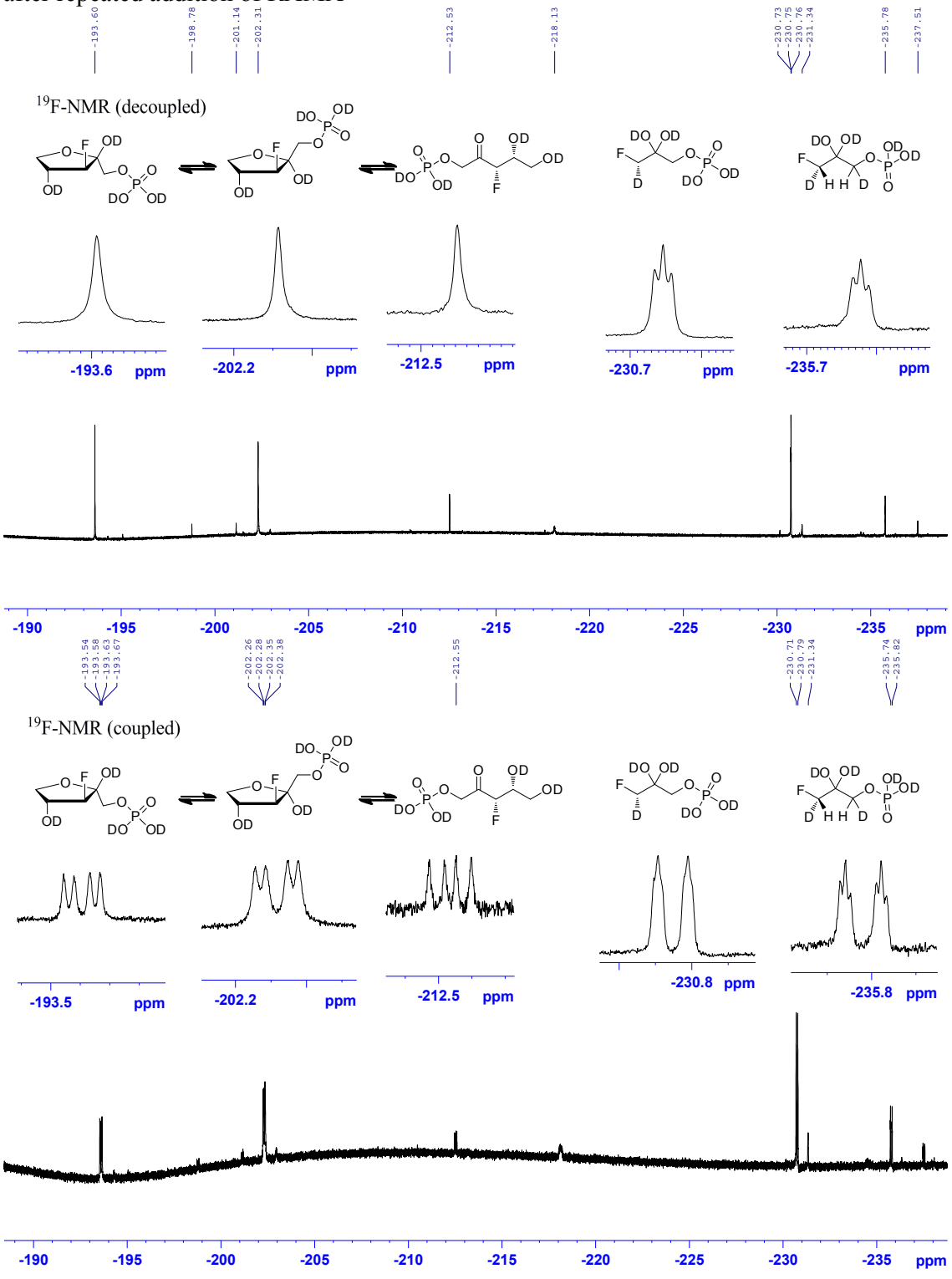


Figure 5. ^{19}F-NMR spectra: RAMA catalyzed reaction of FHAP with glycolaldehyde in D_2O: 3d after repeated addition of RAMA



S28

Figure 6. ^{1}H-NMR (CD_3OD): RAMA catalyzed reaction of FHAP with glycolaldehyde in D_2O after dephosphorylation with acid phosphatase.

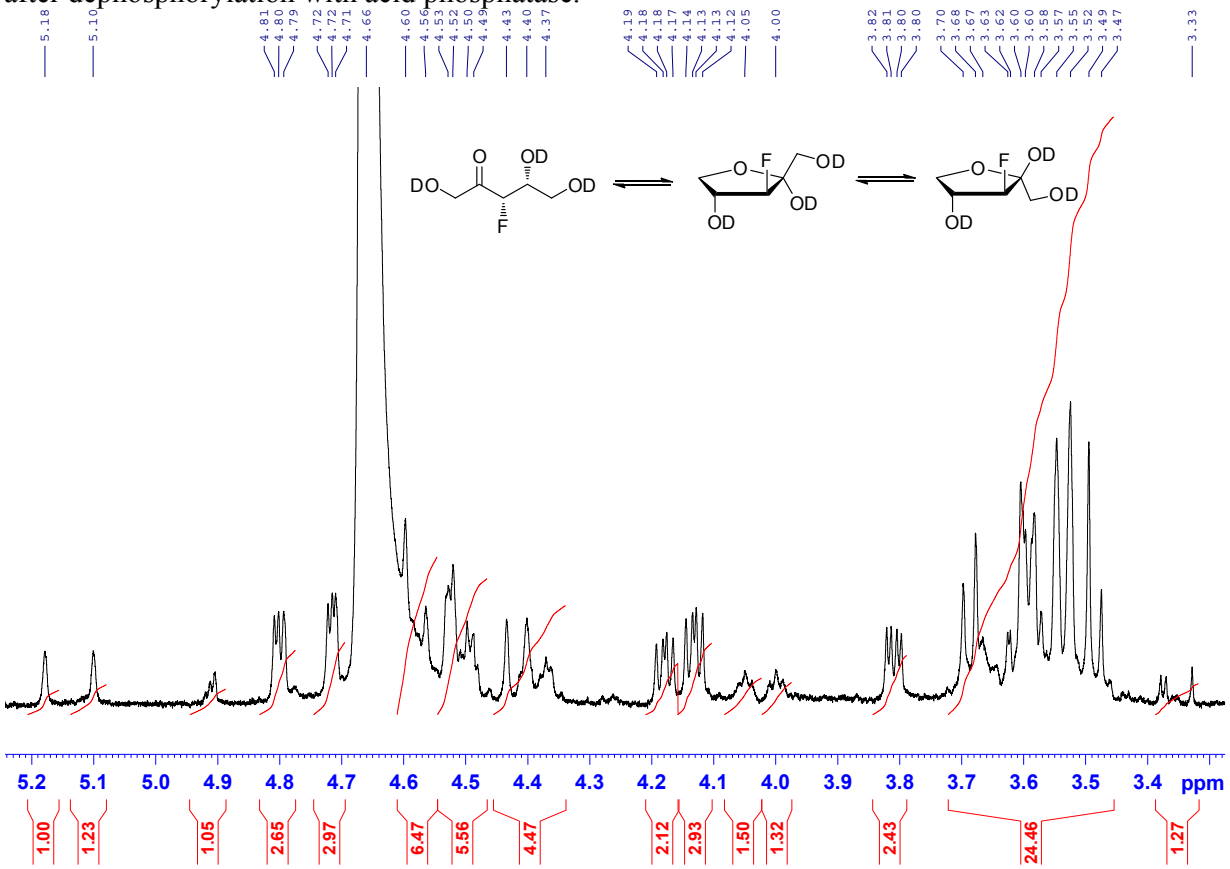


Figure 7. ^{19}F-NMR (decoupled; CD_3OD): RAMA catalyzed reaction of FHAP with glycolaldehyde in D_2O after dephosphorylation with acid phosphatase.

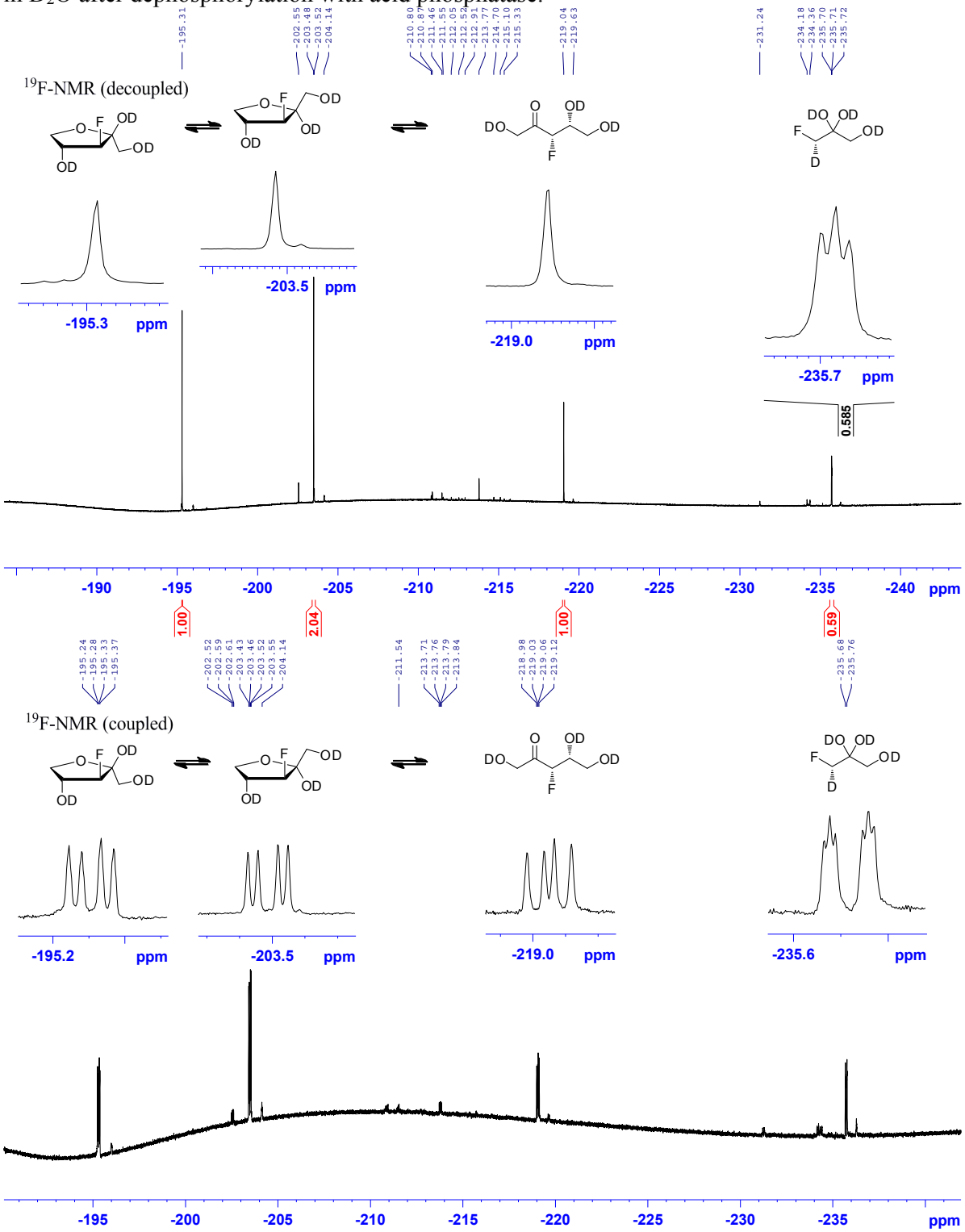
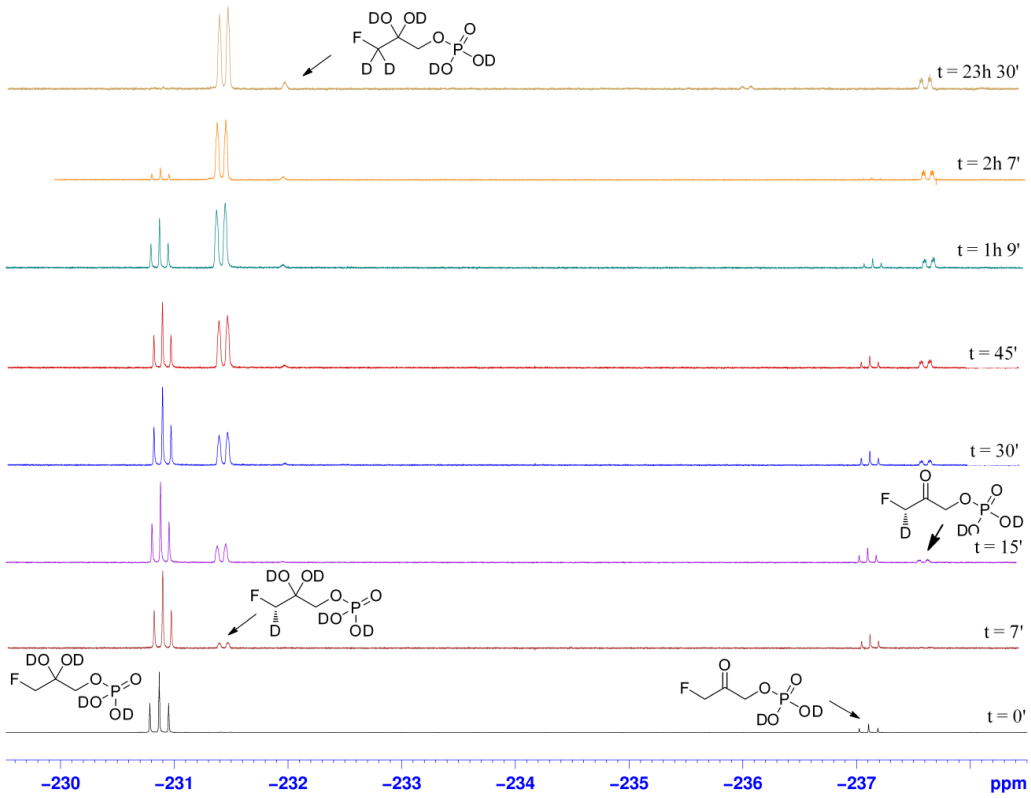


Figure 8. ^{19}F-NMR spectra: RAMA catalyzed incorporation of deuterium from D_2O into FHAP[a]



[a]experimental conditions: A solution of FHAP mono sodium salt (4 mg, 0.021 mmol) in D_2O (0.6 mL) was adjusted with small quantities of 1M NaOD to pD = 7.3. RAMA (4 U) was added and the reaction progress was monitored by ^{1}H-NMR and ^{19}F-NMR. (*S*)-3-D-FHAP: ^{1}H NMR ($CDCl_3$, 400 MHz): δ^{keto} 5.32 (d, J = 46.4 Hz, 1H) 4.53 (d, J = 7.0 Hz, 2H) $\delta^{hydrate}$ 4.36 (d, J = 46.8 Hz, 1H) 3.84 (dd, J = 8.1, 2.2 Hz, 2H). ^{19}F NMR: $\delta^{hydrate}$ –230.7 (dt, J = 46.7, 6.3 Hz); δ^{keto} –237.5 (dt, J = 46.4, 6.3 Hz). HRMS (ESI-neg. mode) calcd for $C_3H_3O_5D_2FP$ (M-D): 172.9984 found, 172.9988 (Sample was measured in D_2O).

Figure 9. ^{1}H NMR: RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 2h 17' after addition of RAMA

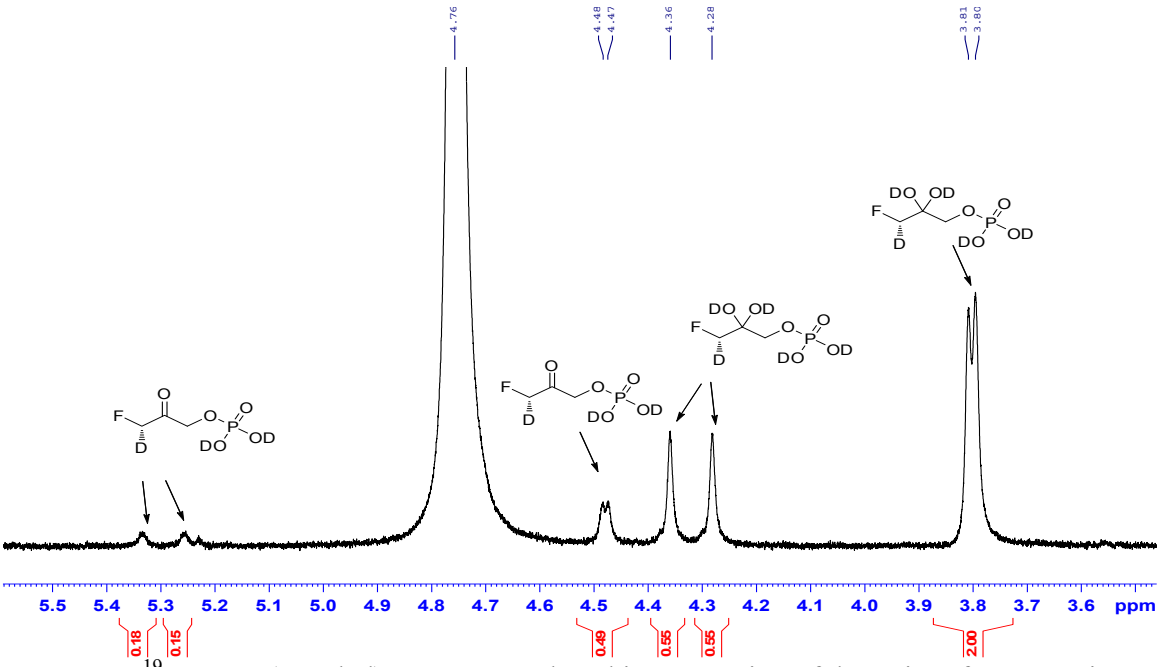


Figure 10. ^{19}F-NMR (coupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 2h 7' after addition of RAMA

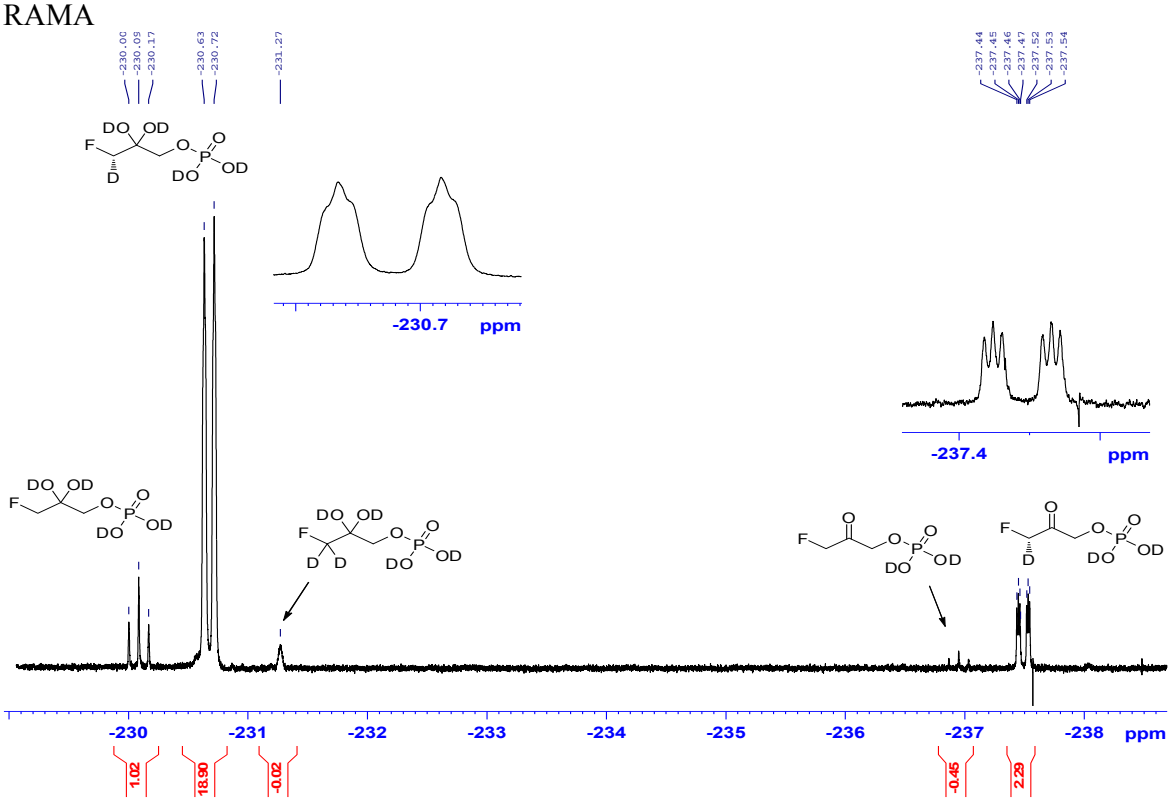


Figure 11. ^{19}F-NMR (decoupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 2h 17' after addition of RAMA

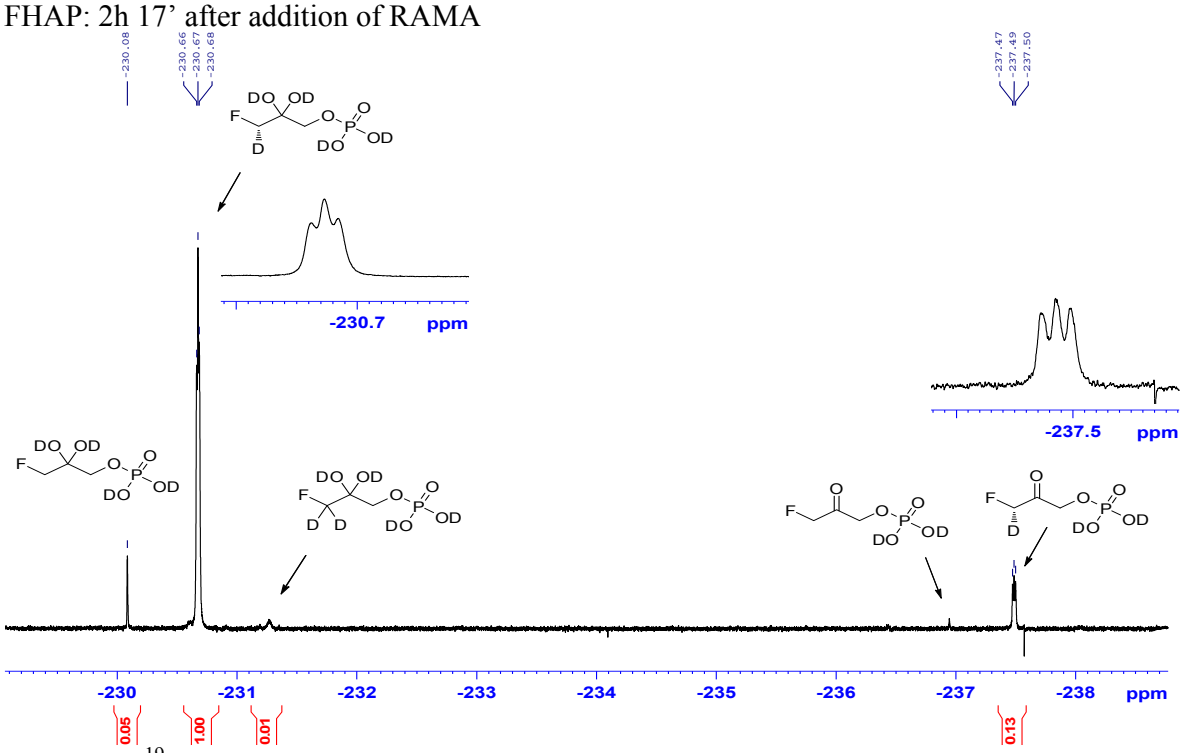
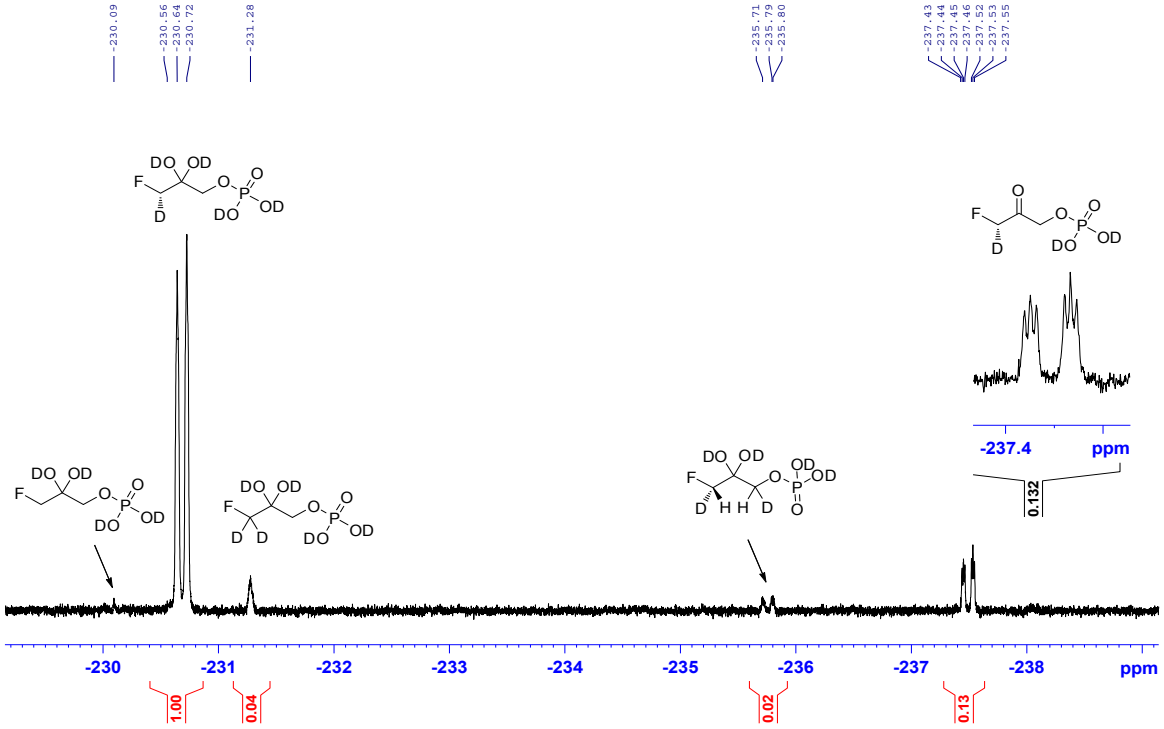


Figure 12. ^{19}F-NMR (coupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 23h 33' after addition of RAMA



S33

Figure 13. ^{19}F-NMR (decoupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 3 days after repeated addition of RAMA

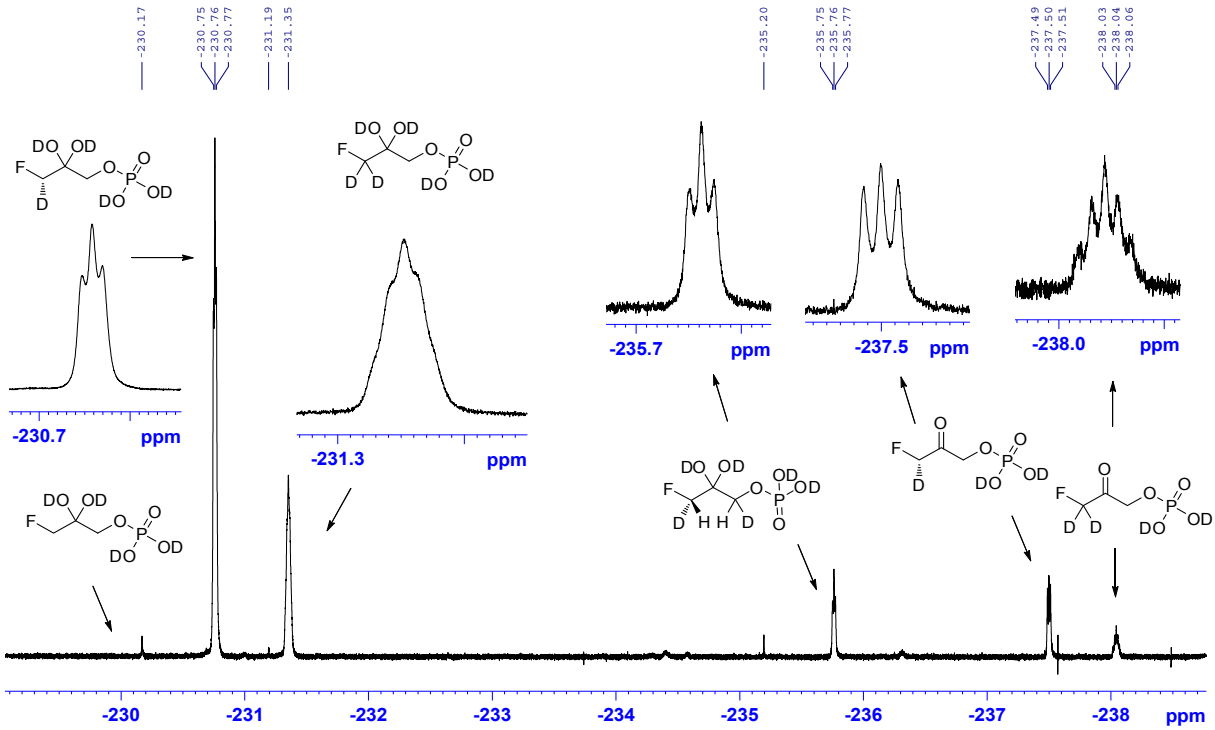
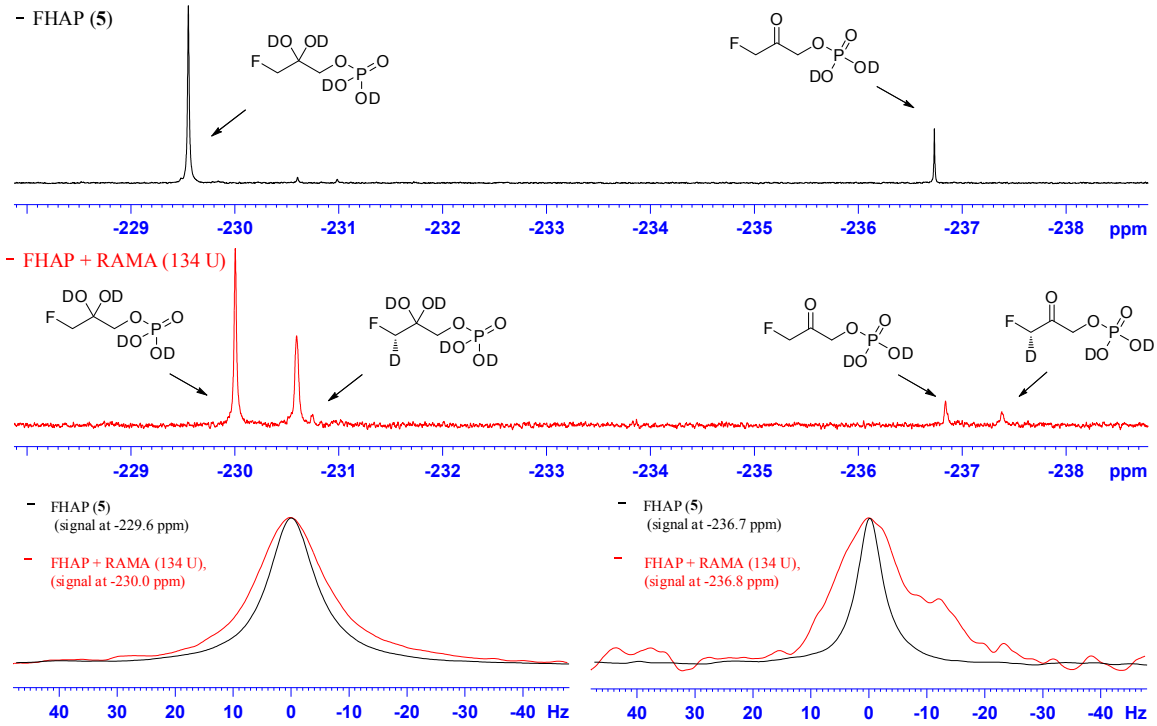
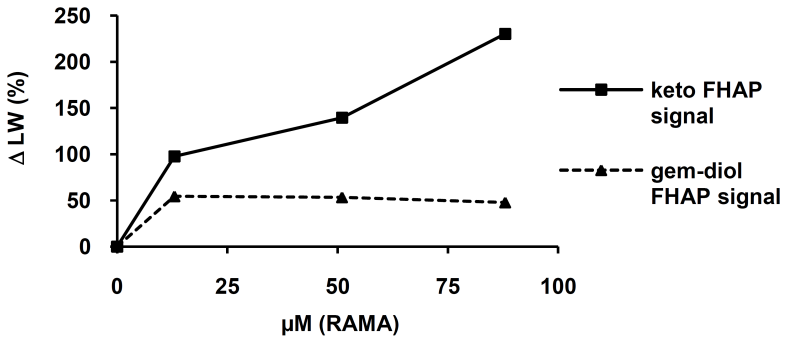


Figure 14. ^{19}F-NMR spectra (decoupled): ^{19}F-NMR binding study of FHAP with RAMA: Change of line width of ^{19}F NMR signals[a]



[a]experimental conditions: FHAP (0.7 mg, 3.6 µmol) was dissolved in 450 µL H_2O and 50 µL D_2O and the pH was adjusted to 7.3 by 1 M NaOH (black line). RAMA was repeatedly added [1st addition: 1.2 mg, 14 U, in 70 µL D_2O; 2nd addition: 4.3 mg, 49 U in 100 µL D_2O; 3rd addition: 6.2 mg, 71 U, in 150 µL D_2O (red line)]



^{19}F-NMR binding study of FHAP with RAMA: Changes in line width (LW) of ^{19}F-NMR resonances – graphical representation of data from table 1

Table 1. ^{19}F-NMR binding study of FHAP with RAMA: Change of line width of ^{19}F NMR signals[a]

FHAP (mg)	RAMA (µM)	Line width (Hz) *FHAP (gem-diol)*	ΔLW (%)	Line width (Hz) *FHAP (keto)*	ΔLW (%)
0,7	0	9,0	-	4,3	-
0,7	13	13,9	54	8,5	98
0,7	51	13,8	53	10,3	140
0,7	88	13,3	48	14,2	230

References:

[1] J. Villieras, M. Rambaud, *Synthesis* **1982**, 924–926.

[2] T. H. Fife, T. C. Bruice, *J. Phys. Chem.* **1961**, *65*, 1079–1080.

[3] The spectroscopical data were identical with those reported previously: Fischer, M; Schmölzer, S.; Nowikow, T.; Schmid., W. *Eur. J. Org. Chem.* **2011**, *9*, 1645–1651.

[4] ^{1}H-NMR, ^{13}C-NMR and HRMS were in full agreement with previously published data: a) T. Vuorinen, A. S. Serianni, *Carbohydr. Res.* **1990**, *209*, 13–31. b) D. de Wit, F. van Rantwijk, L. Maat, A. P. G. Kieboom, *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 271–274.

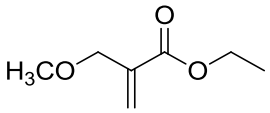
[5] Crystal Structure Visualisation: "*Mercury CSD 2.0 - New Features for the Visualization and Investigation of Crystal Structures"* C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, *J. Appl. Cryst.* **2008**, *41*, 466–470.

Unpublished work:

Following compounds were synthesized according to literature procedures:

- Ethyl 2-(hydroxymethyl)acrylate (**54**)[27]
- Ethyl 2-((trimethylsilyl)methyl)acrylate (**55**)[24]
- Ethyl 2-(diethoxyphosphoryl)-3-(trimethylsilyl)propanoate (**56**)[24]
- Ethyl 2-(bromomethyl)acrylate (**58**)[27]
- Dibenzyl phosphorylchloride (**62**)[28]
- 1,2-*O*-Isopropylidine-D-glyceraldehyde (**64**)[29]
- 1,1-Dimethoxypropan-2-one (**74**)[32a]
- 1,1-Dimethoxypropan-2-ol (**75**)[32a]
- 2-Hydroxypropanal (**76**)[32a]
- But-3-en-2-ol (**77**)[32b]
- 1,2,4,5-Di-*O*-isopropylidene-β-D-fructose (**82**)[33]

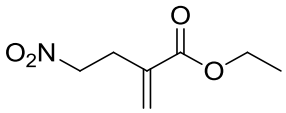
Ethyl 2-(methoxymethyl)acrylate (59):



59

Ethyl 2-(bromomethyl)acrylate (**58**) (60 mg, 0.31 mmol) was dissolved in dry methanol (3 mL) and cesium fluoride (94 mg, 0.93 mmol) was added. The reaction flask was heated at 70°C until TLC monitoring showed complete consumption of the starting materials (~ 28 hours). The reaction mixture was quenched by addition of water (1 mL) and the aqueous phase was extracted with dichloromethane (5 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give ethyl 2-(methoxymethyl)-acrylate (**59**) (20 mg, 45 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.29 (m, 1H, CH) 5.83 (m, 1H, CH) 4.21 (q, *J* = 7.1 Hz, 2H, CH_2) 4.13 (t, *J* = 1.4 Hz, 2H, CH_2) 3.39 (s, 3H, CH_3) 1.30 (t, *J* = 7.1 Hz, 3H, CH_2); ^{13}C-NMR (400 MHz, $CDCl_3$): δ =166.0, 137.5, 125.7, 70.9, 60.8, 58.7, 14.3.

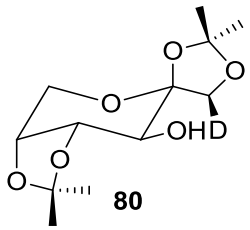
Ethyl 2-methylene-4-nitrobutanoate (60):



60

Ethyl 2-(bromomethyl)acrylate (**58**) (2 g, 0.01 mol) was dissolved in nitromethane (30 mL) and potassium fluoride (1.2 g, 0.02 mol) was added. After addition of water (~0.5 mL), the reaction mixture was heated at 70°C until TLC monitoring showed complete consumption of the starting materials (~ 14 hours). The reaction mixture was quenched by addition of water (20 mL) and the aqueous phase was extracted with dichloromethane (3 x 15 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give ethyl 2-methylene-4-nitrobutanoate (**60**) (1.64 g, 92 %). ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 6.30 (s, 1H, CH) 5.72 (m, 1H, CH) 5.58 (t, *J* = 6.8 Hz, 2H, CH_2) 4.24 (q, *J* = 7.1 Hz, 2H, CH_2) 2.99 (dt, *J* = 3.5 Hz, *J* = 0.87 Hz, 2H, CH_2) 1.32 (t, *J* = 7.1 Hz, 3H, CH_2).

[(*S*)-1-H^2]-1,2,4,5-di-*O*-isopropylidene-β-D-fructose (80):



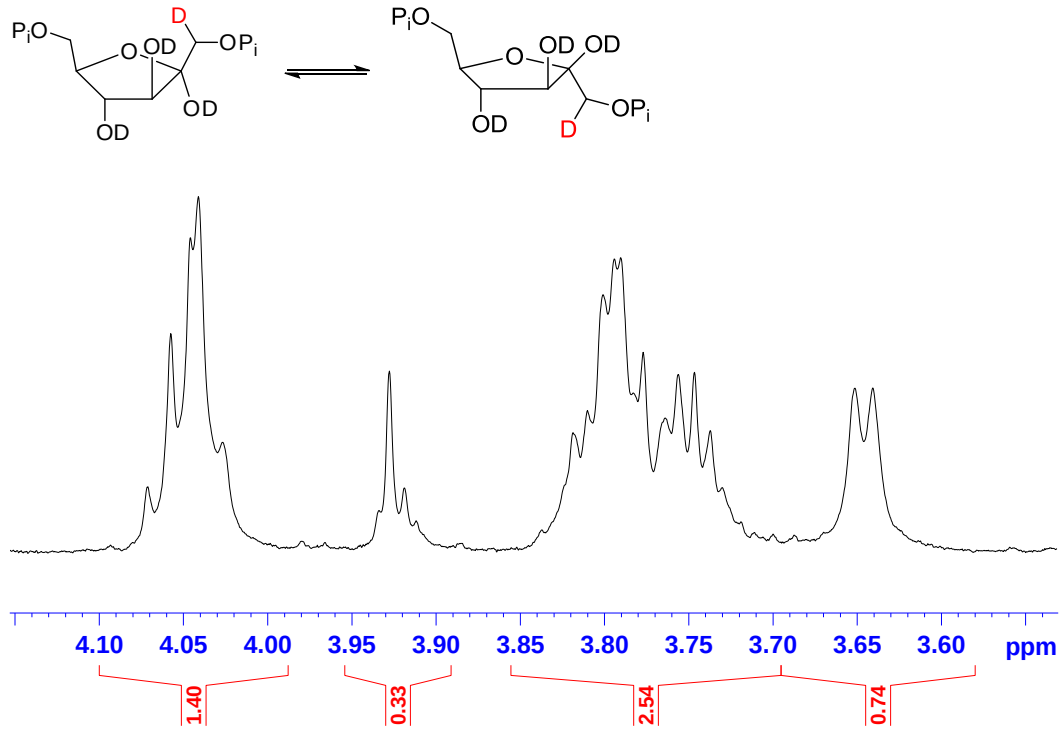
80

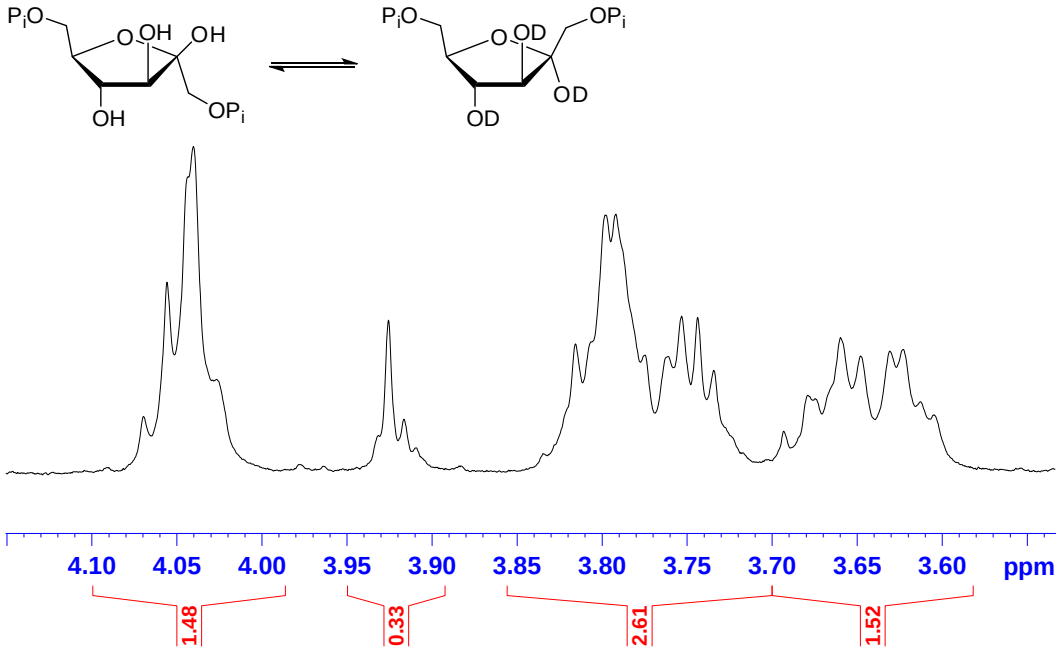
Fructose-1,6-bisphosphate monocalcium salt (75%, 252 mg, 0.5 mmol) was dissolved in D_2O (5 mL) and amberlyst 15 H^+ was added. After 1.5 hours, the resin was filtrated, rinsed with D_2O and the pD was adjusted to pD = 7 with a 1 M NaOD solution. RAMA (3.7 U) was added and the reaction mixture was slowly stirred over night. Since the fructose-1,6-bisphosphate was not fully converted to deuterated fructose-1,6-bisphosphate (monitored by ^{1}H-NMR spectroscopy), RAMA (7.2 U) was added again and the reaction mixture stirred over night. After full conversion, acid phosphatase (11 U) was added over two days in equal portions (the reaction progress was monitored by TLC). Then the pH was readjusted to pH = 6.8 and the solvent was removed under reduced pressure. The crude product was purified by anion exchange chromatography (dowex 50 Cl^-). The deuterated fructose was eluted with H_2O and concentrated under reduced pressure (the unreacted deuterated fructose-1,6-bisphosphate with a 3 M HCl solution). The deuterated fructose fraction was dissolved in dry acetone (2.6

mL) and conc. H_2SO_4 (13 µL) was added. After stirring the reaction mixture over night, the pH was adjusted to pH = 9-10 with an ice-cold aqueous solution of NaOH (2 M). The acetone was removed under reduced pressure and the aqueous phase was extracted with $CHCl_3$ (3 x 5 mL). The combined organic layers were washed with H_2O (1 mL), dried over $MgSO_4$ and concentrated. The crude product was recrystallized in a mixture of diethyl ether and hexane (1/1) to give [(*S*)-1-H^2]-1,2,4,5-di-*O*-isopropylidene-β-D-fructose **(80)** (39 mg, 30%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 4.20 (dd, *J* = 5.8 Hz, *J* = 2.3 Hz, 1H, CH) 4.15-4.08 (m, 2H, CH, CH_2) 4.03-3.84 (m, 2H, CH_2, CHD) 3.66 (dd, *J* = 8.1 Hz, *J* = 6.9 Hz, 1H, CH) 1.96 (d, *J* = 8.3 Hz, 1H, OH) 1.53 (s, 3H , CH_3) 1.51 (s, 3H , CH_3) 1.44 (s, 3H , CH_3) 1.37 (s, 3H , CH_3); ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 111.9, 109.6, 104.7, 77.4, 73.6, 72.3 (t, *J* = 23.0 Hz) 70.7, 61.1, 28.2, 26.7, 26.5, 26.2.

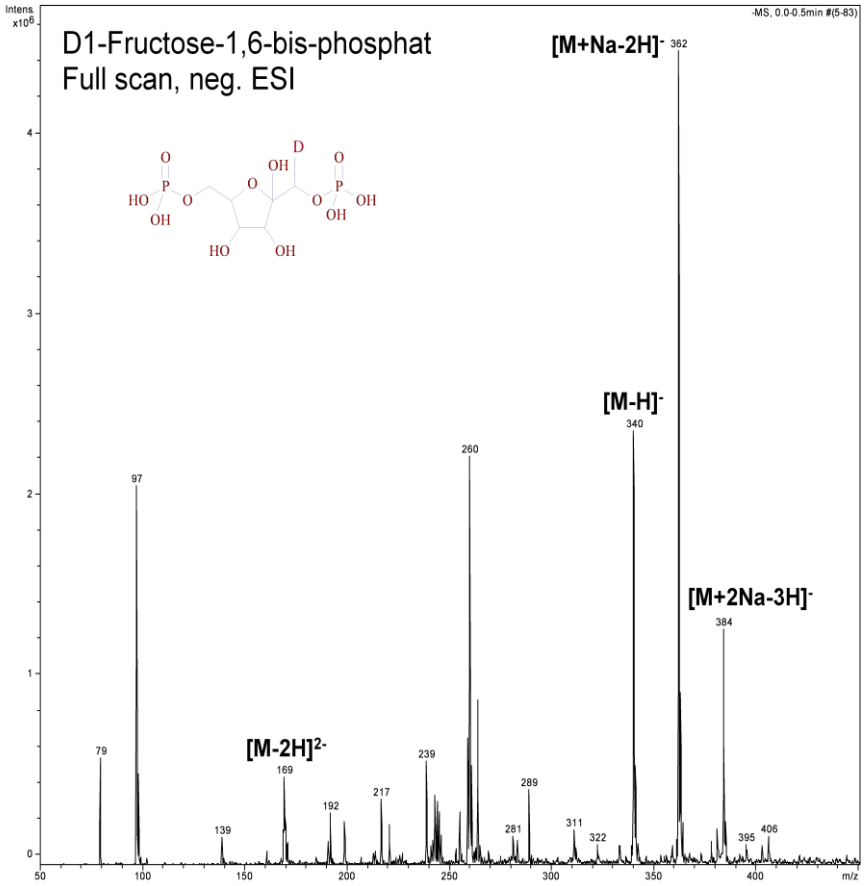
Selected spectra:

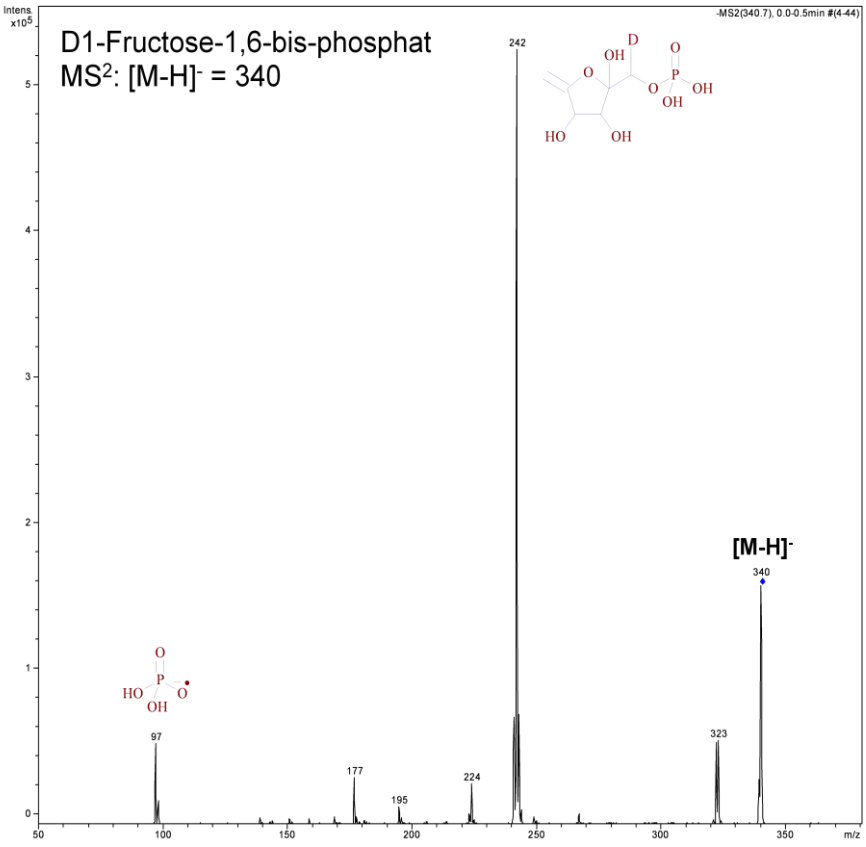
^{1}H-NMR spectra of [1-^{2}H]-D-FBP (**78**) and D-FBP (**4**):

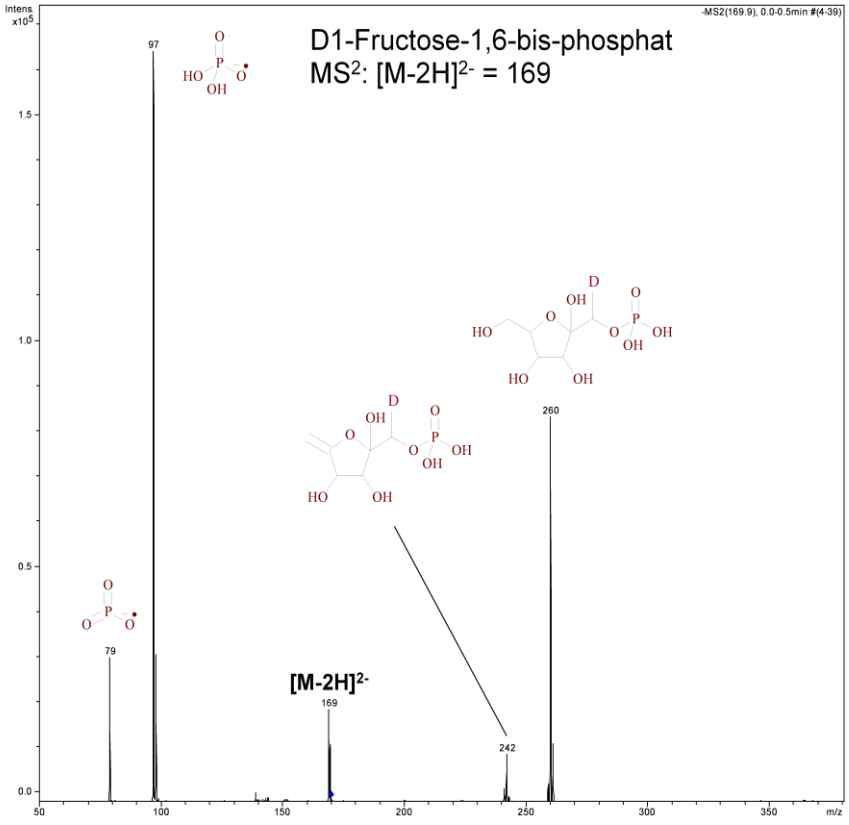




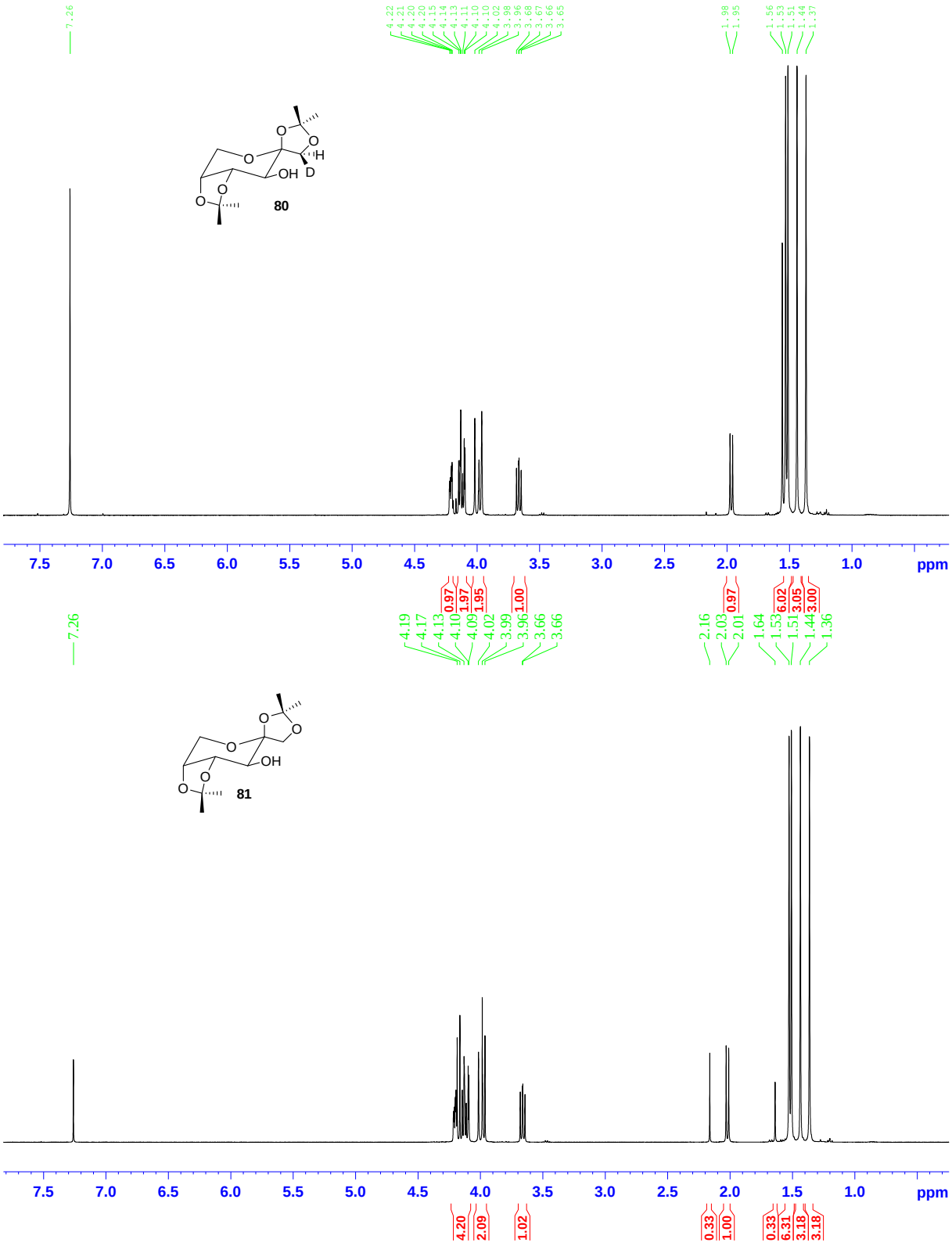
MS-and Ms^2-Spectra of 1D-FBP (**78**):



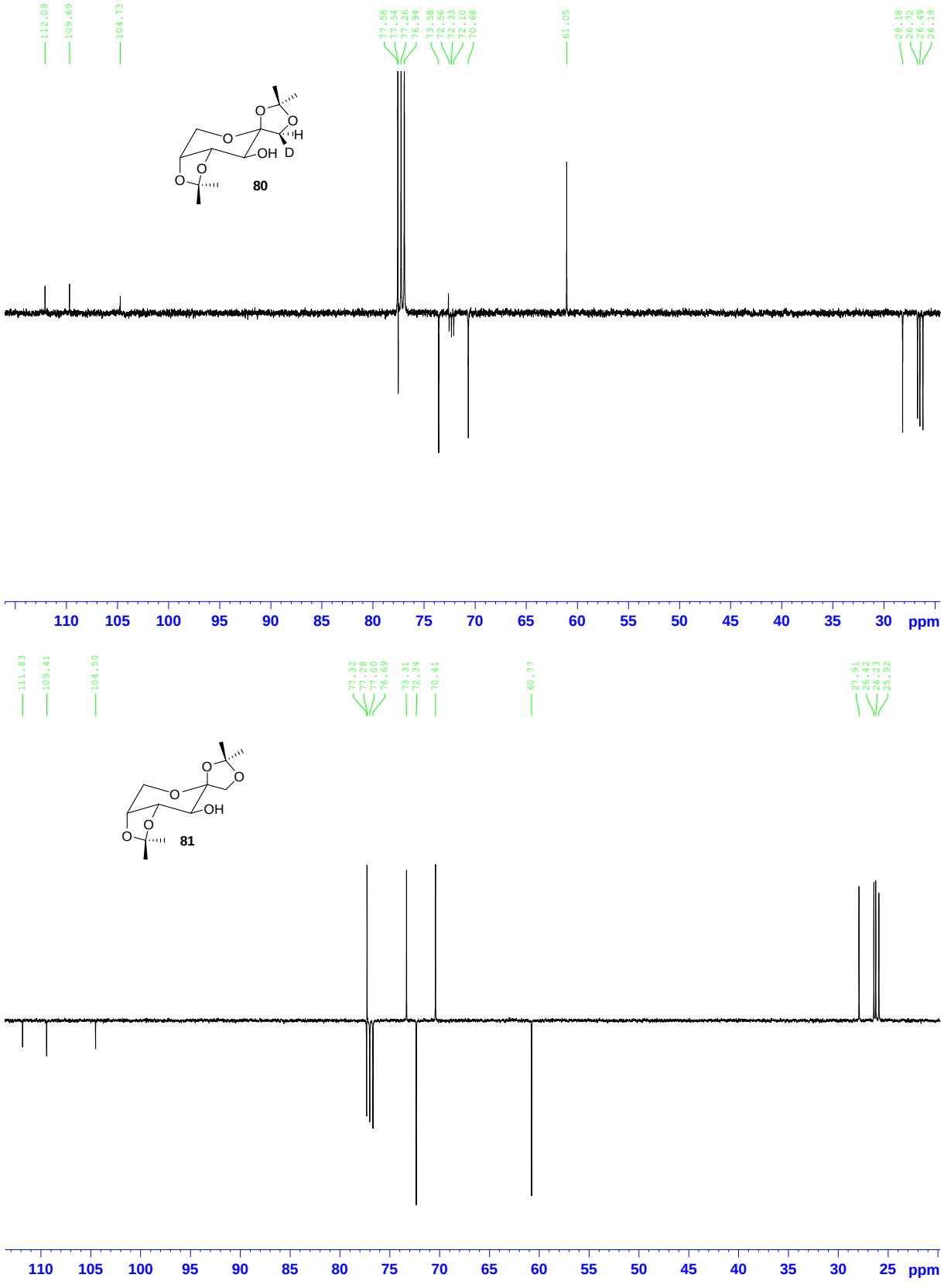



Intens.
x10^5
-MS2(169.9), 0.0-0.5min #(4-39)
D1-Fructose-1,6-bis-phosphat
MS2: [M-2H]2- = 169
97
79
[M-2H]2-
169
242
260
m/z

Comparison of ^{1}H-NMR spectra of [(*S*)-1-H^{2}]-1,2,4,5-di-*O*-isopropylidene-β-D-fructose (**80**) with 1,2,4,5-di-*O*-isopropylidene-β-D-fructose (**81**):



Comparison of ^{13}C-NMR spectra of [(*S*)-1-H^2]-1,2,4,5-di-*O*-isopropylidene-β-D-fructose (**80**) with 1,2,4,5-di-*O*-isopropylidene-β-D-fructose (**81**):



Part B: Preliminary Experiments Regarding the Synthesis of Fluorohydroxyacetone for Organocatalyzed Aldol Reactions

B.1. Abstract

A short synthesis for fluorohydroxyacetone (FHA) was developed. The structure of FHA was characterized by NMR spectroscopy in solution, since pure FHA is highly instable and cannot be isolated. The initial idea behind this project was to explore the organocatalyzed aldol addition of various aldehydes to FHA for the synthesis of fluorinated carbohydrates and carbohydrate derived compounds.

B.2. Introduction and Background

Asymmetric Organocatalysis

Although the Hajos-Parrish-Eder-Sauer-Wiechert reaction was known for over 30 years,[36] asymmetric organocatalysis only recently emerged into a rapidly expanding and exciting field of organic chemistry.[37] After the seminal publication of B. List *et al*. in year 2000,[38] an enormous research effort arose in the organic synthetic community.[39] An experimentally simple proline-catalyzed asymmetric aldol reaction between acetone and various aldehydes was used for the synthesis of β-hydroxyketones with high enantiomeric excess (Scheme 30, entry 1). An historical overview over the organocatalyzed aldol reaction and frequently applied organocatalyst are depicted in scheme 30 and figure 7 respectively. Shortly after, hydroxyacetone (**91**) was shown to regioselectively add to various aldehydes under proline-catalysis to give *anti*-configurated α,β-dihydroxyketones with high diastereo- and enantioselectivities (Scheme 30, entry 2).[40] The diastereoselectivity of this reaction can be changed to the *syn*-products by applying catalyst **85** (Scheme 30, entry 5). Like hydroxyacetone, fluoroacetone (**92**) regioselectively reacted with aldehydes under the

formation of β-hydroxy-α-fluoroketones, albeit in lower yield, diastereo- and enantioselectivities (Scheme 30, entry 3).[41] Cyclic derivatives of DHA like **93** proved to be superior for asymmetric aldol additions in terms of yield, enantio- and diastereoselectivities compared to the DHA (**15**) and non-cyclic protected DHA derivatives **94** (Scheme 30, entries 4,6-10).[42] Interestingly only the cyclic DHA derivatives were reactive under proline catalysis and exclusively yielded anti-configurated products. DHA (**15**) and non-cyclic protected DHA derivatives gave under the catalysis of **87** and **88** the *syn*-aldol products, proline (**82**) was not a catalyst for these donor substrate.[43] It has to be emphasized that all reaction procedures mentioned strongly depend on the aldehyde and are not generally applicable to every acceptor substrate. The most consistent results were published from Enders *et al*. and will be discussed in more detail (Scheme 31). Enders *et al*. used the more complex D-glyceraldehyde (**65**) and L-Garner's aldehyde (**95**) as acceptor aldehydes in proline catalyzed aldol additions.[44] L-Proline (**82**) was the catalyst of choice for the synthesis of protected 5-amino-5-deoxy-L-psicose (**96**) from L-garner aldehyde (**95**) in high yield, excellent anti/syn selectivity and high *ee* values (matched case). 5-Amino-5-deoxy-L-tagatose (**96**) could be obtained using D-proline in high *ee* and *de* values (mismatched case), however in lower yield. Consequently, D-proline was the appropriate catalyst for D-glyceraldehyde **65** (matched case, Scheme 31).

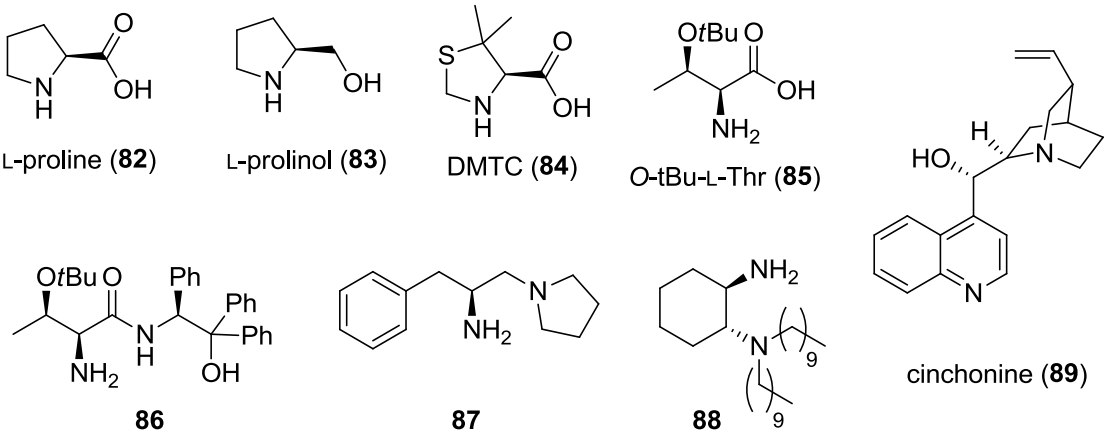
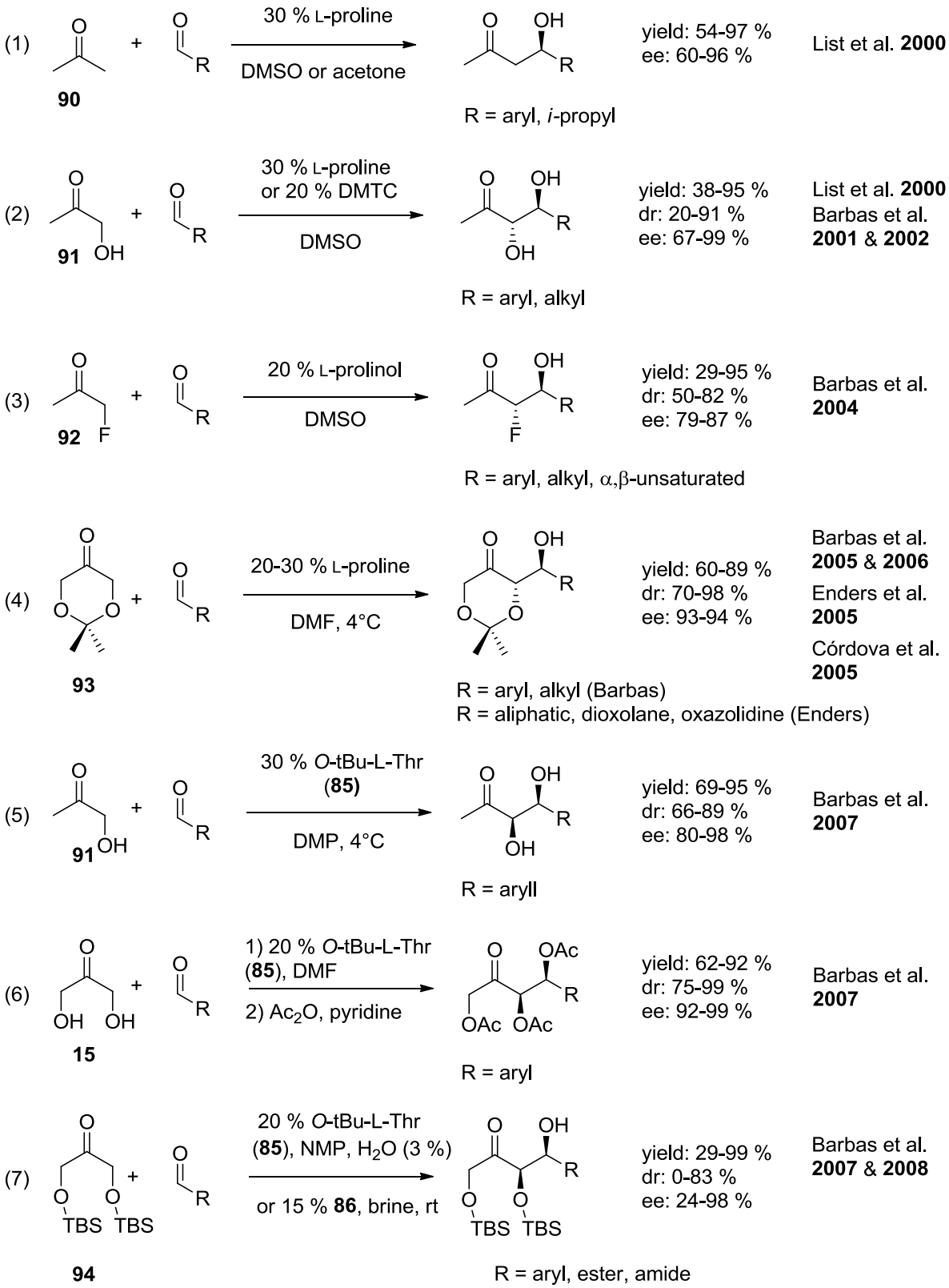
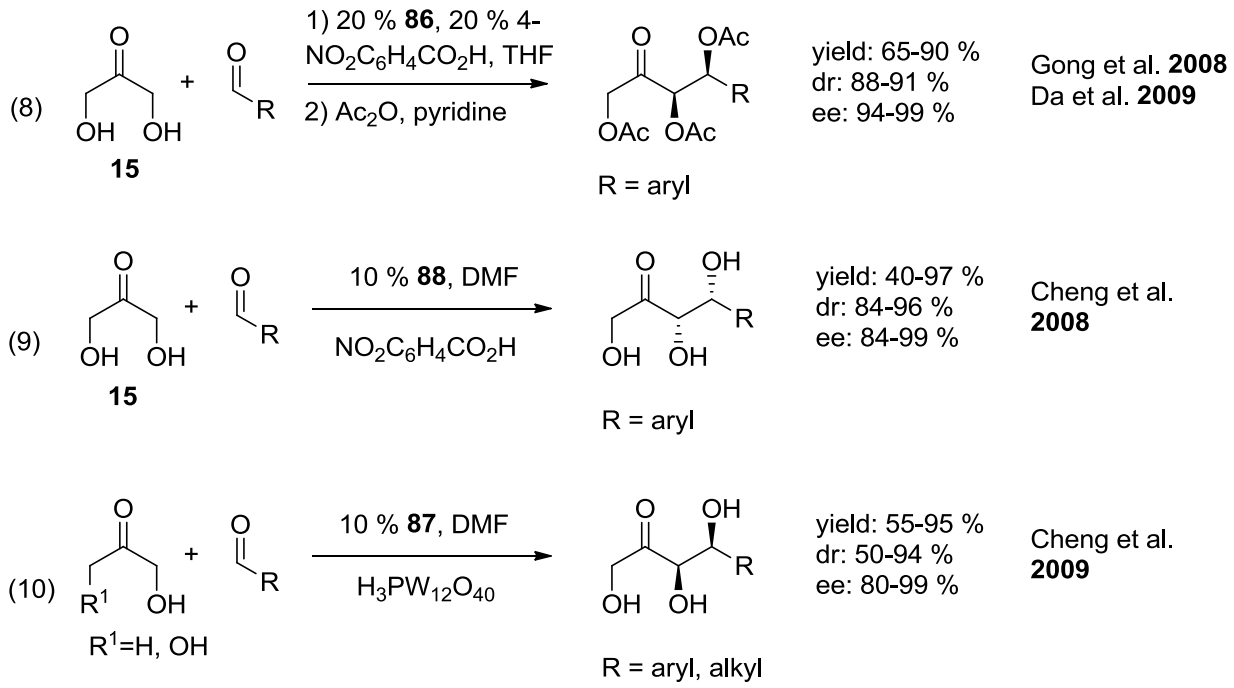


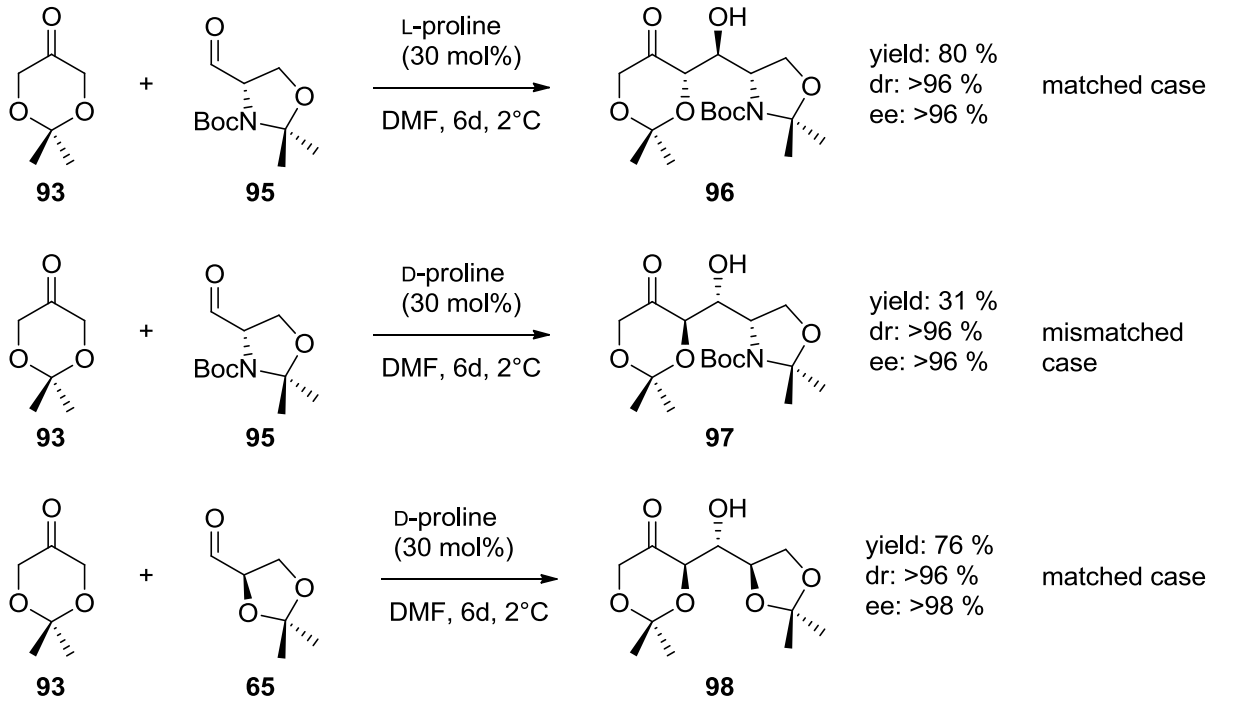
Figure 7. Selected amine- and organo-catalysts for asymmetric aldol reactions



Scheme 30. Organocatalyzed aldol reactions - historical overview[38-44]



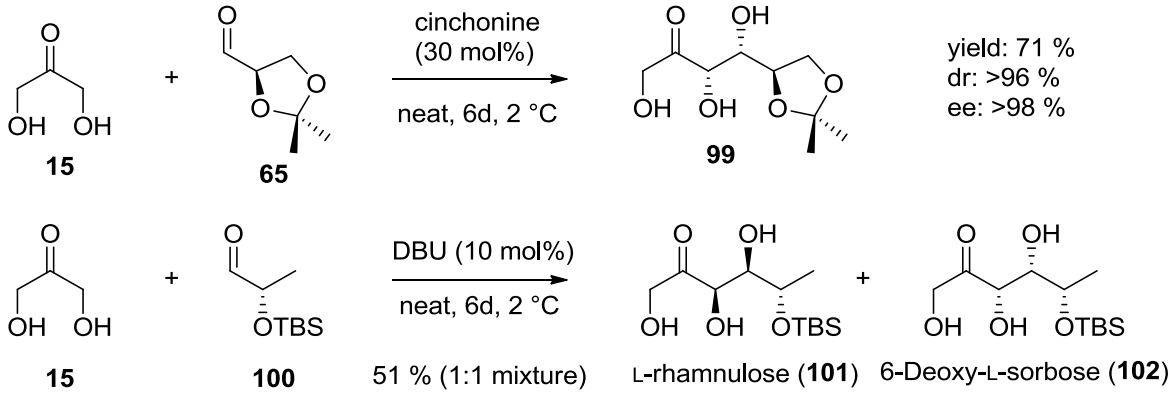
Scheme 30. Organocatalyzed aldol reactions - historical overview (continued).[38,40,44]



Scheme 31. Proline-catalyzed asymmetric aldol reaction of dioxanone with L-garneraldehyde (95) and D-glyceraldehyde (65) by Enders *et al*.[44]

In the year 2008 Mahrwald *et al*. found that tertiary amines like cinchonine are capable of catalyzing the asymmetric addition of DHA (**15**) to D-glyceraldehyde (**65**) to yield D-fructose in high yield, enatio- and diastereoselectivity (Scheme 32).[45] By using non chiral bases like

DBU and TBDMS-protected (*S*)-lactate, a one to one mixture of L-rhamnulofuranose (**101**) and 6-deoxy-L-sorbose (**102**) was isolated.



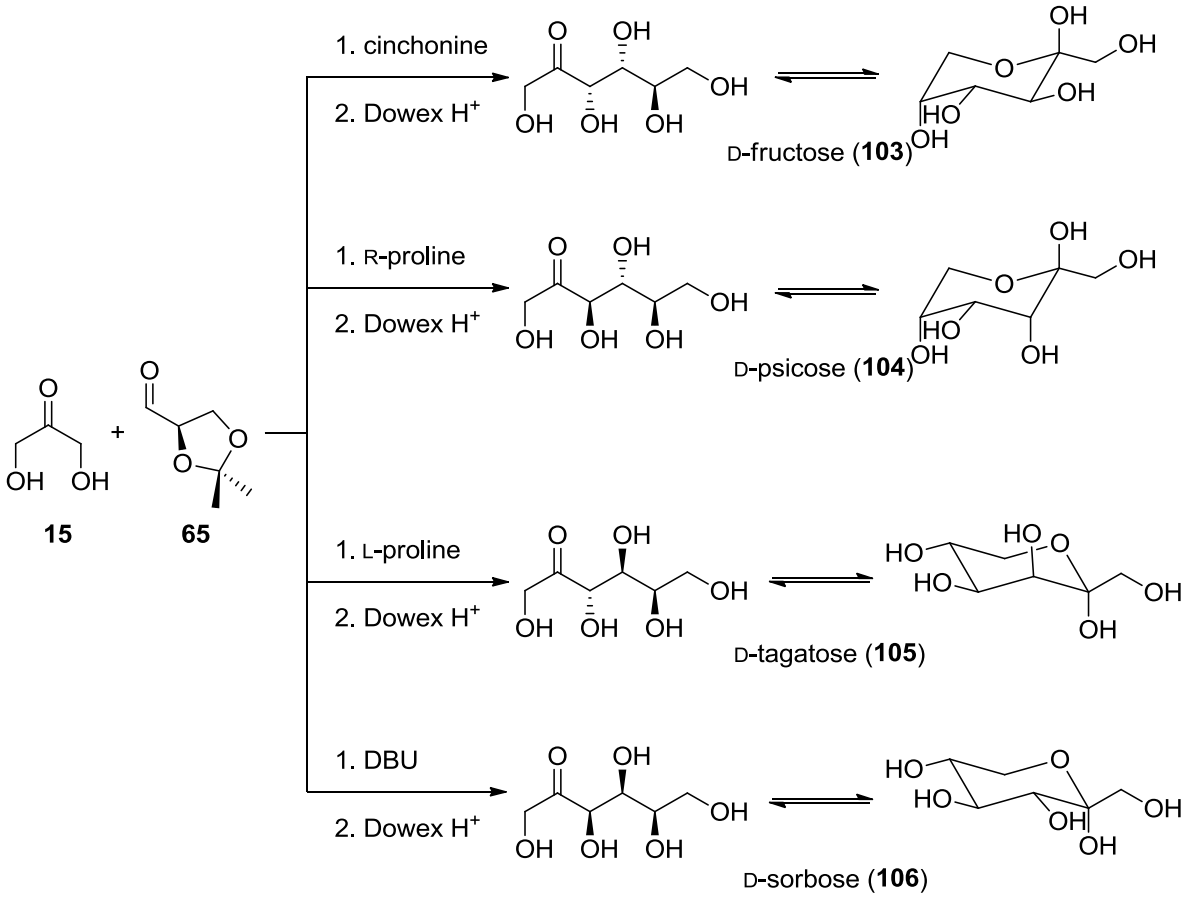
Scheme 32. Amine-catalyzed aldol addition by Mahrwald *et al*. [45]

As a consequence of these research efforts, every desired D-hexopyranose (**103-106**) can be synthesized from D-glyceraldehyde (**65**) by choosing the right catalyst (Scheme 33).[46]

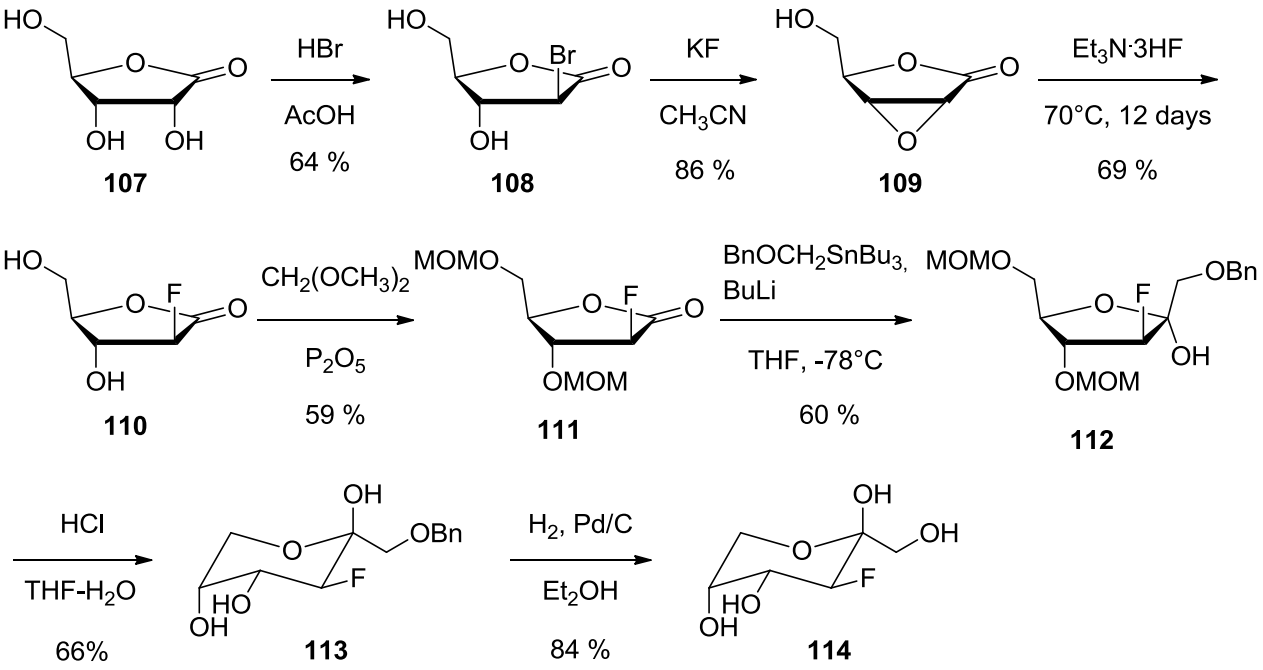
Deoxofluoro Carbohydrates

The importance of deoxyfluoro carbohydrates is reflected by their manifold applications for studying biological systems.[47] The ability of fluorine to mimic hydrogen or hydroxyl groups made it a perfect tool for medicinal diagnostics, since many fluorinated metabolites especially deoxofluoro carbohydrates are processed by the same transporting pathways as their unfluorinated counterparts. These properties, combined with the artificial isotope fluorine-18, made the positron emission tomography (PET)[48] to one of the most powerful probes in medicinal diagnostics of cancer, Parkinson and Alzheimer's diseases by using 2-deoxy-2-[^{18}F]-fluoro-glucose as reporter.[49] In addition, deoxyfluoro sugars have been used for the elucidation of enzyme mechanisms[50] and for mapping of subsides of monoclonal anti-carbohydrate antibodies.[51] Gylcosylfluorides[52] are well established glycosyldonors for the synthesis of oligo-, polysaccacharides and glycans. These carbohydrates are essential in many biological processes like cell-cell interaction/adhesion,[53] inflammation, blood group determination,[54] energy metabolism and storage. The synthesis of fluorinated carbohydrates is often hampered by the difficulties in stereoselective incorporation of fluorine at specific position of the carbohydrate skeleton.[55] Often numerous side reactions[56] can occur and

therefore multistep procedures applying numerous protection- and deprotection steps are necessary (for representative example see Scheme 34).



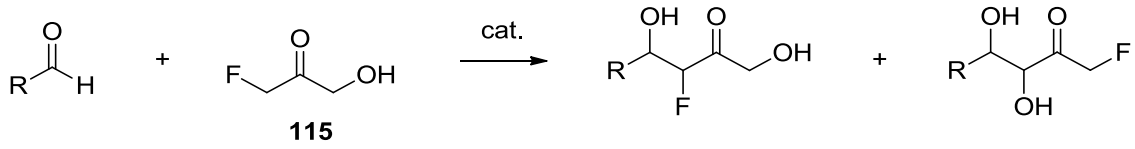
Scheme 33. ***De novo*** **synthesis of carbohydrates by Mahrwald** ***et al.***[46]



Scheme 34. Synthesis of 3-deoxy-3-fluoro-D-fructose (114)[57]

Concept and Strategy

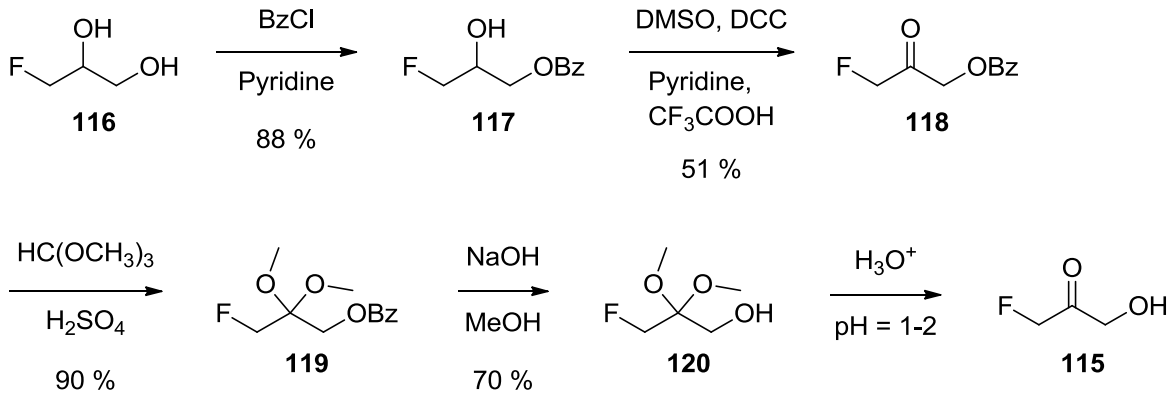
Besides FHAP (**45**) for enzyme catalyzed aldol addition, FHA (**115**) would be a highly desirable donor substrate for organo-, antibody- and fructose 6-phosphate aldolase-catalyzed aldol additions.[58] If FHA (**115**) can be efficiently synthesized, an enormous variety of biological interesting compounds should be easily accessible by the strategy depicted in scheme 35. An organocatalytic approach towards 3-deoxy-3-fluoro-uloses and/or 1-deoxy-1-fluoro-uloses would be highly desirable, since laborious protecting and deprotection steps could be avoided. In addition, the regioselectivity of these reactions may provide information on the relative nucleophilic behavior of α-fluoro to α-hydroxyl carbanions. If the diastereo- and enantioselectivity can be controlled by the organocatalyst or tertiary amine base, all theoretically possible diastereomers are synthesizable in analogy to the work of Barbas, Enders, and Mahrwald *et al*. with DHA (**15**).



Scheme 35. Strategy for the organocatalytic synthesis of fluorinated ketoses

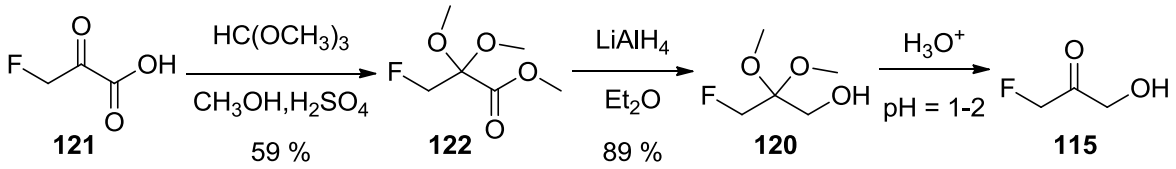
Previous Synthesis of FHA (115)

The first synthesis of FHA (**115**) was reported by Fondy *et al*.,[59] starting from 3-fluoropropan-1,2-diol (**116**) (Scheme 36). After regioselective protection of the primary hydroxyl group with benzoylchloride, the secondary alcohol **117** was oxidized by a Pfitzner-Moffatt oxidation.[60] In the subsequent step the keto functionality of **118** was protected as dimethoxy ketal to enable the alkaline removal of the benzoyl protecting group. An isolation of FHA (**115**) was not possible due to the instability of FHA (**115**) which was obtained after acidic deketalisation of **120**. The main drawback of this synthesis is the usage of only in milligram quantities available expensive diol **116** (153 € / 250 mg) as starting material.[61]



Scheme 36. Synthesis of FHA (115) by Fondy *et al.*[59]

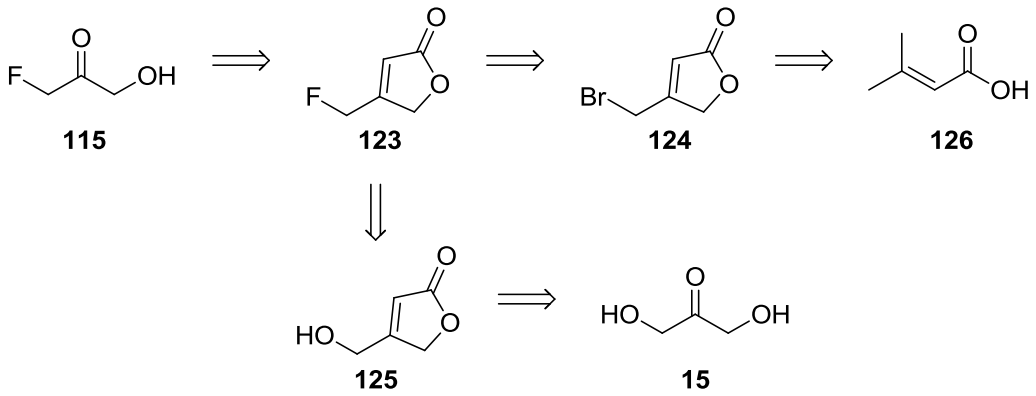
Kozarich *et al*. reported a very concise synthesis of FHA (**115**) (Scheme 37).[62] Starting from commercially available 3-fluoropyruvic acid (**121**), the α-keto acid functionalities were simultaneously protected as dimethyl ketal and methyl ester using trimethylorthoformate. After reduction of the ester **122** with $LiAlH_4$, Fondy's final intermediate **120** was isolated in high yield. Again FHA (**115**) was only obtained in solution after acid catalyzed deketalisation. An obvious drawback of this elegant synthesis is the expensive starting material 3-fluoropyruvic acid (147 €/g).



Scheme 37. Synthesis of FHA (115) by Kozarich *et al.* [62]

Retrosynthetic Analysis

Ideally the hydroxyl and the keto functionalities of FHA (**115**) are generated at a late stage of the synthesis, since free α-hydroxy ketones tend to be unstable.[63] Therefore we thought that a simultaneous liberation of these functionalities should be possible by ozonolysis of the γ-lactone **123** (Scheme 36). The fluorine atom should be introduced by a halogen-halogen exchange with allylic bromide **124** or by fluorination of allylic alcohol **125**. As starting material 3,3-dimethylacrylic acid (**126**) (0.27 €/g) or dihydroxyacetone (**15**) (0.28 €/g) can be used. This strategy would allow a short, cheap and protecting group free synthesis of FHA (**115**).

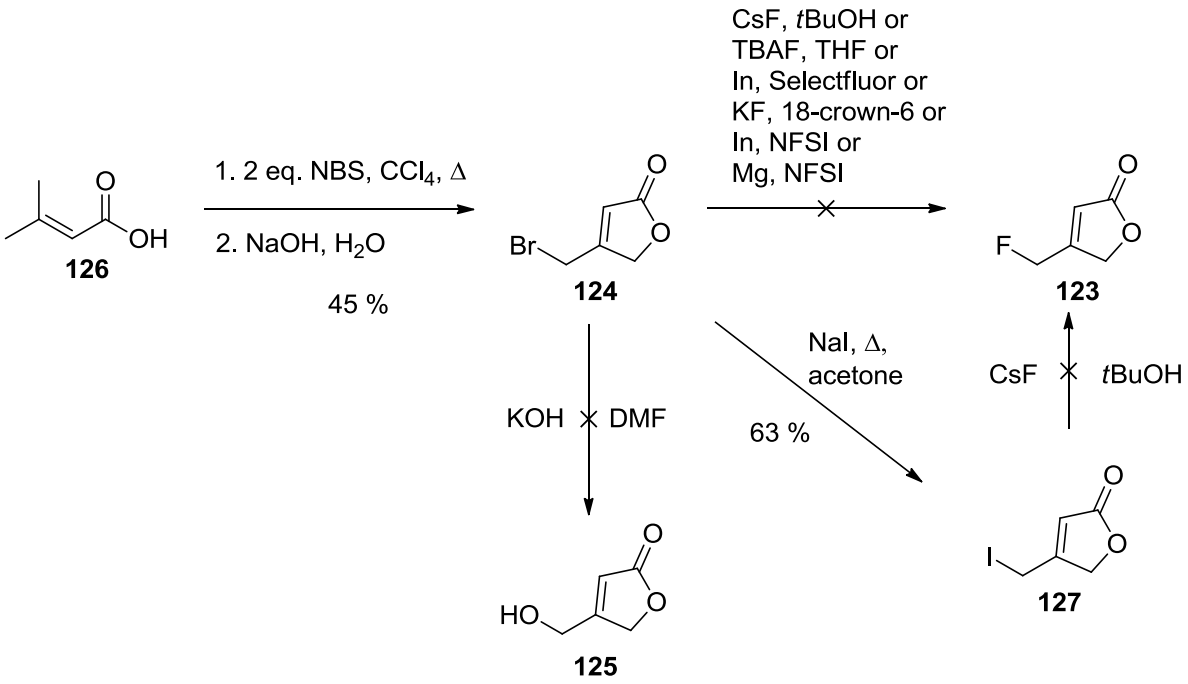


Scheme 38. Retrosynthetic analysis of FHA (115).

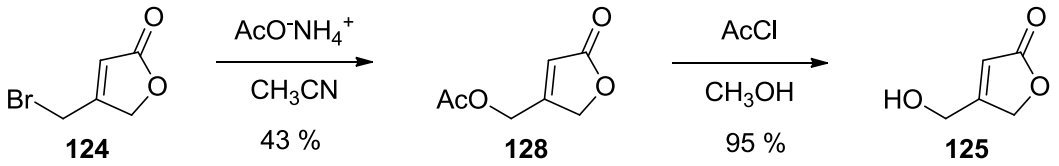
B.4. Results and Discussion

To avoid dangerous DAST, the halogen-halogen exchange approach was investigated first. 3,3-dimethylacrylic acid **126** was converted into furanone **124** by radical di-bromination of the allylic methyl groups with two equivalents of N-bromsuccinimid (NBS) followed by alkaline workup.[64] All attempts to displace the bromine of **124** either by nucleophilic fluorine reagent (CsF, TBAF, KF) or by electrophilic fluorine source (Selectfluor, NFSI) after halogen metal exchange[65] failed (Scheme 39). The corresponding iodo furanone **127**, which was obtained after halogen-halogen exchange with sodium iodide in acetone, was unreactive as well. After these disappointing results we decided to further elaborate the DAST mediated fluorination. A direct conversion of allylic bromide **124** into allylic alcohol **125** with KOH in DMF was unsuccessfully. The desired hydroxy furanone **125** was finally obtained after displacement of allylic bromide with ammonium acetate followed by acid catalyzed cleavage of the acetyl group in moderate yield (Scheme 40).[66]

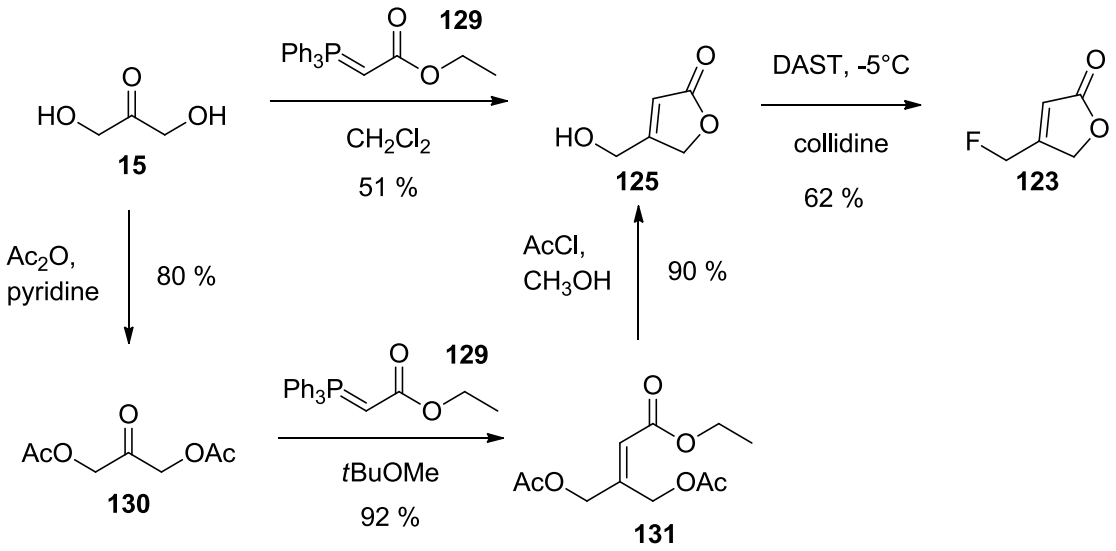
Since this sequence was inefficient, the allylic alcohol **125** was prepared by two slightly different strategies starting from DHA (**15**) (Scheme 41).[66,67] A Wittig reaction of DHA (**15**) with phosphor ylid **129** in dichloromethane afforded **125** in moderate yield. Alternatively DHA (**15**) was acetylated with acetanhydride, followed by the same Wittig reaction. The furanone **125** was obtained after acid catalyzed cleavage of the acetyl protecting groups and lactonisation. The ylid **129** was prepared from ethyl bromoacetate (**132**) and triphenylphosphine (Scheme 42).[69]



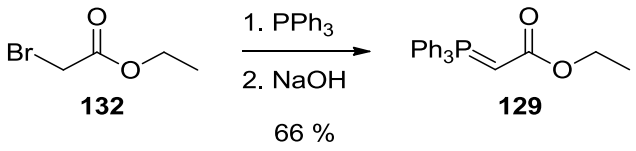
Scheme 39. Halogen-halogen exchange approach for the synthesis of furanone 123[66,68]



Scheme 40. Synthesis of allylic alcohol 125[66]

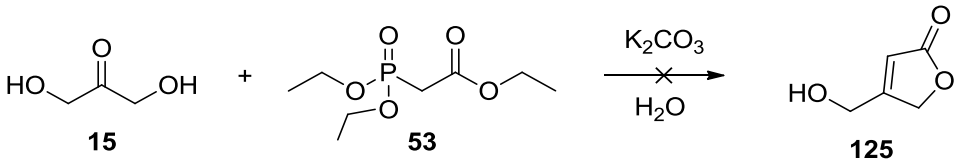


Scheme 41. DAST-mediated synthesis of furanone 123.[67]



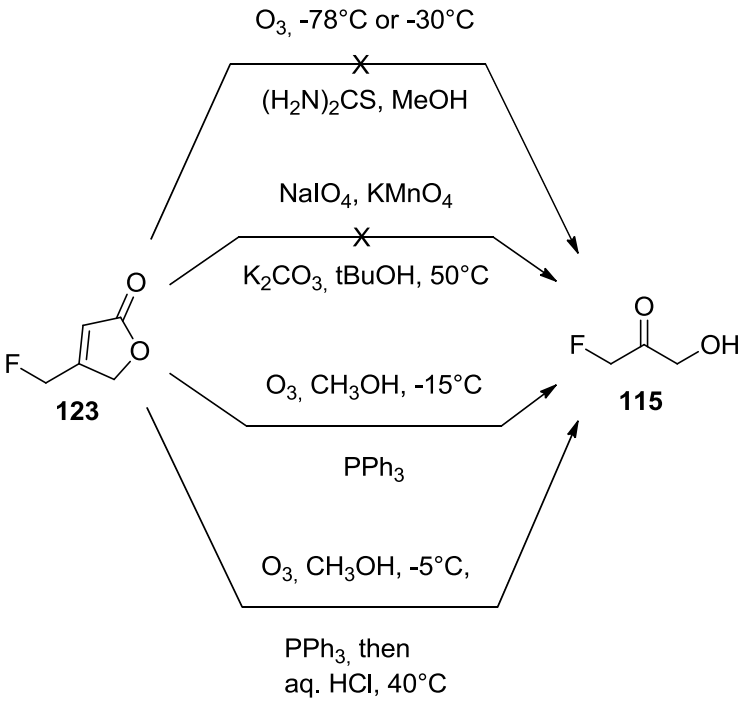
Scheme 42. Ylid formation[69]

To circumvent the ylide formation a Horner-Wadsworth Emmons (HWE) reaction between DHA (**15**) and trietyhlphosphonoacetate (**53**) was attempted (Scheme 43). Interestingly, DHA (**15**) was completely unreactive under these conditions.



Scheme 43. Unsuccessful HWE reaction

The fluorination with DAST at -5°C worked smoothly to afford the desired fluoro furanone **123** in good yield.[67a] Surprisingly the double bond of **123** could not be ozonized under standard conditions using dichloromethane or methanol at -78°C or methanol at -30°C (Scheme 44). Only unreacted starting material could be isolated although the inlet time of ozone was prolonged substantially and the color of the reaction mixture was deep blue for several minutes. Alternatively the double bond was tried to oxidize by a Lemieux-von-Rudloff-Oxidation. Again only unreacted starting material could be isolated. Finally FHA (**115**) was observed as mixture of several compounds via careful ozonolysis of **123** at -15°C in methanol. Elevating the temperature to -5°C and the exposure of the reaction mixture to a diluted aqueous HCl solution, after destroying the ozonides and peroxides with triphenylphosphine gave cleaner spectra. All effort to isolate FHA (**115**) or to purify the crude product by silica chromatography were unsuccessful and can be attributed to its inherent instability as published by Fondy and Kozarich *et al*.[59,62] A precise determination of the FHA (**115**) yield was yet not possible and a more reproducible procedure has to be elaborated for performing the reaction on a larger scale. For these reasons the planned organocatalytic aldol additions were not implemented.



Scheme 44. Synthesis of FHA (115) by ozonolysis of furanone 123

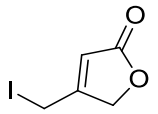
B.4. Experimental Procedures

Following compounds were synthesized according to literature procedures:

- 4-(Fluoromethyl)furan-2(5H)-one (**123**)[67a]:

  Spectroscopical data: ^{1}H-NMR (400 MHz): δ = 6.09-6.05 (m, 1H, CH,) 5.31 (dm, $J_{H\text{-}F}$ = 46.1 Hz, 2H, CH_2) 4.88-4.85 (m, 2H, CH_2); ^{13}C-NMR (400 MHz): δ = 172.6, 163.6 (d, *J* = 20.3 Hz) 116.5 (d, *J* = 6.4 Hz) 78.2 (d, *J* = 169.8 Hz) 70.5 (d, *J* = 5.7 Hz); ^{19}F-NMR (600 MHz): δ = -226.2 (t, *J* = 46.1 Hz).
- 4-(Bromomethyl)furan-2(5H)-one (**124**)[64]
- 4-(Hydroxymethyl)furan-2(5H)-one (**125**)[67a]
- Ethyl (triphenylphosphoranyliden)acetate (**129**)[69]
- 1,3-Dihydroxyacetone diacetate (**130**)[67b]
- Ethyl 4-acetyloxy-3-(acetyloxy)methyl-but-2-enoate (**131**)[67a]

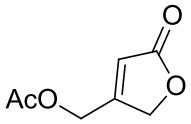
4-(Iodomethyl)furan-2(5H)-one (127):



127

4-(Bromomethyl)furan-2(5H)-one (**124**) (100 mg, 0.56 mmol) was dissolved in dry acetone (3 mL) and to secure absoluteness molecular sieve (3Å) was added. After ten minutes, sodium iodide (169 mg, 1.13 mmol) were added and the reaction mixture was refluxed until ^{1}H-NMR monitoring showed consumption of the starting material. The reaction mixture was quenched by addition of H_2O (2 mL) and extracted with diethyl ether (2 x 3 mL). The combined organic phases were washed with aqueous $NaHSO_3$ solution (2 %) and dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure 4-(iodomethyl)furan-2(5H)-one (**127**) was isolated (80 mg, 63 %). ^{1}H-NMR (250 MHz, $CDCl_3$): δ = 6.13-6.09 (m, 1H, CH,) 5.02-4.98 (m, 2H, CH_2) 4.17-4.12 (m, 2H, CH_2); ^{13}C-NMR (400 MHz, $CDCl_3$): δ = 165.0, 117.7, 72.4, -8.1.

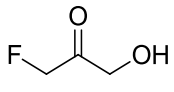
4-Acetyloxymethylfuran-2(5H)-one (128):



128

4-(Bromomethyl)furan-2(5H)-one (**124**) (50 mg, 0.28 mmol) was dissolved in dry acetonitrile (2 mL) and ammonium acetate (26 mg, 0.35 mmol) was added. Since TLC monitoring showed unconsumed starting materials after 24 hours, ammonium acetate (13 mg, 0.175 mmol) was added again and the reaction mixture was stirred for another 12 hours. The reaction was quenched by addition of H_2O (1 mL) and diethyl ether (2 mL). The phases were separated and the organic layer was extracted with diethyl ether (2 x 5 mL). The combined organic phases were dried over $MgSO_4$, concentrated and purified by silica chromatography (toluene/hexane = 3/1) to yield 4-(acetyloxy methyl)furan-2(5H)-one (**128**) (19 mg, 43%). The spectroscopical data were identical with those reported.[66]

Fluorohydroxyacetone (115):

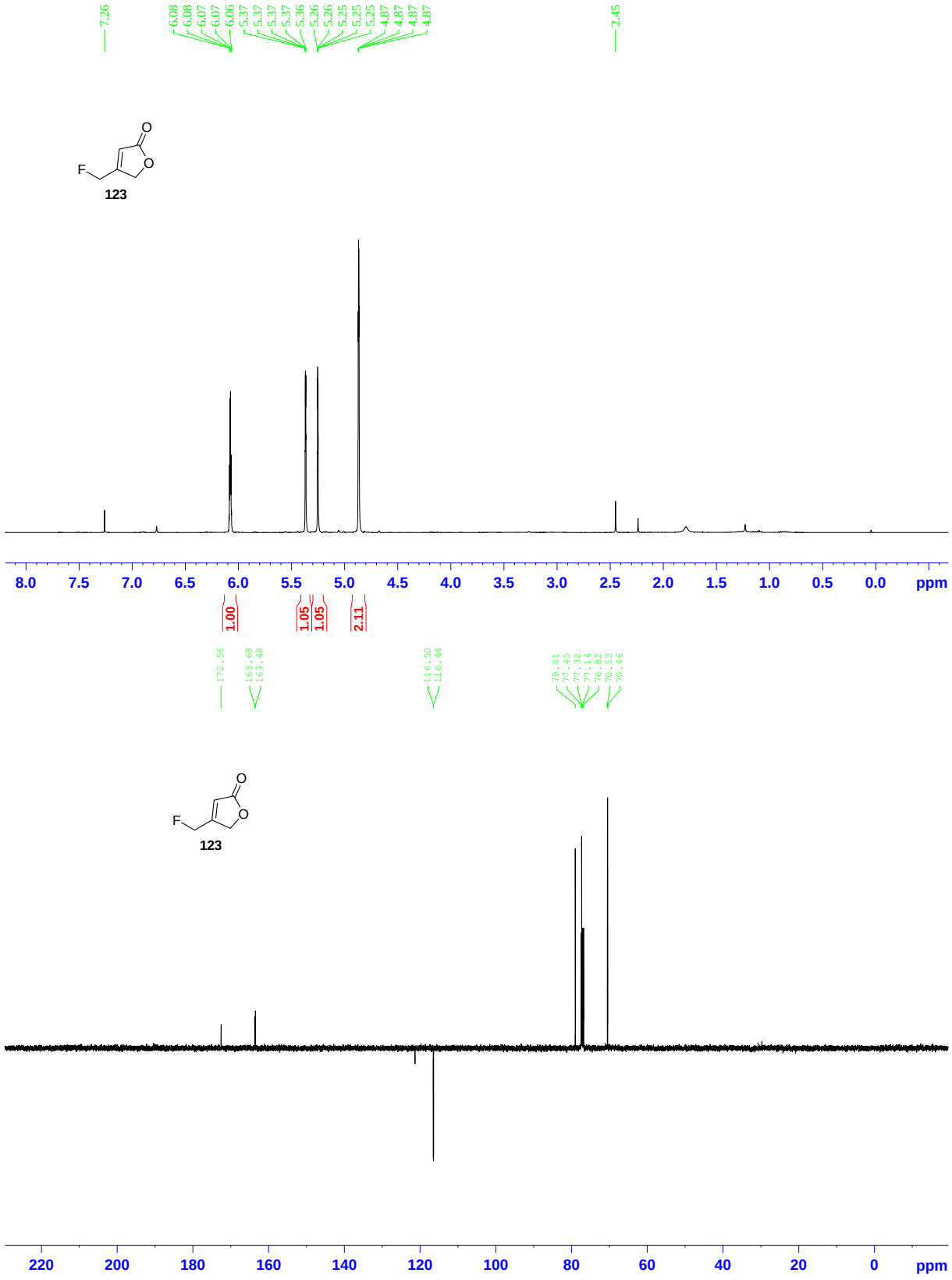


115

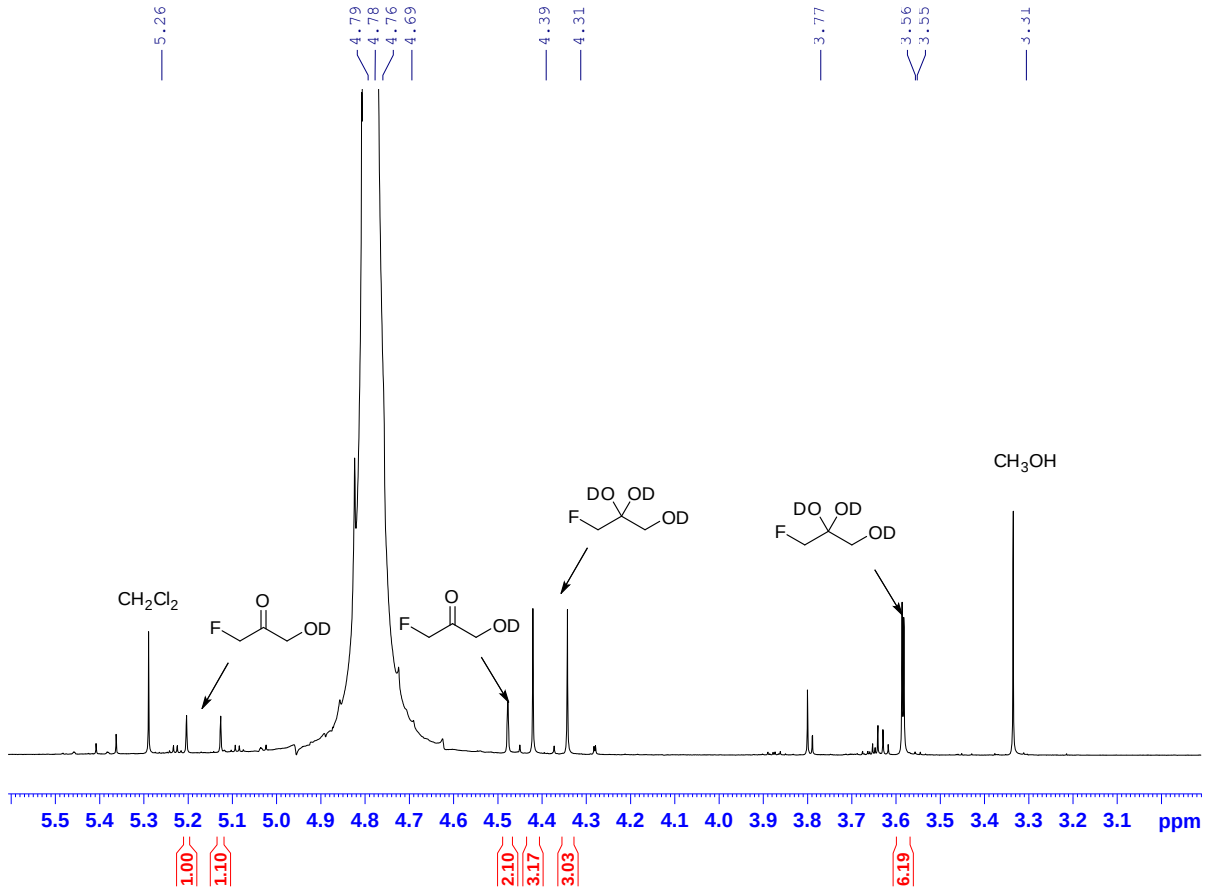
Ozone was bubbled through a solution of 4-(Fluoromethyl)furan-2(5H)-one (**123**) (29 mg, 25 mmol) in dry methanol (2.5 mL) at -5°C for seven minutes . Dry air was bubbled through the solution (~ 4 minutes) and PPh_3 (82 mg, 0.31 mmol) was added. The mixture was allowed to reach room temperature by stirring over night. To remove the methanol, D_2O (1 mL) was added and the solvent was evaporated under reduced pressure (not to dryness!). After repeating this procedure once again, the white solid was removed by filtration over absorbent cotton, rinsed with D_2O and the aqueous solution was acidified by addition of a DCl solution (1 M). After heating the reaction mixture over night at 40°C, Fluorohydroxyacetone (**115**) could be identified by ^{1}H-NMR in good purity. The spectroscopical data were identical with those reported.[62]

Selected spectra:

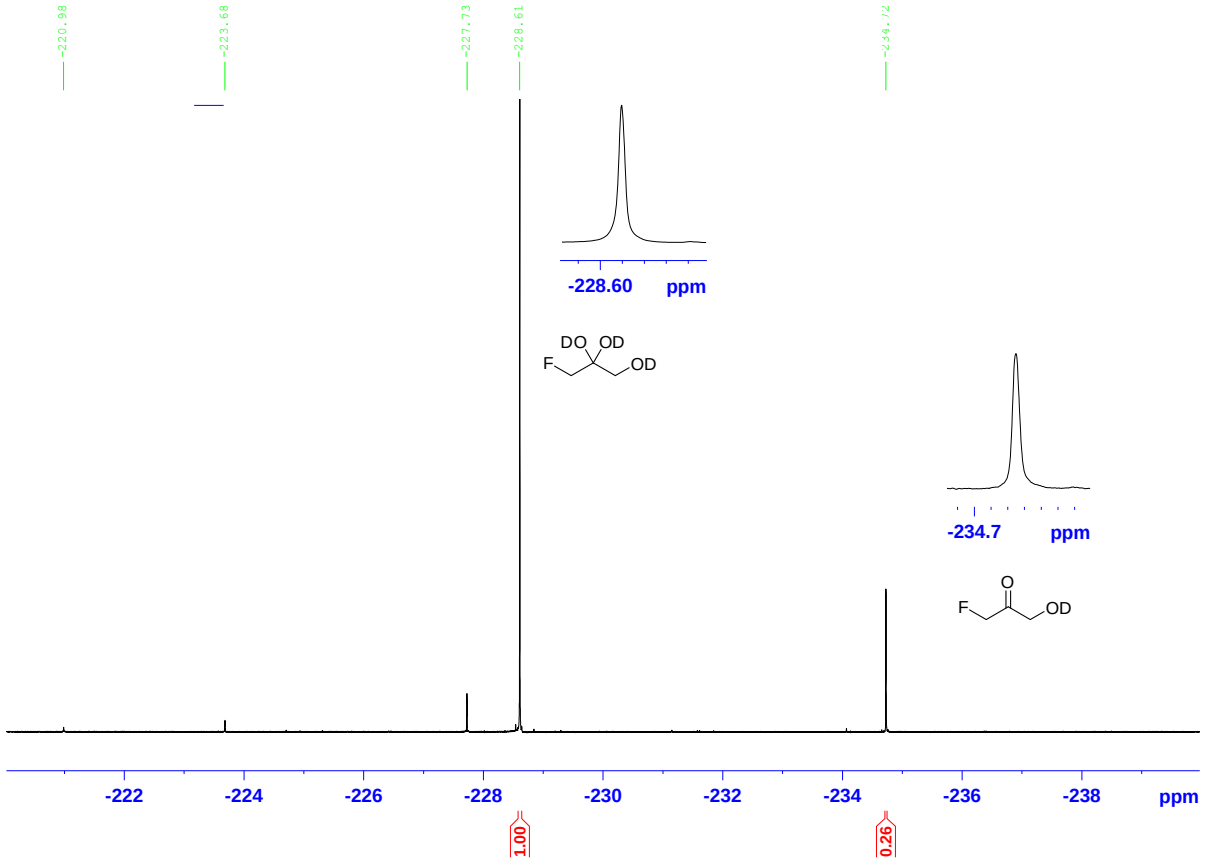
4-(Fluoromethyl)furan-2(5H)-one (**123**):



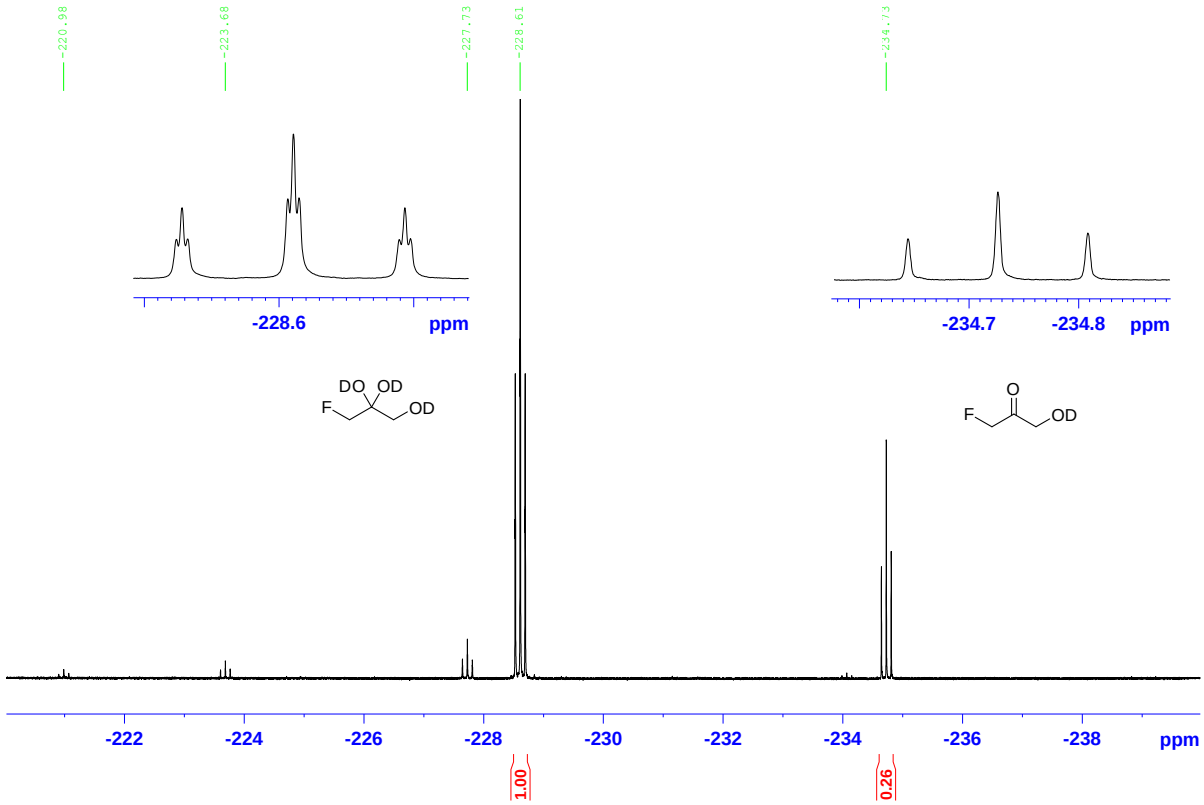
^{1}H-NMR of FHA (**115**):



^{19}F-NMR (decoupled) of FHA (**115**):



^{19}F-NMR (coupled) of FHA (**115**):



PART C: Indium-Mediated Allenylation of Aldehydes with 4-Bromo-2-butyn-1-ols: Versatile α-Hydroxyacetyl Anion Equivalents

C.1. Abstract

We developed a facile and convenient preparation and application of α-hydroxyacetyl anion equivalents for carbon chain elongations. A two-step sequence of an indium mediated allenylation of aldehydes with 4-bromo-2-butyn-1-ols followed by ozonolysis of the resulting allene was used to generate the desired substituted dihydroxyacetone fragments. The regioselectivity of the indium-promoted C-C bond forming reaction can be controlled by the hydroxyl protecting group on 4-bromo-2-butyn-1-ol, preferentially yielding either allenes or alkynes. Compared to established protocols, the necessary amount of indium for the allenylation was decreased by a factor of two to four. The versatility of this strategy was demonstrated by the stereoselective and efficient synthesis of D-*erythro*-2-pentulose (D-ribulose) and 1-deoxy-D-ribulose.

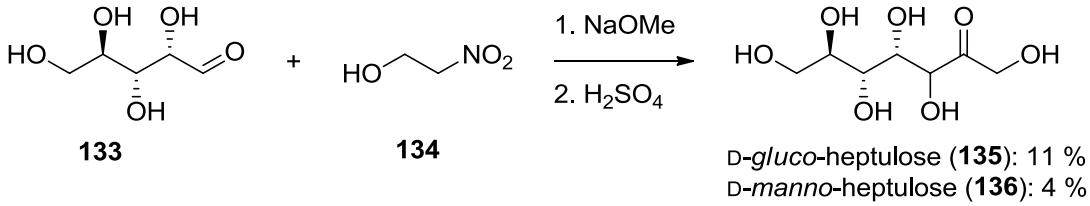
C.2. Introduction and Background

The concept of reactivity "umpolung" proved to be a significant advancement in organic synthesis.[70] Among acyl anion equivalents, α-hydroxyacetyl anions are of increasing interest, although mild chemical methods for creating them are relatively scarce so far.[71,72] The addition of α-hydroxyacetyl anion equivalents to aldehydes and ketones gives access to a variety of natural products, like keto sugars, cortical steroids and anthracycline antibiotics.[73] Recently, indium-mediated reactions of substituted propargyl bromides with aldehydes have been established for the regioselective formation of either homopropargylic or allenylic alcohols.[74,75] Especially α-hydroxy allenes have proven to be valuable synthetic inter-

mediates due to their variability in chemical applications.[76] Following the previous synthetic employed α-hydroxyacetyl anion equivalents, the mechanism and application of indium-mediated allenylation/propargylation reactions will be discussed.

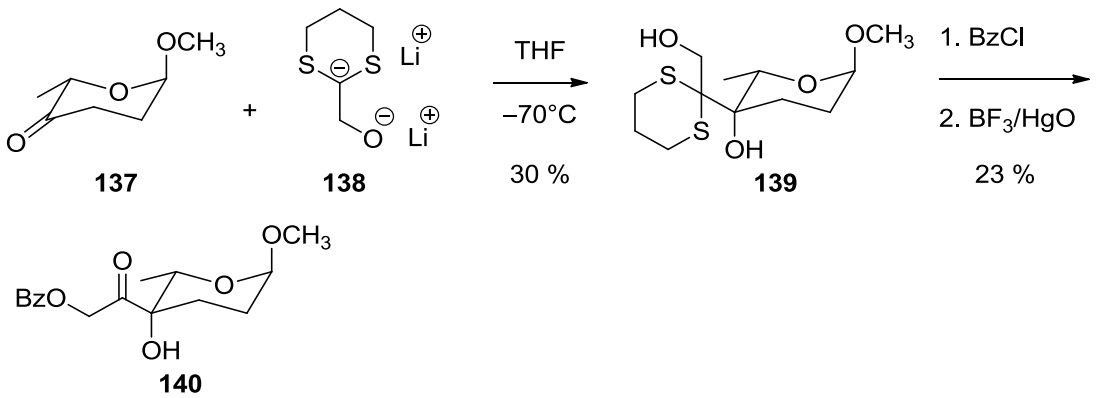
α-Hydroxyacetyl Anion Equivalents

Since decades organic chemists search for efficient methods which permit a direct interconversion of aldoses into the C2 elongated ketose homologues. 2-Nitroethanol was one of the first α-hydroxyacetyl anion equivalents used for this purpose. In 1950 Swoden *et al.*[71h,77] condensed arabinose **133** with 2-nitroethanol **134** under basic conditions and after addition of sulfuric acid (Nef reaction) D-*gluc*o- and D-*manno*-heptulose (**135** and **136**) were isolated in low yield (Scheme 45).



Scheme 45. 2-Nitroethanol as α-hydroxyacetyl anion equivalent[77]

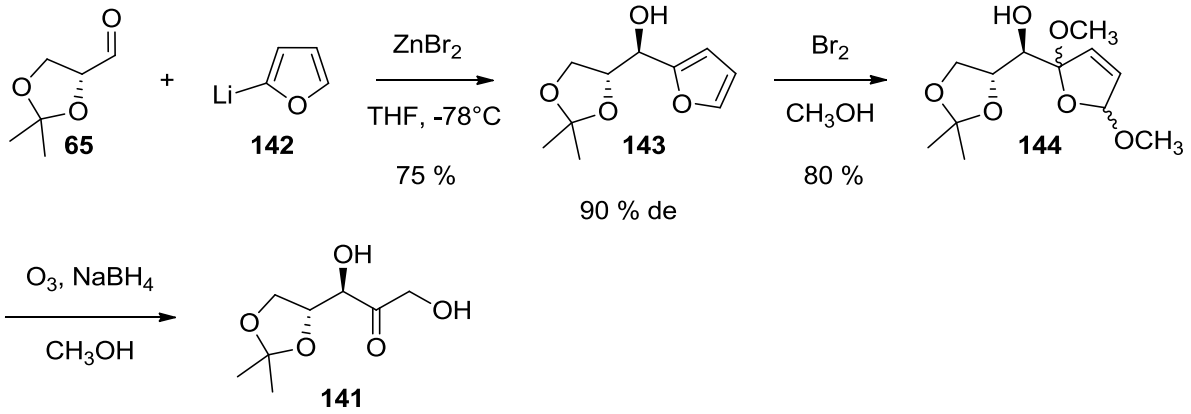
In 1971 Paulsen *et al*. published a simple synthesis of pillarose, using the dianion of 1,3-dithian-2-methanol as an α-hydroxyacetyl anion equivalent.[71e,78] The dithiane was desulfuricated by borontrifluoride in presence of mercury oxide in low yield (Scheme 46).



Scheme 46. 1,3-Dithian-2-methanol dianion (138) as α-hydroxyacetyl anion equivalent[71e,78]

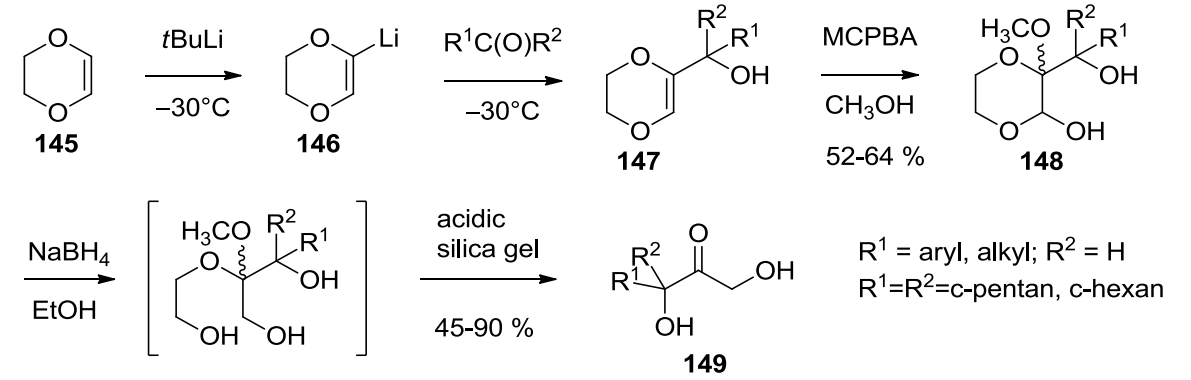
In 1981 Mukaiyma *et al*. described an efficient method for the synthesis of D-erythro-2-pentulose (**141**) by a stereoselective addition of 2-furyllithium (**142**) to 2,3-*O*-isopropylidene-

D-glyceraldehyde (**65**) (Scheme 47).[71a] The intermediate furfurylalcohol (**143**) was converted into the desired pentulose by a bromination/ozonolysis sequence.



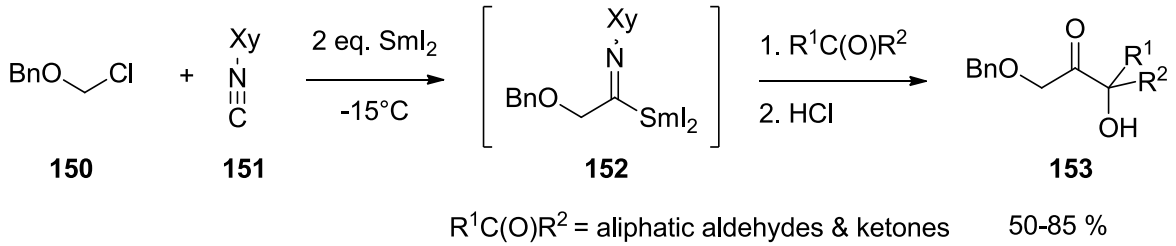
Scheme 47. 2-Furyllithium (142) as α-hydroxyacetyl anion equivalents[71a]

Dihydro-1,4-dioxin-2-yl lithium (**146**) was used for the synthesis of various α,α-dihydroxyketones by Fetizon *et al*. (Scheme 48).[71b] The hydroxy intermediate **147** was converted to the desired substituted dihydroxyacetone **149** by an oxidation/reduction sequence.



Scheme 48. Dihydro-1,4-dioxin-2-yl lithium (146) as α-hydroxyacetyl anion equivalent[71b]

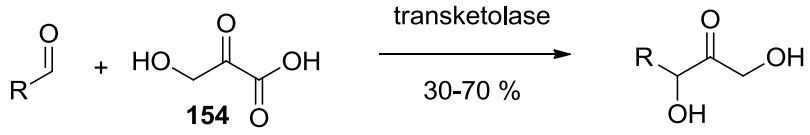
In 1990 Ito *et al*. reported a facile samarium(II)iodide coupling of **152** with aldehydes and ketones (Scheme 49).[72c,d] The resulting imines were hydrolyzed under acidic conditions to give various substituted 1-*O*-benzyl-protected dihydroxyacetones **153**. This strategy was used in the short synthesis of D-erythro-2-pentulose.



Scheme 49. Samarium(II)iodide coupling of aldehydes with 152[77c,d]

All approaches for the generation of an α-hydroxyacetyl anion equivalent mentioned above suffer either from low yield or from harsh reaction conditions, which are not applicable to unprotected carbohydrates.

In nature, the transfer of an α-hydroxyketone subunit from one substrate to another is catalyzed by transketolases in presence of thiamine pyrophosphate and magnesium ions. Synthetically β-hydroxypyruvic acid (HPA) **154** is used as a ketol donor, making the reaction irreversible due to decarboxylation of **154**. The isolated yields differ strongly with respect to the acceptor aldehydes (Scheme 50).[72]

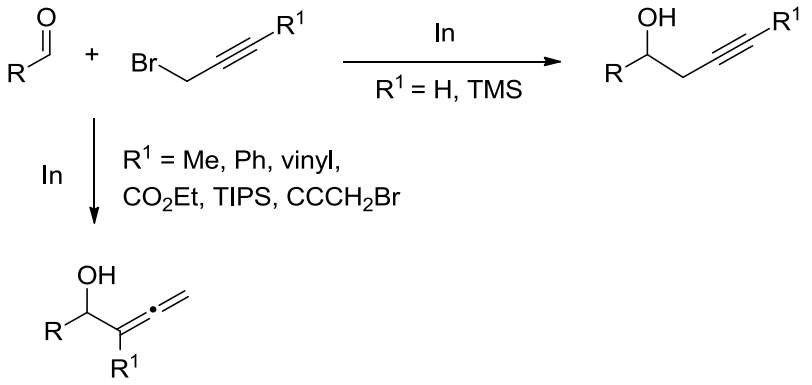


Scheme 50. Transketolase catalyzed synthesis of substituted dihydroxyacetones[72]

Indium-Mediated Allenylation/Propargylation of Aldehydes with Substituted Alkynes

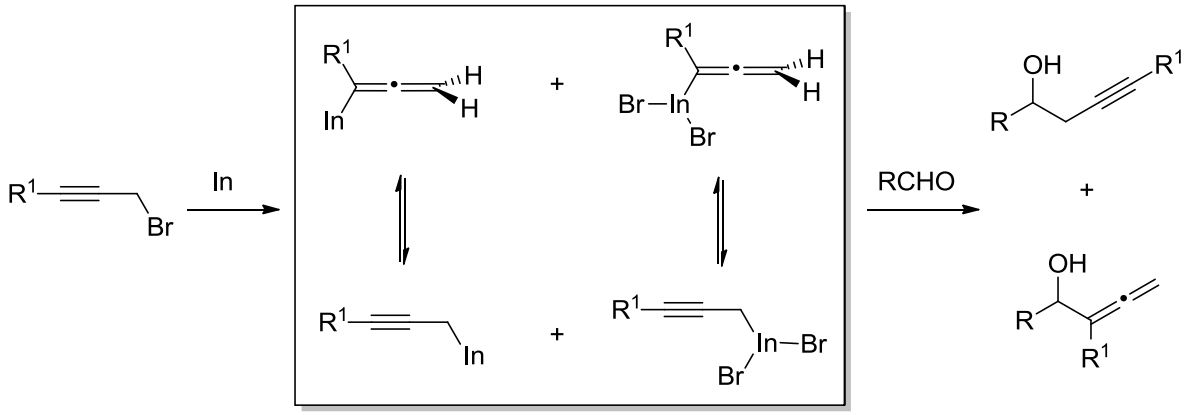
Albeit one example of an indium mediated propargylation was already mentioned in the seminal publication on indium mediated allylations of carbonyl compounds by Araki *et al.*,[79] it took another seven years until the first preparatively useful study on indium-mediated couplings of aldehydes with propargyl bromides was published.[80] Substituted propargylbromides preferentially yielded the allene products, whereas unprotected propargylbromides gave the alkyne products (Scheme 51). Trialkysilyl propargylbromides afforded mixtures of regioisomers under aqueous reaction conditions. In 2003 Loh *et al.* showed that the regioselectivity of propargylbromides can be controlled by employing trimetylsilyl or bulkier triisopropylsilyl propargylbromides under Lewis acid catalysis.[81] The change in regioselectivity was attributed to chelation of silicon to the reactive indium

intermediate, which is not possible for sterical reasons for the triisopropylsilyl propargylbromides.



Scheme 51. Indium-Mediated Allenylation/Propargylation of Aldehydes with Substituted Alkynes[80-82,85,88]

The active intermediate of indium-mediated allenylation/propargylation in solution is still a matter of debate.[82] Chan *et al*. observed an indium(I) species in aqueous solution and a mixture of indium(I) and indium(III) species in tetrahydrofuran (Scheme 50).[82a] The system (^{1}H-spectra) was further complicated by the substituent on the propargylbromides. For unsubstituted prop-2-ynyl bromides the allenylindium structures dominated, due to their higher stability. This result was verified by either HF or B3LYP calculations. After addition of *p*-chlorobenzaldehyde, the corresponding homopropargylic alcohol was isolated. Substituents at the alkyne strongly influenced the equilibrium between the allenyl and the alkenylindium species, favoring the latter one due to steric repulsion. Addition of *p*-chlorobenzaldehyde to these systems yielded allenylic alcohols. All findings were in agreement with an S_E2' reaction, a pathway generally observed by addition of allenyl/propargyl organometallics to carbonyl compounds (Figure 8).



Scheme 52. Mechanism of the indium-mediated propargylation/allenylation of aldehydes with substituted propargylbromides by Chan *et al*.[82]

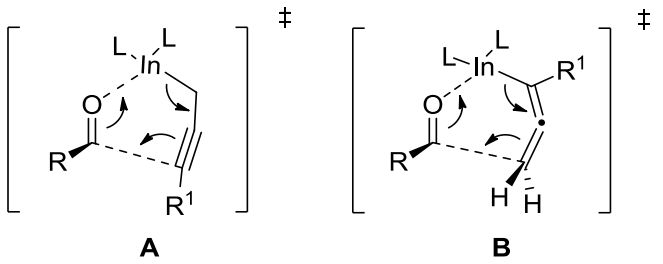


Figure 8. Possible transition states for the allenylation (A) and propargylation reaction (B)

In 2006 Hammond *et al*. crystallographically characterized the first In-C-C≡C complex (Figure 9).[83] Two alkynyl ligands, one bromine and two molecules of DMSO coordinated to central indium atom. By reaction of similar substituted complexes with electrophiles, they demonstrated that the ratio of allene to alkyne products solely depend on the reactivity of the electrophile (Scheme 53).

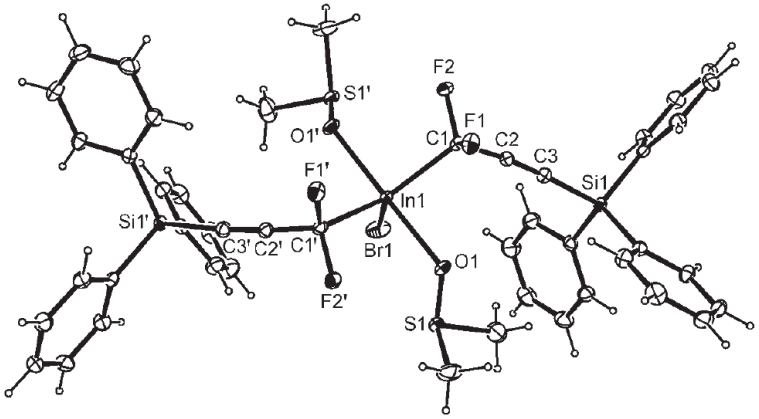
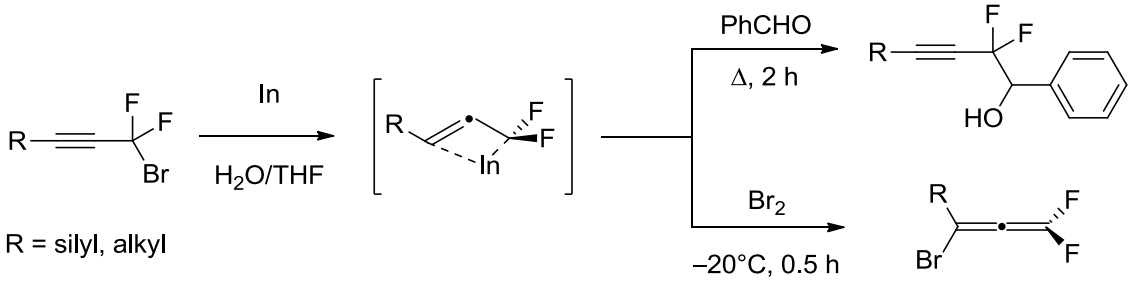


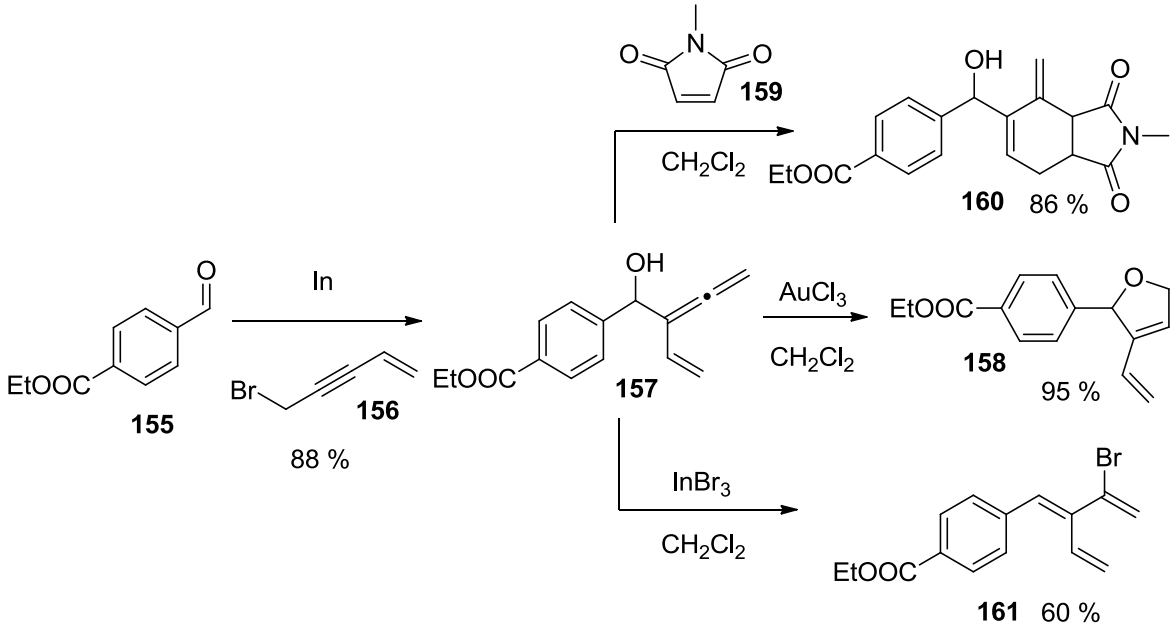
Figure 9. Crystal structure of first In-C-C≡C complex[83]



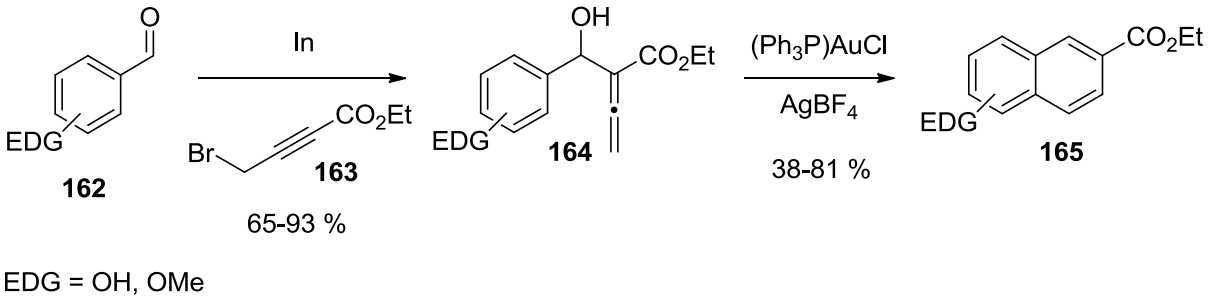
Scheme 53. Indium-mediated difluroallenylation and propargylation[83]

Recently allenes have been established as versatile synthetic intermediates.[84] Especially transition metal catalyzed cyclizations by gold or platinum are synthetically valuable transformations. Lee *et al*. published two consecutive papers on combining indium mediated

allenylations with gold-catalyzed cyclizations (Scheme 54 and 55).[85] Alternatively the allenes can be further converted to other synthetically useful intermediates by Diels-Alder reactions or by Lewis acid catalysis.

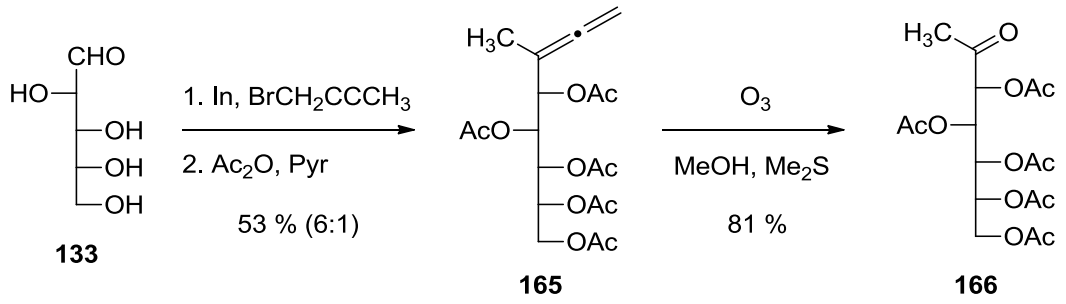


Scheme 54. Allenes as synthetic intermediates[85]

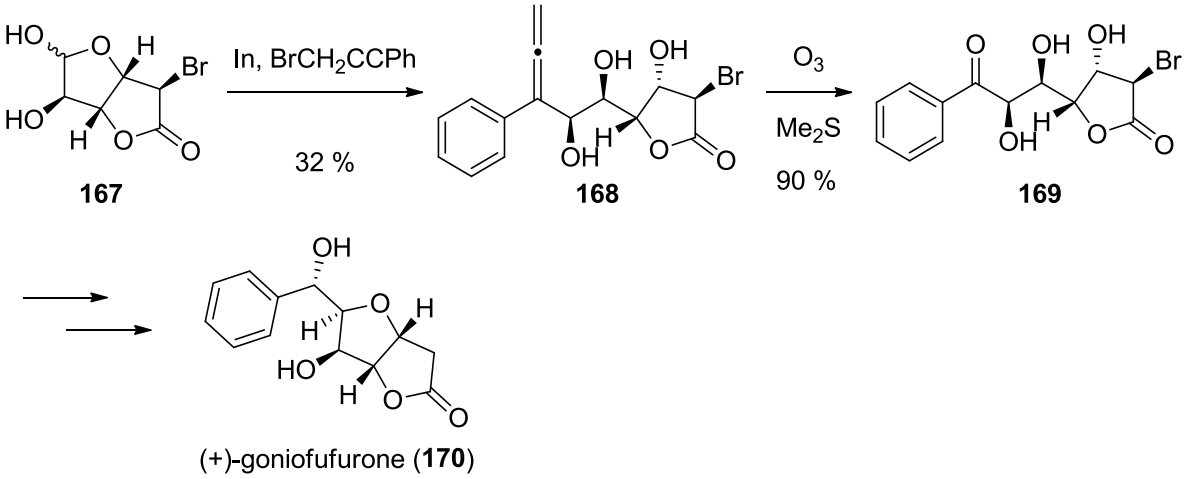


Scheme 55. Allenes as synthetic intermediates[85]

The conversion of allenes into ketones by ozonoylsis[86] is straight forward and was reported for the synthesis of 1-deoxy-heptuloses by Whitesides *et al*.[87] and the natural product (+)-goniofufurone (**170**) by Li *et al*. (Scheme 56 and 57).[88]



Scheme 56. Synthesis of 1-deoxy-heptuloses by Whitesides *et al*.[87]



Scheme 57. Synthesis of (+)-goniofufurone by Li *et al*.[88]

Concept and Strategy

The idea of this project was the conversion of aldoses into ketoses by a C2 elongation. Ideally, naturally occurring aldoses are used to avoid the normally necessary protection and deprotection steps in carbohydrate chemistry. A novel equivalent to an α-hydroxyacetyl anion had to be designed for this transformation, since no general applicable method existed for this C2 synthon. Our retrosynthetic considerations directed us to 4-bromo-2-butin-1-ol for a synthetic equivalent for an α-hydroxyacetyl anion (Figure 10). For this substituted propargylbromide we expected that allenes **173** are formed by an indium-mediated allenylation reaction with aldehydes. Ozonolysis of the allene moiety of **173** afforded the desired C2 elongated ketoses (Scheme 58).

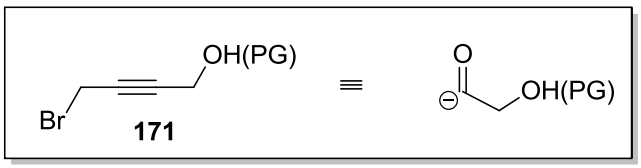
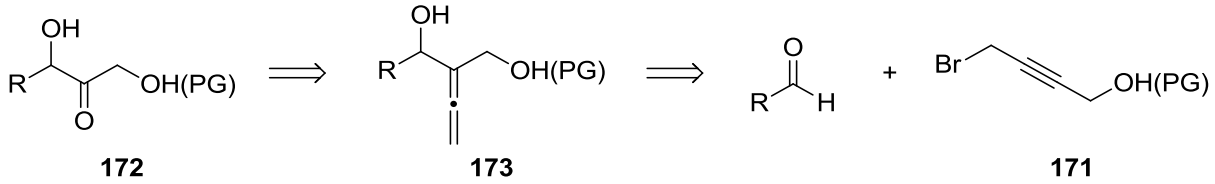


Figure 10. 4-bromo-2-butin-1-ols as α-hydroxyacetyl anion equivalents.



Scheme 58. Retrosynthetic analysis.

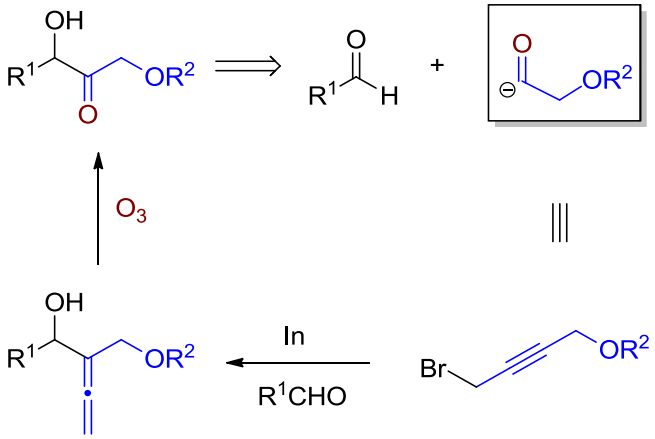
C.3. Results and Discussion

Published Work:

Indium-mediated Allenylation of Aldehydes and its Application in Carbohydrate Chemistry: Efficient Synthesis of D-Ribulose and 1-Deoxy-D-ribulose

M. Fischer, C. Schmölzer, C. Nowikow, W. Schmid, *Eur. J. Org. Chem*. **2011**, 1645-1651.

Graphical Abstract:



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Indium-Mediated Allenylation of Aldehydes and Its Application in Carbohydrate Chemistry: Efficient Synthesis of D-Ribulose and 1-Deoxy-D-ribulose

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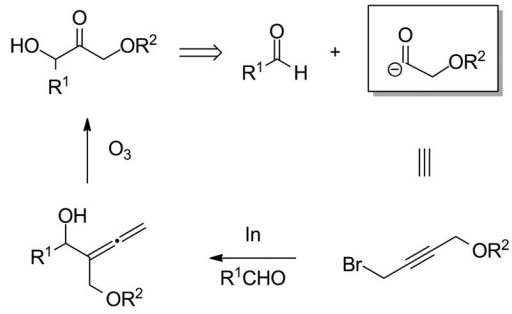
Keywords: Indium / Allenes / Carbohydrates / Acyl anion / Water chemistry

A two-step reaction sequence starting with the indium-mediated allenylation of aldehydes with 4-bromo-2-butyn-1-ols and subsequent ozonolysis of the resulting allenylic product was developed to generate a variety of dihydroxyacetone derivatives. The regioselectivity of the indium-promoted C–C bond-forming reaction can be manipulated through hydroxy protecting groups on 4-bromo-2-butyn-1-ol, yielding either allenes or alkynes as preferred products. Compared to established protocols, the necessary amount of indium for this type of allenylation can be decreased by a factor of two to four. The versatility of this strategy was demonstrated in the stereoselective and straightforward synthesis of D-ribulose and 1-deoxy-D-ribulose.

Introduction

Indium-mediated reactions, such as the addition of substituted propargyl bromides to aldehydes, have gained substantial interest in synthetic organic chemistry.[1,2] This reaction enables the regioselective formation of either homopropargylic or allenylic alcohols, the latter being extremely valuable synthetic intermediates due to their variability in chemical applications.[3] Suitably substituted propargyl bromides can be retrosynthetically seen as a source of α-hydroxyacetyl anion equivalents (Scheme 1), which play important roles in the concept of "umpolung" reactivity in synthetic strategies.[4] However, simple and mild methods for generating α-hydroxyacetyl anions are relatively scarce[5,6] although their application in the synthesis of a variety of natural products,[7] like cortical steroids, anthracycline antibiotics, or keto sugars, have impressively demonstrated their synthetic potential.

This publication reports on a facile synthetic sequence, generating an α-hydroxyacetyl anion equivalent from 4-bromo-2-butyn-1-ols and utilizing this synthon in an indium-mediated allenylation reaction of various aldehydes. The resulting allenes are converted into keto moieties by ozonolysis, thus forming the desired substituted dihydroxyacetone derivative. Because the regioselectivity of the indium-mediated reaction can be manipulated by the nature of the hydroxy protecting group of 4-bromo-2-butyn-1-ol, the ratio of the allene and alkyne products can be influenced within a certain range. We used this strategy for a stereoselective and efficient synthesis of D-*erythro*-2-pentulose (D-ribulose) and its 1-deoxy-D-*erythro*-2-pentulose analogue.



Scheme 1. Retrosynthetic concept.

Results and Discussion

Our initial studies focused on the addition of 1-(benzyloxy)-4-bromo-2-butyne (**2**) to 2,3-*O*-isopropylidene-D-glyceraldehyde[8] (**1**) under indium promotion. Compound **1** was used as the starting aldehyde due to its synthetic importance as a starting material in carbohydrate chemistry and total syntheses.[9] The yields obtained are summarized in Table 1.

Reaction of **1** with the in situ generated organoindium reagent in water or THF did not yield significant amounts of products (Table 1, Entries 1 and 2).[10] In contrast, by addition of LiI or LiCl in THF the reaction proceeded smoothly and afforded good yields of desired allene **3a** (Table 1, Entries 3 and 8).[11,12] The use of THF/water mix-

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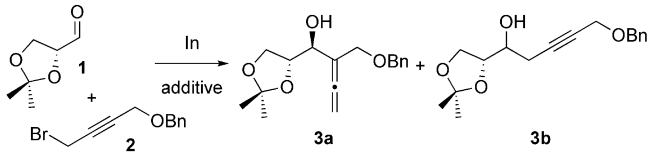
Table 1. Indium-mediated reactions of 2,3-*O*-isopropylidene-D-glyceraldehyde (**1**) with 1-(benzyloxy)-4-bromo-2-butyne (**2**).



1 + **2** → **3a** + **3b**

Entry[a]	Indium [equiv.]	Additive [equiv.]	Solvent	Yield [%][b]	**3a/3b**[c]
1	1.0		H_2O	0	nd
2	1.0		THF	ca. 5	nd
3	1.0	LiI (1.0)	THF	74	73:27
4	1.0	LiI (1.0)	THF/H_2O	58	52:48
5	1.0	LiI (3.0)	DMF	70	52:48
6	1.0	LiI (2.0)	THF	71	69:31
7	1.0	LiBr (2.0)	THF	46	70:30
8	1.0	LiCl (2.0)	THF	84	70:30
9	1.0	LiF (2.0)	THF	0	nd
10	0.55	LiI (1.0)	THF	69	78:22
11	0.55	LiCl (2.0)	THF	nd[d]	nd
12	1.0	*n*Bu_4NI (1.0)	THF	58	69:31
13	1.0	*n*Bu_4NCl (1.0)	THF	0	nd
14	1.0	$LiNO_3$ (2.0)	THF	0	nd
15	1.0	NaCl (2.0)	THF	35	68:32

[a] Except where indicated, all reactions were carried out on 0.5-mmol scale with aldehyde/bromide/indium = 1:1:1 under ultrasonic conditions. [b] Isolated combined yields of allenes and alkynes. [c] nd = not determined. [d] Starting materials were not fully consumed.

tures or DMF as solvent led to a decrease in the combined yields and regioselectivity (Table 1, Entries 4 and 5). The best results were obtained by addition of **1** to a suspension of indium (1 equiv.), propargyl bromide **2** (1 equiv.), and either LiI (1 equiv.) or LiCl (2 equiv.) in anhydrous THF under ultrasonic conditions (Table 1, Entries 3 and 8).[13] However, nearly half of the metallic indium remained unchanged in the reaction mixture after consumption of starting materials **1** and **2**.[14] Reducing the amount of indium to 0.55 equiv. resulted in nearly the same combined yields of allene **3a** and alkyne **3b** (Table 1, Entry 10), whereas the regioselectivity shifted towards desired allene **3a**. In this case, by substituting LiI with LiCl (Table 1, Entry 11) only trace amounts of products were observed. Additionally, a further decrease in the amount of indium to 0.2 equiv. led to incomplete consumption of starting materials **1** and **2**. For practical reasons, we used the optimized reaction conditions of Entry 10 (Table 1) for further studies.

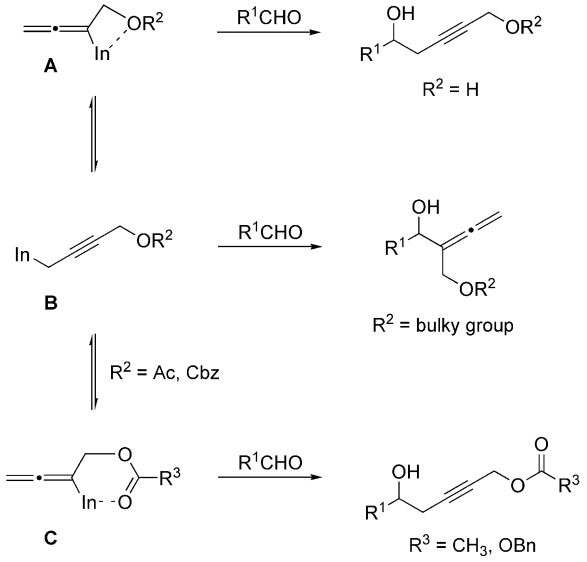
Interestingly, the allenylation of **1** was highly diastereoselective (>96%*de*), whereas the propargylation gave mixtures of diastereomers ranging from 4:1 to 2:1 (*erythro*/*threo*).[15] The configuration of the stereocenter formed in product **3a** was unambiguously assigned by transformation of the allenylic alcohol into the corresponding D-*erythro*-2-pentulose (**25**).[16] The observed *anti* relationship between the newly generated hydroxy group and the C2 hydroxy group of starting aldehyde **1** can be easily rationalized by the Felkin–Anh model.[17]

The organoindium species involved in this type of reaction was the subject of numerous investigations.[18,19] The data summarized in Table 1 suggest some basic mechanistic considerations. In general, propargyl iodides proved to be more reactive than bromides, albeit leading to lower yields.[20] The addition of LiI to the reaction mixtures accelerated the formation of the reactive organoindium species, presumably by in situ generation of the corresponding propargyl iodides.[21] The addition of LiCl instead of LiI beneficially influenced the combined yield of alkyne- and allene-type products (Table 1, Entries 6 and 8).[22] However, only in the case where LiI was applied could the amount of indium be decreased significantly (Table 1, Entries 10 and 11). Using tetrabutylammonium salts instead of lithium salts only in the case of *n*Bu_4NI led to product formation (Table 1, Entries 12 and 13).[23] In general, Grignard-type reaction conditions were superior to Barbier-type ones.[12b]

Because indium chelates to oxygen functionalities,[24] we hypothesized that the hydroxy protecting group of compound **2** is crucial for the regioselective formation of **3a** and **3b** (Scheme 2).[25] The results are summarized in Table 2. In analogy to the investigations of Lin et al. on siliconated propargyl bromides,[2a] we observed that bulky protecting groups favor the formation of the allenylic alcohols (i.e., **3a** and **8a**; Table 2, Entries 1 and 6) presumably via intermediate **B** (Scheme 2). As expected, changing the protecting group from benzyl to methyl decreased the regioselectivity for the formation of **4a** while increasing the combined yield (Table 2, Entry 2). Unprotected 4-bromo-2-butyn-1-ol (**5**; Table 2, Entry 3) reversed the regioselectivity of the reaction presumably by formation of intermediate **A** (Scheme 2), thus yielding mainly homopropargylic alcohol **5b**. In addition, acetyl- or Cbz-protected 4-bromo-2-butyn-1-ol may stabilize allenylic intermediate **C** by formation of a six-membered transition state, thus yielding propargylic derivatives (i.e., **6b** and **7b**; Table 2, Entries 4 and 5) preferentially. Further evidence for intermediate **C** was obtained by the following observations: Compounds with an oxygen atom too distant for chelating the allenylic intermediate or lacking an oxygen atom (Table 2, Entries 7 and 8) almost exclusively gave allenylic products **9a** and **10a**.

These results led us to examine the relative reactivity of 1-(benzyloxy)-4-bromo-2-butyne (**2**) towards a variety of different aldehydes (Table 3). In summary, citronellal gave lower yields than aromatic and α,β-unsaturated aldehydes (Table 3, Entries 1–4). 2-Phenylpropionaldehyde and the serine-derived "Garner aldehyde" gave good to excellent diastereoselectivity (Table 3, Entries 5 and 6).[26] As observed for 4-hydroxybenzaldehyde, a free hydroxy group is tolerated in carbonyl substrates (Table 3, Entry 3).

Furthermore, we examined the potential reactivity of 1-(benzyloxy)-4-bromo-2-butyne (**2**) towards water-soluble substrates (Table 3, Entries 7–11). In these cases, we added an aqueous solution of the corresponding aldehydes to the preformed indium intermediate, resulting in THF/water mixtures of 2:1. As depicted in Table 3, a freshly prepared solution of formaldehyde reacted smoothly with **2**, yielding compound **17a** in excellent yield. In analogy, aqueous solu-



Scheme 2. Allenylation vs. propargylation: influence of hydroxy protecting groups on regioselectivity. The corresponding organoindium(III) species are not shown.[19]

Table 2. Indium-mediated reactions of 2,3-*O*-isopropyliden-D-glyceraldehyde (**1**) with substituted propargyl bromides.

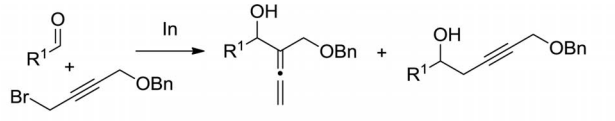
Entry[a]	$BrCH_2CCR^1$, R^1 =	Yield [%][b]	Allene/Alkyne
1	CH_2OCH_2Bn (**2**)	69	78:22 (**3a/3b**)
2	CH_2OCH_3 (**4**)	80	60:40 (**4a/4b**)
3	CH_2OH (**5**)	70	28:72 (**5a/5b**)
4	CH_2OAc (**6**)	70	45:55 (**6a/6b**)[c]
5	CH_2OCbz (**7**)	65	49:51 (**7a/7b**)
6	$CH_2OTBDPS$ (**8**)	48	67:33 (**8a/8b**)
7	CH_3 (**9**)	75	98:2 (**9a/9b**)
8	$CCCH_2OH$ (**10**)	40	>99:1 (**10a/10b**)

[a] All reactions were carried out on 0.5-mmol scale with aldehyde/bromide/LiI/indium = 1:1:1:0.55 under ultrasonic conditions. [b] Isolated combined yield. [c] Partial acetyl migration in allenylic product **6a** was observed (1-OAc/3-OAc = 56:44).

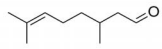
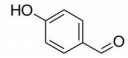
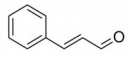
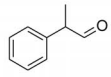
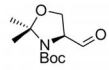
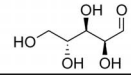
tions of glycolaldehyde and chloroacetaldehyde were used and again satisfactory yields were obtained (Table 3, Entries 8 and 9). With unprotected D-glyceraldehyde, the *syn* allenylation product was obtained in high diastereoselectivity (Table 3, Entry 10).[27] Unprotected D-arabinose produced an inseparable mixture of corresponding allene **21a** and alkyne **21b** in low yield (Table 3, Entry 11).[28] The change in regioselectivity of Garner's aldehyde and D-glyceraldehyde (Table 3, Entries 6 and 10) in comparison to the other aldehydes used is remarkable, although we do not have a satisfactory explanation of this phenomenon at the moment.

Subsequently, as proof of concept for the present methodology, allene **3a** was converted into corresponding pentulose **25** (Scheme 3). Ozonolysis of the allene functionality of **3a** at –78 °C in dichloromethane gave 2-keto derivative **22** in excellent yield. Deprotection of the benzyl and acetonide protecting groups was achieved by standard procedures to yield D-*erythro*-2-pentulose (**25**).[29]

Table 3. Indium-mediated allenylation of aldehydes with 1-(benzyloxy)-4-bromo-2-butyne (**2**).

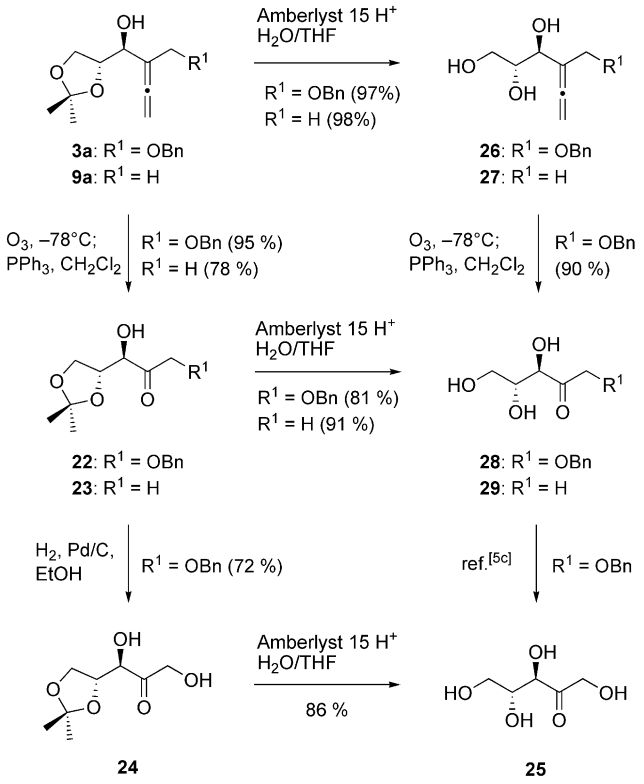


2 **11a–21a** **11b–21b**

Entry[a]	Aldehyde [equiv.]	Indium [equiv.]	Yield [%][b]	Allene/Alkyne[d]
1		0.55	58	78:22 (**11a/11b**)
2		0.55	90	76:24 (**12a/12b**)
3		0.55	60	76:24 (**13a/13b**)
4		0.55	88[c]	78:22 (**14a/14b**)[e]
5		0.55	83	70:30 (**15a/15b**)
6		1	77[c]	35:65 (**16a**:**16b**)
7		0.66	87	74:26 (**17a/17b**)
8		0.55	86	61:39 (**18a/18b**)
9		0.55	58	83:17 (**19a/19b**)[f]
10		1	53	38:62 (**20a/20b**)
11		1	5	50:50 (**21a/21b**)[g]

[a] Except where indicated, all reactions were carried out on 1.0-mmol scale with aldehyde/bromide/LiI = 1:1:1 under ultrasonic conditions. Solvents: THF (Entries 1–6), THF/H_2O = 2:1 (Entries 7–11). [b] Isolated combined yield. [c] Yield is adjusted to account for recovered starting materials. [d] Diastereomeric ratios of allenylic products (*anti*/*syn*): **15a** (1:10), **16a** (40:1), **20a** (1:8), and **21a** (1:20). [e] Cinnamyl aldehyde only gave regiospecific 1,2-addition products. [f] Chloride was substituted by iodide in a ratio of 1:3 in the allenylic products. [g] Instead of LiI, LiCl (2 equiv.) was used.

The versatility of the present methodology was further demonstrated by an efficient synthesis of 1-deoxy-D-ribulose (**29**).[30,31] In a three-step synthesis, **29** was obtained in an overall yield of 51% starting from **1** and by using 1-bromo-2-butyne as an acetyl anion equivalent.[32] In comparison to the literature,[33] when adding LiI the applied quantities of 1-bromo-2-butyne and indium were decreased by a factor of two and four, respectively. Because **3a** is a fully orthogonally protected precursor, the sequence of deprotection steps can be easily altered, giving rise to various derivatives of pentulose (**24**, **26**, and **28**).



Scheme 3. Synthesis of D-*erythro*-2-pentulose (**25**) and 1-deoxy-D-*erythro*-2-pentulose (**29**).

Conclusions

Hydroxy-protected 4-bromo-2-butyn-1-ols (i.e., **2**, **4**, **6**–**8**) are versatile synthetic equivalents for α-hydroxyacetyl anions. This fact is demonstrated by their application in a short and efficient indium-mediated synthesis of D-*erythro*-2-pentulose (**25**) in 32% overall yield. The regioselectivity of the indium-promoted C–C bond-forming reaction can be influenced by the protecting group on 4-bromo-2-butyn-1-ol (for **2**, **4**–**8**). Due to chelation control, the addition of unprotected 4-bromo-2-butyn-1-ol (**5**) to aldehydes yields mainly alkyne-type products, whereas the addition of 1-(benzyloxy)-4-bromo-2-butyne (**2**) leads to allene-type derivatives preferentially. Compared to established synthetic protocols, the amount of indium was decreased by a factor of two to four. Furthermore, the reaction presented tolerates aqueous reaction conditions, thus significantly broadening the scope of this method. The applicability of this concept is further demonstrated by a three-step synthesis of 1-deoxy-D-*erythro*-2-pentulose (**29**).

Experimental Section

General Considerations: All allenylations were carried out in oven-dried Erlenmeyer flasks under an argon atmosphere. Ultrasonication was performed in a conventional ultrasound cleaning bath at room temperature. Indium (powder, 100 mesh) was purchased by Sigma–Aldrich. Anhydrous THF was distilled from potassium under an atmosphere of argon. ^{1}H and ^{13}C NMR spectra were recorded with either a Bruker Avance DRX 400 MHz or DRX 600 MHz spectrometer. Unless otherwise stated, all NMR spectra were measured either in $CDCl_3$ solutions and referenced to the residual $CHCl_3$ signal (^{1}H, δ = 7.26 ppm; ^{13}C, δ = 77.16 ppm) or in D_2O solutions (HDO, ^{1}H, δ = 4.79 ppm). High-resolution mass spectrometry (HRMS) was performed with a Finnigan MAT 900 with resolution of 10000. Optical rotations were measured with a P341 Perkin–Elmer polarimeter. Flash chromatography was performed by using Merck silica gel 60 (0.004–0.063 mm). TLC monitoring was done on Merck plates (silica gel 60 F_{254}), and compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (48 g) and $Ce(SO_4)_2$ (2 g) in 10% H_2SO_4 (1 L), followed by heating, or with an aqueous $KMnO_4$ solution. 2,3-*O*-Isopropylidene-D-glyceraldehyde and *O*-protected 4-bromobut-2-yn-1-ol were prepared by following literature procedures.[8,25] For the preparation of the formalin solution, paraformaldehyde (1.08 g, 36 mmol) was heated at reflux in water (20 mL) containing concentrated H_3PO_4 (200 μL) for 1.5 h until a clear solution was obtained.

Standard Conditions for Allenylation (Method A): Synthesis of Compounds 3a–21a and 3b–21b

Representative Procedure of the Reaction of Aldehydes with Indium and Substituted Propargyl Bromides: To a suspension of powdered indium (32 mg, 0.275 mmol) and LiI (67 mg, 0.5 mmol) in dry THF (4 mL) was added 1-(benzyloxy)-4-bromo-2-butyne (**2**; 120 mg, 0.5 mmol). After the mixture was sonicated for 20 min under an argon -atmosphere, 2,3-*O*-isopropylidene-D-glyceraldehyde (**1**; 65 mg, 0.5 mmol) was added. The reaction mixture was further sonicated until TLC monitoring (hexane/ethyl acetate = 3:2) showed complete consumption of the starting material. The reaction was quenched by the addition of a saturated solution of $NaHCO_3$ (5 mL). The aqueous phase was separated and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with thiosulfate solution (2%) and brine, dried with $MgSO_4$, filtered, and concentrated. The regioisomers were separated by silica gel column chromatography (hexane/ethyl acetate = 5:1) to yield **3a** (78 mg, 54%) and **3b** (22 mg, 15%). The yield of the reaction was not decreased by scaling up to 2 mmol.

1-*O*-Benzyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (3a): $[\alpha]_D^{20}$ = +20.2 (*c* = 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.27 (m, 5 H, Ar-H), 4.97–4.93 (m, 2 H, CH_2), 4.56 (d, ^2J = 11.9 Hz, 1 H, CH_2), 4.53 (d, ^2J = 11.9 Hz, 1 H, CH_2), 4.36–4.30 (m, 1 H, CH), 4.26 (dt, ^2J = 11.3 Hz, ^5J = 2.2 Hz, 1 H, CH_2), 4.22 (m, 1 H, CH), 4.14 (dt, ^2J = 11.3 Hz, ^5J = 1.8 Hz, 1 H, CH_2), 4.05 (dd, ^2J = 8.4 Hz, ^3J = 6.3 Hz, 1 H, CH_2), 4.01 (dd, ^2J = 8.4 Hz, ^3J = 6.1 Hz, 1 H, CH_2), 2.64 (d, ^3J = 4.4 Hz, 1 H, OH), 1.43 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 207.2 (C), 137.8 (C), 128.6 (2 CH), 128.1 (2 CH), 128.0 (CH), 109.4 (C), 100.4 (C), 77.9 (CH_2), 77.5 (CH), 72.5 (CH_2), 71.2 (CH), 69.8 (CH_2), 65.8 (CH_2), 26.7 (CH_3), 25.3 (CH_3) ppm. IR (film): $\tilde{\nu}$ = 3433, 2931, 1957, 1062, 738, 698 cm^{-1}. HRMS (ESI): calcd. for $C_{17}H_{22}O_4Na$ $[M + Na]^+$ 313.1416; found 313.1411.

(2*R*)-7-(Benzyloxy)-1,2-*O*-isopropylidenehept-5-yne-1,2,3-triol (3b): Mixture of two diastereomers: major/minor = 2.8:1.0. ^{1}H NMR (400 MHz, $CDCl_3$, major isomer): δ = 7.37–7.27 (m, 5 H, Ar-H), 4.59 (s, 2 H, CH_2), 4.19–4.15 (m, 2 H, CH_2), 4.11–4.04 (m, 2 H, CH_2), 4.01–3.94 (m, 1 H, CH), 3.80–3.74 (m, 1 H, CH), 2.63–2.48 (m, 2 H, CH_2), 1.42 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (400 MHz, $CDCl_3$, major isomer): δ = 137.6 (C), 128.6 (2 CH), 128.2 (2 CH), 128.0 (CH), 109.5 (C), 82.3 (C), 79.2 (C), 77.5 (CH), 71.9 (CH_2), 70.4 (CH), 66.0 (CH_2), 57.8 (CH_2), 26.8 (CH_3), 25.3 (CH_3), 24.1 (CH_2) ppm. IR (film): $\tilde{\nu}$ = 3377, 2825, 2240, 1062, 747. 699 cm^{-1}. HRMS (ESI): calcd. for $C_{17}H_{22}O_4Na$ $[M + Na]^+$ 313.1416; found 313.1406.

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Typical Procedure for Ozonolysis (Method B): Synthesis of Compounds 22, 23, and 28

1-*O*-Benzyl-4,5-*O*-isopropylidene-D-*erythro*-2-pentulose (22): Ozone was bubbled through a solution of compound **3a** (145 mg, 0.5 mmol) in CH_2Cl_2 (25 mL) at –78 °C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of PPh_3 (157 mg, 0.6 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure, and the crude material was purified by flash chromatography (hexane/ethyl acetate = 4:1) to yield compound **22** (133 mg; 95%). $[\alpha]_D^{20}$ = –57.3 (*c* = 0.3, CH_2Cl_2).^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.26 (m, 5 H, Ar-H), 4.62 (s, 2 H, CH_2), 4.57 (d, ^2J = 18.2 Hz, 1 H, CH_2), 4.29 (d, ^2J = 17.9 Hz, 1 H, CH_2), 4.17 (dd, ^2J = 6.7 Hz, ^3J = 6.4 Hz, 1 H, CH_2), 4.12–3.93 (m, 3 H, 2 CH, CH_2), 3.29 (d, ^3J = 5.8 Hz, 1 H, OH), 1.42 (s, 3 H, CH_3), 1.33 (s, 3 H, CH_3) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.0 (C), 137.1 (C), 128.7 (2 CH), 128.3 (CH), 128.2 (2 CH), 110.4 (C), 76.2 (CH), 75.3 (CH), 73.7 (CH_2), 73.6 (CH_2), 67.1 (CH_2), 26.7 (CH_3), 25.2 (CH_3) ppm. IR (film): $\tilde{\nu}$ = 3386, 2934, 1729, 1067, 739, 698 cm^{-1}. HRMS (ESI): calcd. for $C_{15}H_{20}O_5Na$ $[M + Na]^+$ 303.1208; found 303.1206.

1-Deoxy-4,5-*O*-isopropylidene-D-*erythro*-2-pentulose (23): Method B was applied to compound **9a** (92 mg, 0.5 mmol) for ozonolysis to yield product **23** (68 mg; 78%). $[\alpha]_D^{20}$ = –90.6 (*c* = 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.14–4.01 (m, 3 H, CH CH_2), 3.95–3.87 (m, 1 H, CH), 3.47 (d, ^3J = 5.4 Hz, 1 H, OH), 2.3 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 209.1 (C), 110.4 (C), 77.6 (CH), 76.4 (CH), 67.5 (CH_2), 27.7 (CH_3), 26.8 (CH_3), 25.4 (CH_3) ppm. IR (film): $\tilde{\nu}$ = 3424, 2989, 1716, 1069 cm^{-1}. HRMS (ESI): calcd. for $C_8H_{14}O_4Na$ $[M + Na]^+$ 197.0790; found 197.0794.

4,5-*O*-Isopropylidene-D-*erythro*-2-pentulose (24): Pentulose derivative **22** (21 mg, 0.075 mmol) was dissolved in ethanol (2 mL) and hydrogenated in the presence of 10% palladium-on-charcoal (1 mg) with a balloon of hydrogen. The catalyst was removed by filtration, and the solvent was removed under reduced pressure to yield compound **24** (10 mg, 72%). $[\alpha]_D^{20}$ = –71.5 (*c* = 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.68 (dd, ^2J = 20.1 Hz, ^3J = 5.0 Hz, 1 H, CH_2), 4.40 (dd, ^2J = 20.0 Hz, ^3J = 3.5 Hz, 1 H, CH_2), 4.24 (dd, ^3J = 7.7 Hz, ^3J = 5.2 Hz, 1 H, CH), 4.14 (dd, ^2J = 8.9 Hz, ^3J = 6.3 Hz, 1 H, CH_2), 4.07 (dd, ^2J = 8.9 Hz, ^3J = 5.0 Hz, 1 H, CH_2), 3.99 (ddd, ^3J = 7.8 Hz, ^3J = 6.3 Hz, ^3J = 5.1 Hz, 1 H, CH), 3.24 (d, ^3J = 5.4 Hz, 1 H, OH), 3.05 (t, ^3J = 4.9 Hz, 1 H, OH), 1.49 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ = 210.7 (C), 110.6 (C), 76.0 (CH), 75.4 (CH), 67.6 (CH_2), 67.3 (CH_2), 26.7 (CH_3), 25.1 (CH_3) ppm. IR (film): $\tilde{\nu}$ = 3411, 2925, 1726, 1066 cm^{-1}. HRMS (EI): calcd. for $C_7H_{11}O_6$ $[M - CH_3]^+$ 175.0606; found 175.0610.

Typical Procedure for Isopropylidine Cleavage of Ketoses (Method C): Synthesis of Compounds 25, 28, and 29

D-*erythro*-2-Pentulose (25): To a suspension of 4,5-*O*-isopropylidene-D-*erythro*-2-pentulose (**24**; 9 mg, 0.047 mmol) in water/THF (1:1, 0.8 mL) was added Amberlyst 15 (H^+ form, 6 mg), and the mixture was stirred at room temperature. After TLC monitoring showed conversion of the starting material, the pH of the solution was adjusted to 7 by addition of 0.05 N NaOH. The resin was removed by filtration and washed with water, and the solvent was lyophilized. The crude product was purified by flash chromatography over silica gel (ethyl acetate/MeOH = 8:1) to yield **25** (6.1 mg, 86%). Equilibrium of three compounds: α/β/open = 58:24:18. $[\alpha]_D^{20}$ = –14.0 (*c* = 0.25, H_2O). The spectroscopic data were identical with those reported.[16]

Typical Procedure for Isopropylidine Cleavage of Allenes (Method D): Synthesis of Compounds 26 and 27

1-*O*-Benzyl-2-deoxy-2-*C*-ethenylidene-D-*erythro*-pentitol (26): To a suspension of compound **3a** (34 mg, 0.117 mmol) in water/THF (1:1, 2 mL) was added Amberlyst 15 (H^+ form, 40 mg), and the mixture was stirred at room temperature. After TLC monitoring showed conversion of the starting material, the resin was removed by filtration and washed with methanol, and the solvent was removed under reduced pressure to yield compound **26** (28 mg; 97%). $[\alpha]_D^{20}$ = +14.8 (*c* = 0.25, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 7.58–7.36 (m, 5 H, Ar-H), 5.12 (s, 2 H, CH_2), 4.64 (s, 2 H, CH_2), 4.27–4.13 (m, 3 H, CH, CH_2), 3.89–3.77 (m, 2 H, CH_2), 3.73–3.61 (m, 1 H, CH_2) ppm. ^{13}C NMR (400 MHz, D_2O): δ = 207.0 (C), 137.1 (C), 128.8 (2 CH), 128.7 (2 CH), 128.4 (CH), 100.4 (C), 78.7 (CH_2), 73.2 (CH), 71.8 (CH_2), 70.5 (CH), 67.9 (CH_2), 62.5 (CH_2) ppm. IR (film): $\tilde{\nu}$ = 3377, 2926, 1956, 1055, 745, 698 cm^{-1}. HRMS (ESI): calcd. for $C_{14}H_{18}O_4Na$ $[M + Na]^+$ 273.1103; found 273.1099.

(2*R*,3*S*)-4-Methylhexa-4,5-dien-1,2,3-triol (27): Method D was applied to compound **9a** (46 mg, 0.25 mmol) for deprotection to yield product **27** (35 mg, 98%). $[\alpha]_D^{20}$ = +25.9 (*c* = 0.35, MeOH). ^{1}H NMR (400 MHz, D_2O): δ = 4.88–4.80 (m, 2 H, CH_2), 4.08 (dt, ^3J = 7.5 Hz, ^5J = 1.4 Hz, 1 H, CH), 3.85 (dd, ^2J = 11.7 Hz, ^3J = 2.78 Hz, 1 H, CH_2), 3.81–3.74 (m, 1 H, CH), 3.66 (dd, ^2J = 11.7 Hz, ^3J = 6.6 Hz, 1 H, CH_2), 1.75 (t, ^5J = 3.22 Hz, 3 H, CH_3) ppm. ^{13}C NMR (600 MHz, D_2O): δ = 206.9 (C), 98.5 (C), 76.0 (CH_2), 73.4 (CH), 72.7 (CH), 63.0 (CH_2), 14.0 (CH_3) ppm. IR (film): $\tilde{\nu}$ = 3317, 2926, 1961, 1035 cm^{-1}. HRMS (ESI): calcd. for $C_7H_{12}O_3Na$ $[M + Na]^+$ 167.0684; found 167.0680.

1-*O*-Benzyl-D-*erythro*-2-pentulose (28): Method C was applied to compound **22** (39 mg, 0.139 mmol) for deprotection to yield product **28** (27 mg; 81%), or compound **26** (25 mg, 0.1 mmol) was ozonized in a mixture of MeOH/CH_2Cl_2 (9:1) according to method B to yield product **28** (21.6 mg; 90%). Equilibrium of three compounds: α/β/open = 62:25:13. $[\alpha]_D^{20}$ = –20.7 (*c* = 2.25, MeOH). ^{1}H NMR (600 MHz, CD_3COCD_3+D_2O, α-isomer): δ = 7.39–7.23 (m, 5 H, Ar-H), 4.65–4.46 (m, 2 H, CH_2), 4.22–4.17 (m, 1 H, CH), 4.06 (d, ^3J = 5.3 Hz, 1 H, CH), 3.92 (dd, ^2J = 9.4 Hz, ^3J = 5.3 Hz, 1 H, CH_2), 3.78 (dd, ^2J = 9.3 Hz, ^3J = 3.6 Hz, 1 H, CH_2), 3.52 (d, ^2J = 10.6 Hz, 1 H, CH_2), 3.44 (d, ^2J = 10.2 Hz, 1 H, CH_2) ppm. ^{1}H NMR (600 MHz, CD_3COCD_3+D_2O, β-isomer): δ = 7.39–7.23 (m, 5 H, Ar-H), 4.65–4.46 (m, 2 H, CH_2), 4.45–4.38 (m, 1 H, CH), 4.04 (dd, ^2J = 8.9 Hz, ^3J = 6.3 Hz, 1 H, CH_2), 3.97 (d, ^3J = 5.3 Hz, 1 H, CH), 3.71 (d, ^2J = 10.2 Hz, 1 H, CH_2), 3.64 (dd, ^2J = 8.9 Hz, ^3J = 4.7 Hz, 1 H, CH_2), 3.58 (d, ^2J = 10.2 Hz, 1 H, CH_2) ppm. ^{1}H NMR (600 MHz, CD_3COCD_3+D_2O, open isomer): δ = 7.71–7.55 (m, 2 H, Ar-H), 7.64–7.60 (m, 1 H, Ar-H), 7.57–7.51 (m, 2 H, Ar-H), 4.65–4.46 (m, 4 H, 2 CH_2), 4.23 (d, ^3J = 5.7 Hz, 1 H, CH), 3.89–3.84 (m, 1 H, CH), 3.66 (dd, ^2J = 11.0 Hz, ^3J = 5.3 Hz, 1 H, CH_2), 3.59 (dd, ^2J = 11.2 Hz, ^3J = 5.9 Hz, 1 H, CH_2) ppm. ^{13}C NMR (600 MHz, CD_3COCD_3+D_2O, α-isomer): δ = 139.3 (C), 129.0 (2 CH), 128.2 (2 CH), 128.1 (CH), 103.3 (C), 73.8 (CH_2), 72.2 (CH_2), 71.9 (CH_2), 71.8 (CH), 71.2 (CH) ppm. ^{13}C NMR (600 MHz, CD_3COCD_3+D_2O, β-isomer): δ = 139.2 (C), 128.5 (CH), 128.4 (2 CH), 128.1 (2 CH), 106.1 (C), 76.9 (CH), 74.0 (CH_2), 72.3 (CH_2), 71.9 (CH_2), 71.6 (CH) ppm. ^{13}C NMR (600 MHz, CD_3COCD_3+D_2O, open isomer): δ = 209.7 (C), 139.5 (C), 132.9 (2 CH), 132.6 (2 CH), 132.5 (CH), 77.0 (CH), 74.4 (CH_2), 74.2 (CH), 73.3 (CH_2), 62.9 (CH_2) ppm. HRMS (ESI): calcd. for $C_{12}H_{16}O_5Na$ $[M + Na]^+$ 263.0895; found 263.0893.

1-Deoxy-D-*erythro*-2-pentulose (29): Method C was applied to compound **23** (10 mg, 0.057 mmol) for deprotection to yield product **29** (7 mg; 91%). Equilibrium of three compounds: α/β/open =

14:14:72. $[a]_D^{20}$ = –30 (c = 0.3, H_2O). The spectroscopic data were identical with those reported.[30,31] HRMS (ESI): calcd. for $C_5H_9O_4$ $[M – H]^-$ 133.0501; found 133.0504. HRMS (EI): calcd. for $C_5H_8O_3$ $[M – H_2O]^+$ 116.0473; found 116.0476.

Supporting Information (see footnote on the first page of this article): Experimental and spectroscopic details for compounds **6**–**8**, **10**, **4a**–**21a**, **4b**–**21b**, **30a**, **30b**, **31a**, and **31b** and ^1H and ^{13}C NMR spectra of compounds **6**–**8**, **10**, **22**–**29**, **3a**, **3b**, **4a**–**21a**, **21b**, **30a**, **30b**, **31a**, and **31b**.

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[20] Allenylation of benzaldehyde with 1-(benzyloxy)-4-iodobut-2-yne gave the corresponding allene and alkyne in a combined yield of 76% compared to 90% with 1-(benzyloxy)-4-bromo-2-butyne (**2**). In the case of 1-(benzyloxy)-4-iodo-2-butyne, the self-coupling product could be isolated. Furthermore 1-(benzyloxy)-4-iodo-2-butyne decomposed even at –20 °C after several days. These factors lead to a decrease in yield when using iodo derivatives as a starting material.

[21] After allenylations, milligram quantities of the corresponding alkyne iodides were isolated, in accordance with ref.[12b]

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[28] For the isolated side products see the Supporting Information: 5-[(benzyloxy)methyl]hepta-5,6-dien-1,4-diol (**30a**); 8-(benzyloxy)oct-6-yne-1,4-diol (**30b**); 1-*O*-benzyl-2,3-butadien-1-ol (**31a**); 1-*O*-benzylbut-2-yn-1-ol (**31b**).

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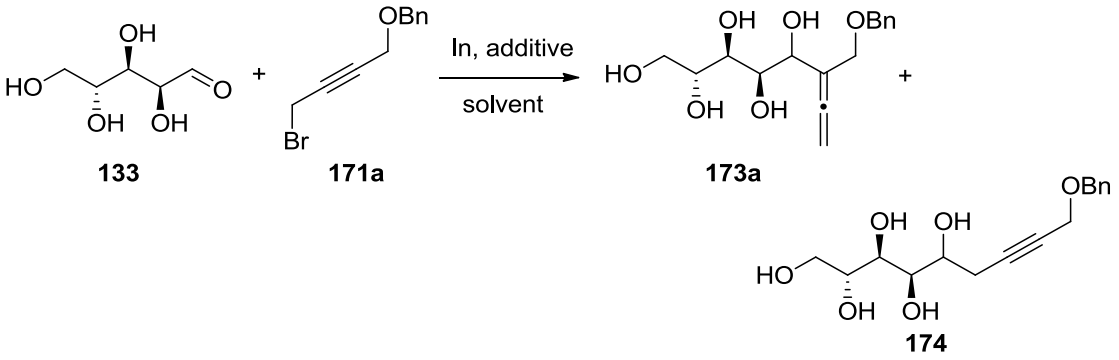
Unpublished Work:

Indium-Mediated Allenylation/Propargylation of Arabinose (122) with 1-(Benzyloxy)-4-bromo-2-butyne (171a):

The indium-mediated allenylation of arabinose was attempted by various procedures as depicted in table 2. Arabinose was the carbohydrate of choice since it is known to be a very reactive aldose for indium-mediated allylation reactions (Scheme 59). The first eight procedures were performed before the crucial addition of either LiI or LiCl for these type of reactions was recognized. It was supposed that solvent systems, which are suitable for indium-mediated allylation reactions of aldoses, are applicable for indium-mediated allenylations as well (entries 1-7). An acceleration of the reaction by diluted hydrochloric acid (entries 1, 6 and 12) or by a saturated ammonium chloride solution (entry 5) was not successful. The addition of tetrabutylammonium iodide, a phase transfer catalyst and a reaction promoter (iodide), did not yield any product either (entry 8). The reaction conditions, which have been effective for various aldehydes, were unproductive for unprotected arabinose (entries 7-12). Only by addition of lithium chloride, traces of product could be observed on TLC plates (entries 13-15). These compounds were isolated in the case of entry 15 and identified as the desired products **173a** and **174.** Obviously arabinose (**133**) was not reactive enough for indium-mediated allenylations with 1-(benzyloxy)-4-bromo-2-butyne (**171a**). The best result (5% yield) was obtained by using two equivalents of arabinose (**133**) and LiCl and one equivalent of indium and 1-(benzyloxy)-4-bromo-2-butyne (**171a**) in a THF/water mixture (1/1).



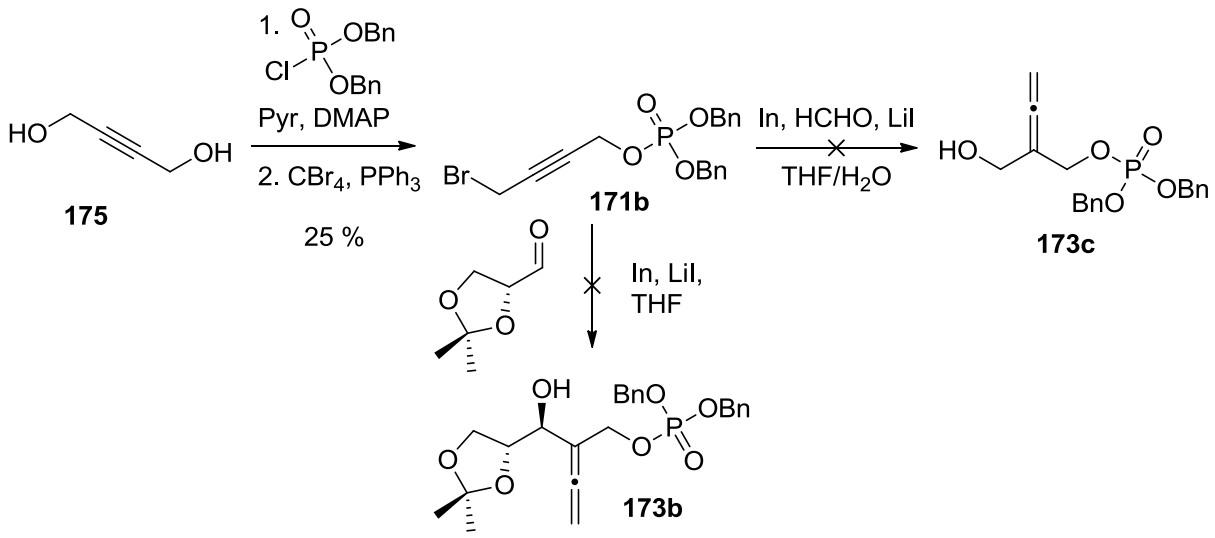
Scheme 59. Indium-mediated allenylation/propargylation of arabinose with 1-(benzyloxy)-4-bromo-2-butyne

Table 2. Indium-mediated allenylation/propargylation of arabinose with 1-(benzyloxy)-4-bromo-2-butyne – applied conditions

entry	arabinose [equiv.]	indium [equiv.]	1-(benzyloxy)-4-bromo-2-butyne [equiv.]	additive [equiv.]	solvent	yield (allene / alkyne)
1	1	2	2		0.1 N HCl/EtOH = 1/9	0
2	1	2	2		EtOH/H_2O = 4/1	0
3	1	2	2		THF/H_2O	0
4	1	2	2		THF/H_2O/DMF	0
5	1	2	2		THF/NH_4Cl_{sat} = 1/5	0
6	1	2	3		0.1 N HCl/EtOH = 1/10	0
7	1	2	2	Bu_4NI	THF/H_2O = 1/10	0
8	1	2	3	LiI [1]	DMF_{abs}	0
9	1	1	1	LiI [1]	THF_{abs}	0
10	1	1	1	LiI [1]	THF/H_2O	0
11	1	1	1	LiCl [2]/ LiI [1]	THF/H_2O	0
12	1.2	1	1	LiCl [2]	THF/0.1 N HCl	0
13	1.2	1	1	LiCl [2]	THF/H_2O = 1/4	<5 % (nd.)
14	2	1	1	LiCl [2]	THF/H_2O = 1/1	<5 % (nd.)
15	2	1	1	LiCl [2]	THF/H_2O = 2/1	5 % (1/1)

Indium-Mediated Allenylation/Propargylation of Aldehydes with Dibenzyl (4-bromobut-2-yn-1-yl) Phosphate (171b):

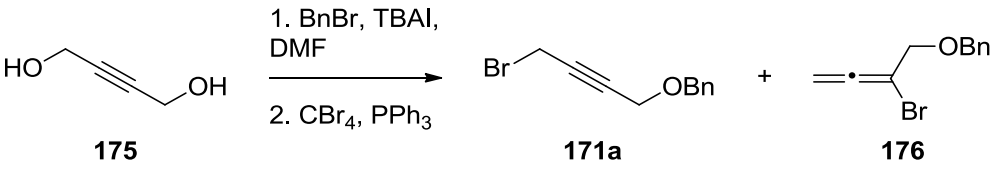
Starting from 2-butyn-1,4-diol, dibenzyl (4-bromobut-2-yn-1-yl) phosphate (**171b**) was synthesized in two steps (Scheme 60). The dibenzyl phosphate was prepared by phosphorylation of one hydroxyl group with dibenzyl phosphorylchloride, followed by displacement of the other hydroxyl group by bromine via an Appel reaction. The indium-mediated allenylation of 1,2-*O*-isopropylidene-D-glyceraldehyde or freshly prepared formaldehyde with alkyne **171b** were unsuccessful and did not yield the product **173b** and **173c**, a potential precursor for DHAP (**1**).



Scheme 60. Attempted indium-mediated allenylation of aldehydes with dibenzyl (4-bromobut-2-yn-1-yl) phosphate (171b)

Reactivity of Alkynyl Bromide 171a vs. Allenyl Bromide 176:

During the preparation of alkyne **171a**, a substantial amount (1.89 g, 16%) of allenyl bromide **176** was isolated (Scheme 61). Allene **176** was only formed, when the starting material for the Appel reaction, 4-(benzyloxy)-but-2-yn-1-ol was not purified by vacuum distillation.



Scheme 61. Synthesis of allenylbromide 176

Subsequently, the reactivity of the alkynylbromide **171a** and the allenylbromide **176** was compared via the indium-mediated allenylation/propargylation of benzaldehyde. Alkynylbromide reacted substantially faster than allenylbromide **176** (Figure 11). In addition, it was demonstrated that the preformation of the reactive indium species (Grignard conditions) was superior to Barbier additions (allene **176** was unreactive applying Barbier conditions).

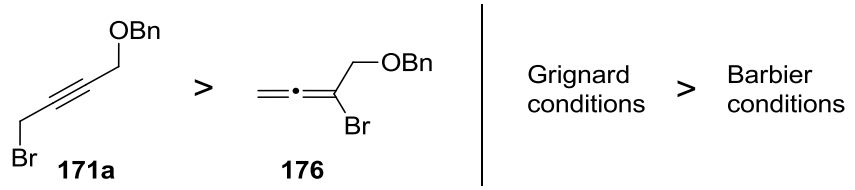


Figure 11. Reactivity of alkyne 171a and allene 176 and comparison of Grignard with Barbier conditions

Proof of Stereochemistry:

An initial major problem of indium-mediated allenylation reactions with 1,2-*O*-isopropylidene-D-glyceraldehyde was the assignment of the stereochemistry of the novel formed stereocenter at C3 of allene **173d**. All primarily attempted conversions of allene **173d** into known pentuloses **177** were unsuccessful (which was later done as published). Even the literature known benzyl cleavage did not yield any assignable structure.[71c] Therefore, we attempted to determine the stereochemistry via some further derivatized structures of compound **173d** either by NOESY experiments or X-ray diffraction (Figure 12).

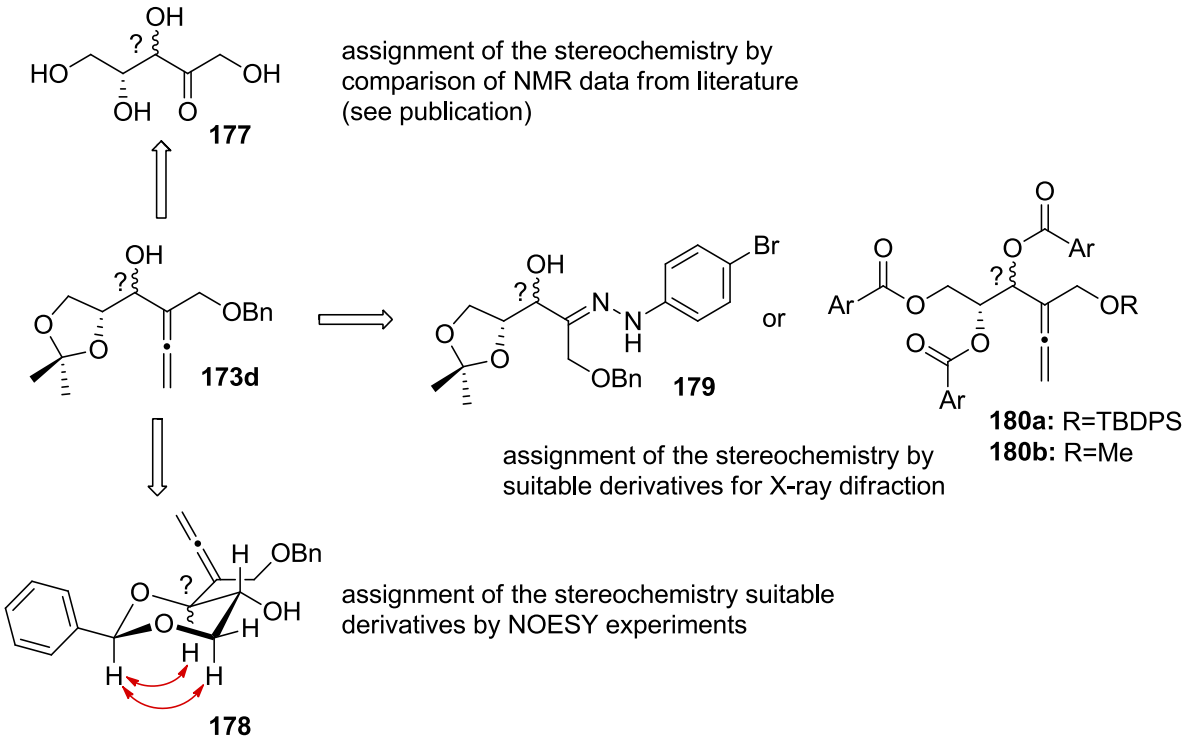
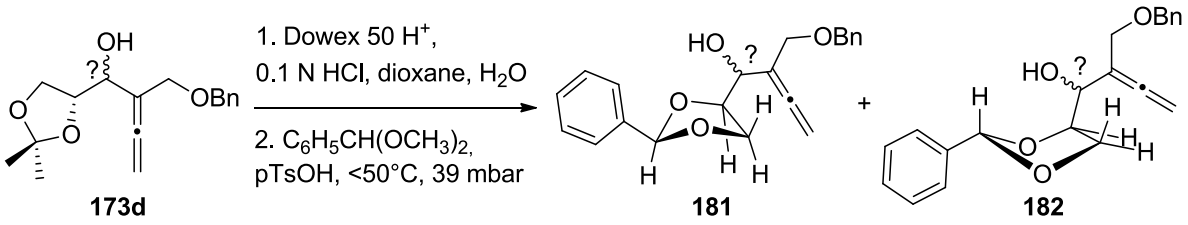


Figure 12. Possible strategies to assign the novel stereocenter at C3 of allene 173d

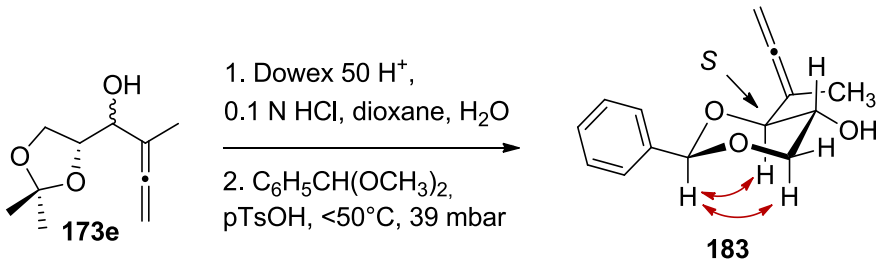
The acetonide protecting group of allene **173d** was quantitatively removed under aqueous acidic conditions. Surprisingly, the protection of the 1,2,3-triol with a benzylidene group exclusively gave five membered 1,2-protected triols **181** and **182** and no 1,3-protected triol

178 was formed (Scheme 62). Usually, benzylidene protecting groups favor the formation of 1,3-dioxane rings under thermodynamic control.[89] A stereochemical determination at C3 was not possible by NOE experiments for the dioxolane rings **181** and **182**.

This strategy was efficient for compound **173e**, which was synthesized by the indium-mediated allenylation of glyceraldehyde **65** with 1-bromo-2-butyne (Scheme 63). By assignment of the NOESY crosspeaks, the stereocenter at C3 was determined to be *S*-configurated. The same result was later obtained by conversion of **173d** into D-*erythro*-pent-2-ulose (see publication).

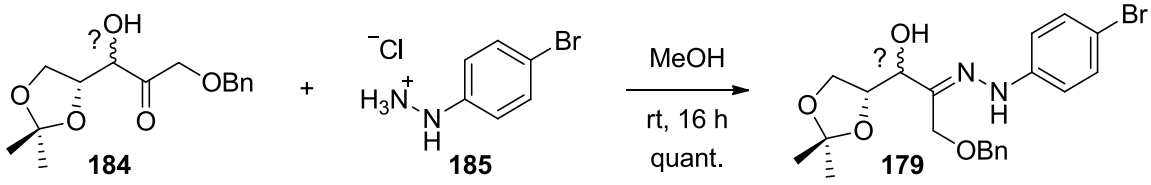


Scheme 62. Formation of dioxolane 181 and 182

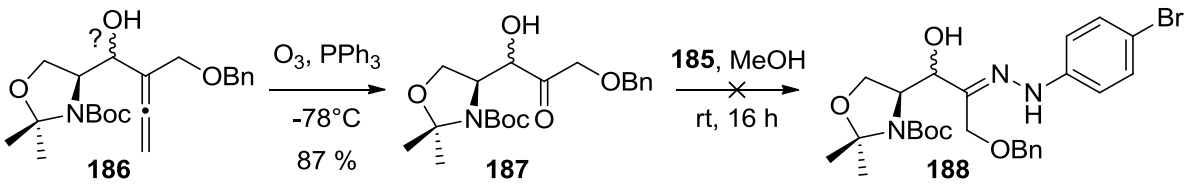


Scheme 63. Determination of the stereochemistry of C3 of compound 183 by NOESY crosspeaks.

All derivatized compounds for X-ray diffraction did not yield any suitable crystals for this methodology. Phenyl hydrazones, well known for their beneficial properties for crystallization, either did not yield any crystals (Scheme 64) or could not be synthesized at all (Scheme 65).

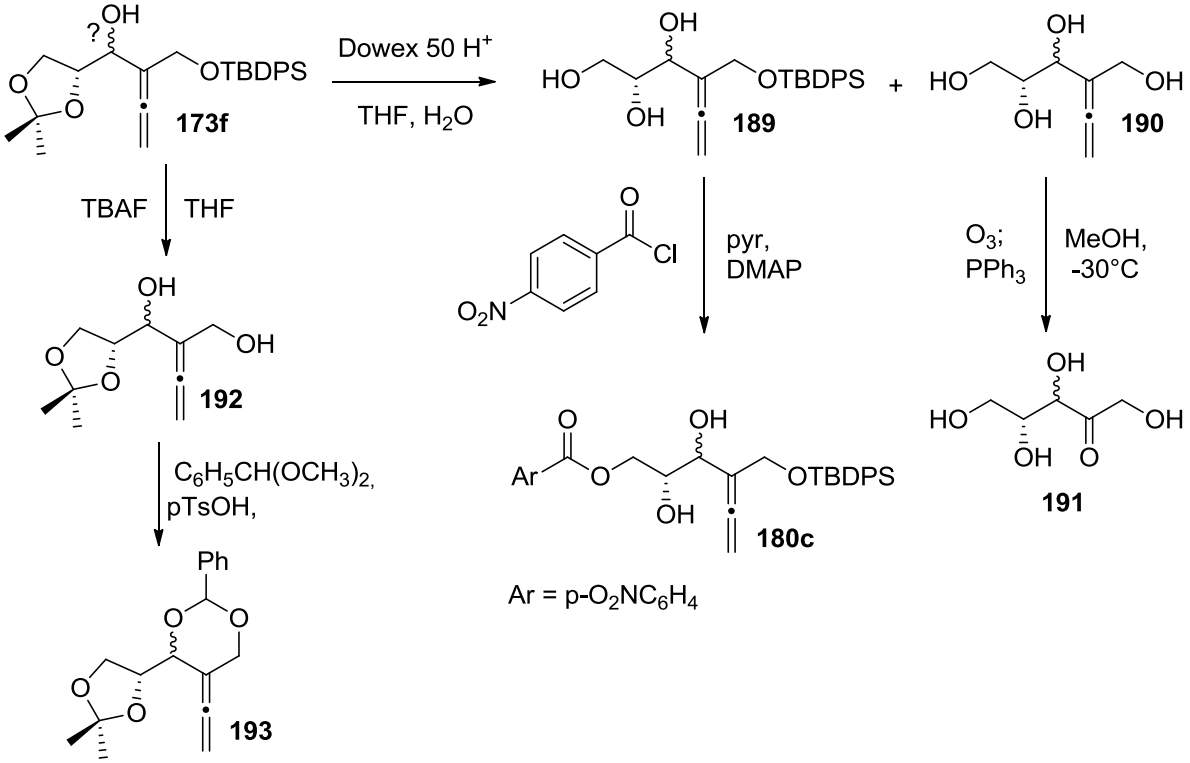


Scheme 64. Hydrazone formation of compound 184



Scheme 65. Attempted hydrazone formation of compound 187.

A *para*-nitrobenzoyl protection of compound **189** or **194** again gave no suitable crystals for X-ray diffraction (Scheme 66 and 67). Only the primary hydroxyl group of allene **189** reacted with *para*-nitrobenzoyl chloride. During the acid catalyzed removal of the acetonide protecting group of **173f**, a partial cleavage of the silyl protecting group was observed. The product **190** was converted into the corresponding pentulose by ozonolysis. A precise determination of the pentulose was not possible, due to impurities. Alternatively, the silyl protecting group of **173f** was selectively removed with TBAF. A benzylidene protection of the free hydroxy groups yielded 1,3-dioxane **193.** Unfortunately, in this case an assignment of the stereochemistry at C3 was not reasonable, because the two six-membered rings **193a** and **193b** could not be distinguished by NOESY experiments (Figure 13).



Scheme 66. *p*-Nitrobenzoyl protection of 189, ozonolysis of 190 and 1,3-dioxan formation of 192.

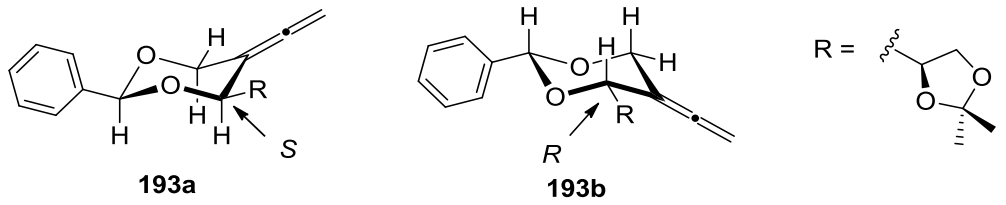
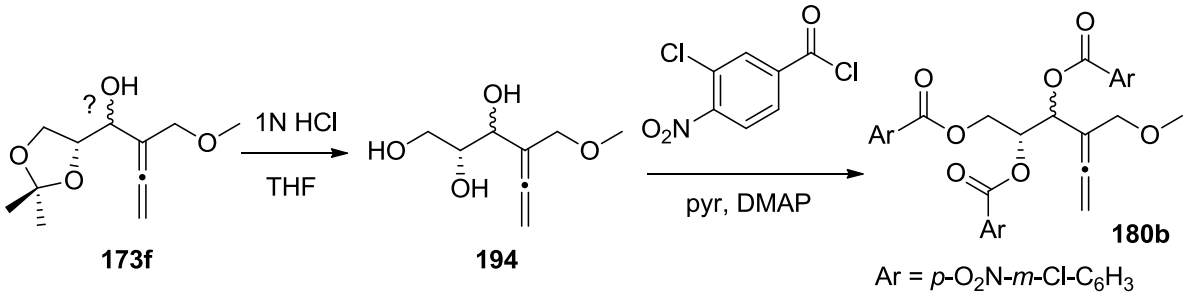


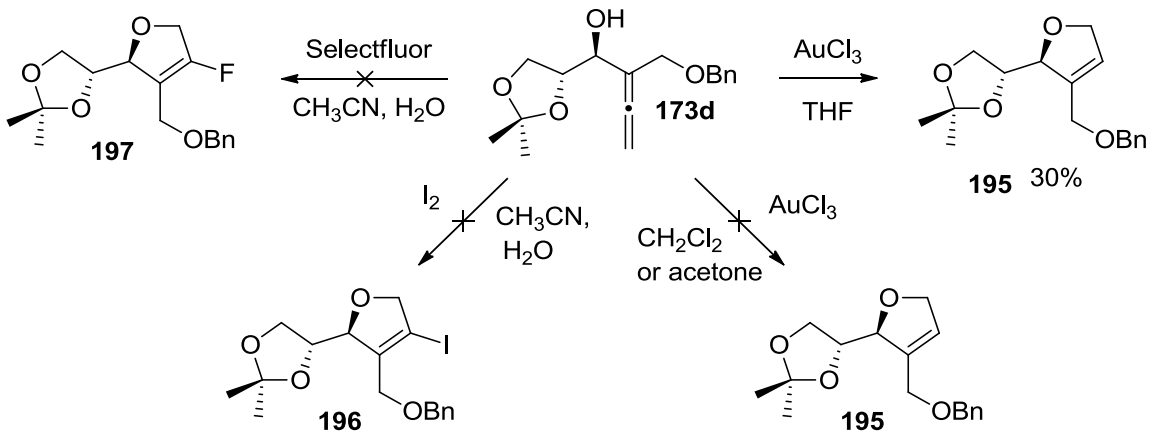
Figure 13. Unfeasible determination of the stereocenter at C3 for compound 193 by NOE experiments.



Scheme 67. 3-Chloro-4-nitrobenzoyl protection of compound 194.

Miscellaneous Experiments:

The free hydroxyl group of allene **173d** is suitable positioned for a 5-*endo-dig* ring closing reaction with the neighboring terminal allene moiety (Scheme 68). Nevertheless, the initial gold catalyzed cyclization failed under standard conditions (dichloromethane as solvent).[84] Using THF as solvent, the desired dihydrofuran **195** was isolated in 30 % yield. Primarily experiments for an iodine- or Selectfluor-catalyzed ring closing reaction for the synthesis of compounds **196** and **197** were unsuccessful. Further studies towards these directions have to be pursued.



Scheme 68. Various conditions for 5-endo-dig cyclizations.

C.4. Experimental Procedures

Published Work:

Indium-mediated Allenylation of Aldehydes and its Application in Carbohydrate Chemistry: Efficient Synthesis of D-Ribulose and 1-Deoxy-D-ribulose

M. Fischer, C. Schmölzer, C. Nowikow, W. Schmid, *Eur. J. Org. Chem*. **2011**, 1645-1651.

Supporting Information:

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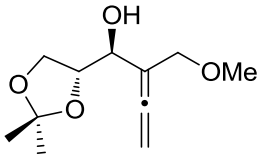
SUPPORTING INFORMATION

DOI: 10.1002/ejoc.201001443
Title: Indium-Mediated Allenylation of Aldehydes and Its Application in Carbohydrate Chemistry: Efficient Synthesis of D-Ribulose and 1-Deoxy-D-ribulose
Author(s): Michael Fischer, Christoph Schmölzer, Christina Nowikow, Walther Schmid*

- **Experimental and spectroscopical data for compounds 6-8, 10; 4a-21a, 4b-21b, 30a, 30b, 31a, 31b.**

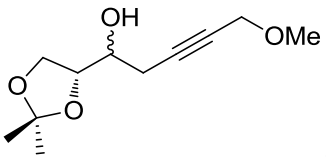
- **^{1}H and ^{13}C NMR spectra of compounds 6-8; 10, 22-29; 3a, 3b; 4a-21a, 21b, 30a, 30b, 31a, 31b.**

2-Deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-1-*O*-methyl-D-*erythro*-pentitol (4a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 1-(methoxy)-4-bromo-2-butyne **4** (82 mg, 0.5 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 4/1) to yield 2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-1-O-methyl-D-*erythro*-pentitol **4a** (51 mg, 48%) and 7-(methoxy)-1,2-O-isopropylidenehept-5-yne-1,2,3-triol **4b** (34 mg, 32 %). **(4a):** $[\alpha]_D^{20}$ = +20.0° (*c* 0.5, EtOAc); ^{1}H NMR (400 MHz; $CDCl_3$): δ=4.95–4.91 (m, 2H; CH_2) 4.28 (dt, ^3J = 5.3 Hz, ^5J = 1.9 Hz, 1H; CH) 4.25-4.18 (m, 1H; CH) 4.14 (dt, ^2J = 11.2 Hz, ^5J = 2.2 Hz, 1H; CH_2) 4.05 (dd, ^2J = 8.5 Hz, ^3J = 6.4 Hz, 1H; CH_2) 4.01 (dt, ^2J = 11.1 Hz, ^5J = 1.9 Hz, 1H; CH_2) 4.00 (dd, ^2J = 8.4 Hz, ^5J = 6.0 Hz, 1H; CH_2) 3.35 (s, 3H; CH_3) 1.43 (s, 3H; CH_3) 1.35 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=207.0 (C), 109.4 (C), 100.3 (C), 77.8 (CH_2), 77.5 (CH), 72.1 (CH_2), 71.2 (CH), 65.7 (CH_2), 58.2 (CH_3), 26.7 (CH_3) 25.3 (CH_3); IR (film) 3411, 2937, 1962, 1067 cm^{-1}; HRMS (EI): *m/z*:$[M\text{-}CH_3]^+$ calcd for $C_{10}H_{15}O_4$: 199.0970, found: 199.0975.



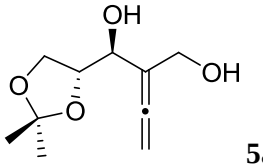
4a

(2*R*)-7-(Methoxy)-1,2-*O*-isopropylidenehept-5-yne-1,2,3-triol (4b): Mixture of diastereomeres: major : minor = 4.0 : 1; ^{1}H-NMR (400 MHz; $CDCl_3$): δ^{major}=4.11-4.05 (m, 4H; 2xCH_2) 4.10-3.93 (m, 1H; CH) 3.81-3.73 (m, 1H; CH) 3.37 (s, 3H; CH_3) 2.64-2.46 (m, 2H; CH_2) 2.15 (d, ^3J = 4.3 Hz, 1H; OH) 1.42 (s, 3H; CH_3) 1.36 (s, 3H; CH_3) ; ^{13}C NMR (400 MHz; $CDCl_3$): δ^{major}=109.6 (C), 81.8 (C), 81.3 (C), 77.5 (CH), 70.5 (CH), 66.1 (CH_2), 57.8 (CH_3), 51.4 (CH_2), 25.3 (CH_3), 24.1 (CH_2); IR (film) 3407, 2935, 1066 cm^{-1}; HRMS (EI): *m/z*:$[M\text{-}CH_3]^+$ calcd for $C_{10}H_{15}O_4$: 199.0970, found: 199.0973.



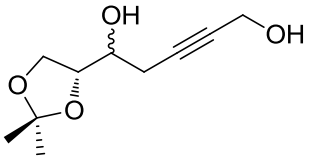
4b

2-Deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (5a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 4-bromo-2-butyn-1-ol **5** (75 mg, 0.5 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 1/1) to yield compounds **5a** (20 mg, 20 %) and **5b** (51 mg, 51 %). **(5a):** $[\alpha]_D^{20}$ = +24.4° (*c* 0.25, CH_2Cl_2); ^{1}H NMR (400 MHz; $CDCl_3$): δ=5.00-4.97 (m, 2H; CH_2) 4.39-4.32 (m, 1H; CH) 4.30-4.24 (m, 2H; CH_2) 4.24–4.18 (m, 1H; CH) 4.09 (dd, ^2J = 8.6 Hz, ^3J = 6.6 Hz, 1H; CH_2) 4.01 (dd, ^2J = 8.4 Hz, ^3J = 6.2 Hz, 1H; CH_2) 2.49 (t, ^3J = 5.4 Hz, 1H; OH) 2.42 (d, ^3J = 4.0 Hz, 1H; OH) 1.45 (s, 3H; CH_3) 1.36 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=205.4 (C), 109.6 (C), 103.9 (C), 79.3 (CH_2), 77.9 (CH), 71.2 (CH), 65.9 (CH_2), 62.6 (CH_2), 26.6 (CH_3), 25.2 (CH_3); IR (film) 3411, 2934, 1962, 1067cm^{-1}; HRMS (EI): *m/z*: $[M\text{-}CH_3]^+$ calcd for $C_9H_{13}O_4$: 185.0814, found: 185.0812.



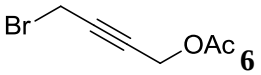
5a

(2*R*)-1,2-*O*-Isopropylidenehept-5-yne-1,2,3,7-tetrol (5b): Mixture of diastereomeres**:** major:minor = 3.5:1; ^{1}H-NMR (400 MHz; $CDCl_3$): δ^{major}=4.65 (t, ^{5}J = 2.0 Hz, 2H; CH_2) 4.10-4.02 (m, 2H; CH_2) 4.01-3.94 (m, 1H; CH) 3.80-3.71 (m, 1H; CH) 2.57 (ddt, ^{2}J = 17.0 Hz, ^{3}J = 4.7 Hz, ^{5}J = 2.3 Hz, 1H; CH_2) 2.49 (ddt, ^{2}J = 17.2 Hz, ^{3}J = 6.5 Hz, ^{5}J = 2.1 Hz, 1H; CH_2) 2.48 (bs, 1H; OH) 2.04 (bs, 1H; OH) 1.41 (s, 3H; CH_3) 1.35 (s, 3H; CH_3) ; ^{13}C NMR (400 MHz; $CDCl_3$) δ^{major}=109.6 (C), 81.8 (C), 81.3 (C) 77.5 (CH), 70.5 (CH), 66.1 (CH_2), 51.4 (CH_2), 26.8 (CH_3), 25.3 (CH_3) 24.1 (CH_2); IR (film) 3369, 2924, 1063 cm^{-1}; HRMS (EI): *m/z*: $[M\text{-}CH_3]^+$ calcd for $C_9H_{13}O_4$: 185.0814, found: 185.0812.



5b

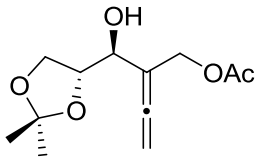
Typical procedure for bromination of mono oxygen substituted 2-butyne-1,4-diol (Method E) – synthesis of compounds 6-8: 1-*O*-Acetyl-4-bromo-but-2-yn-1-ol (6): To a solution of 4-*O*-acetyl-but-2-yne-1,4-diol[1] (1.28 g, 0.01 mol) in anhydrous CH_2Cl_2 (30 mL), CBr_4 (3.32 g, 0.01 mol) was added and cooled to 0°C. In small portions PPh_3 (2.63 g, 0.01 mol) was added over a period of 30 minutes and the reaction mixture was further stirred at room temperature until TLC monitoring showed conversion of the starting material. MeOH (5mL) was added and the solvent were evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 6/1) to yield 1-*O*-acetyl-4-bromo-but-2-yn-1-ol **6** (1.8 g, 95 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=4.72 (t, ^{5}J = 2.0 Hz, 2H; CH_2) 3.92 (t, ^{5}J = 2.0 Hz, 2H; CH_2) 2.09 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=170.2 (C), 81.8 (C), 80.8 (C), 52.3 (CH_2), 20.8 (CH_3), 13.8 (CH_2); HRMS (EI): *m/z*: $[M\text{-}CH_3]^+$ calcd for $C_6H_7BrO_2$: 189.9629, found: 189.9625.



6

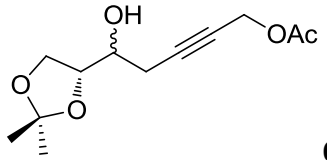
1-*O*-Acetyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (6a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 1-O-acetyl-4-bromo-but-2-yn-1-ol **6** (96 mg, 0.5 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 3/1) to yield compounds **6a** (21 mg, 17 %), **6b** (47 mg, 39 %) and **6c** (17 mg, 14 %). **(6a):** $[\alpha]_D^{20}$ = +38.0° (*c* 0.2, CH_2Cl_2); ^{1}H-NMR (400 MHz; $CDCl_3$): δ=5.00–4.98 (m, 2H; CH_2) 4.73 (dt, ^{2}J = 12.1 Hz, ^{5}J = 2.3 Hz, 1H; CH_2) 4.65 (dt, ^{2}J = 12.3 Hz, ^{5}J = 2.2 Hz, 1H; CH_2) 4.32-4.26 (m, 1H; CH) 4.23-4.16 (m, 1H; CH) 4.04 (dd, ^{2}J = 8.46 Hz, ^{3}J = 6.44

Hz, 1H; CH_2) 3.98 (dd, ^2J = 8.5 Hz, ^3J = 6.2 Hz, 1H; CH_2) 2.40 (d, ^3J = 3.8 Hz, 1H; OH) 2.07 (s, 3H; CH_3) 1.43 (s, 3H; CH_3) 1.35 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.8 (C), 170.9 (C), 109.4 (C), 100.6 (C), 79.5 (CH_2), 77.7 (CH), 70.0 (CH), 65.5 (CH_2), 62.7 (CH_2), 26.6 (CH_3), 25.2 (CH_3), 21.1 (CH_3); IR (film) 3434. 2988, 1956, 1738, 1373, 1260 cm^{-1}; HRMS (EI): *m/z*: $[M\text{-}CH_3]^+$ calcd for $C_{11}H_{15}O_5$: 227.0919, found: 227.0911.



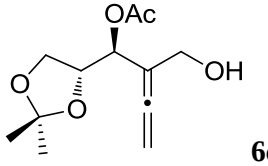
6a

(2*R*)-7-*O*-Acetyl-1,2-*O*-isopropylidene-5-heptyne-1,2,3,7-tetrol (6b): Mixture of diastereomeres: ^{1}H-NMR (400 MHz; $CDCl_3$): δ^{major}=4.65 (t, ^5J = 2.1 Hz, 2H; CH_2) 4.09-4.00 (m, 2H; CH_2) 3.99-3.90 (m, 1H; CH) 3.78-3.70 (m, 1H; CH) 2.56 (ddt, ^2J = 17.0 Hz, ^3J = 4.7 Hz, ^5J = 2.3 Hz, 1H; CH_2) 2.48 (ddt, ^2J = 17.0 Hz, ^3J = 6.7 Hz, ^5J = 2.2 Hz, 1H; CH_2) 2.30 (d, ^3J = 4.7 Hz, 1H; OH) 2.07 (s, 3H; CH_3) 1.39 (s, 3H; CH_3) 1.34 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ^{major}=170.5 (C), 109.5 (C), 83.0 (C), 77.5 (CH), 77.1 (C), 70.3 (CH), 66.0 (CH_2), 52.7 (CH_2), 26.8 (CH_3), 25.3 (CH_3), 24.1 (CH_2), 20.9 (CH_3); IR (film) cm^{-1} 3386. 2927, 1726, 1380, 1230, 1027 cm^{-1}; HRMS (EI): *m/z*: $[M\text{-}CH_3]^+$ calcd for $C_{11}H_{15}O_5$: 227.0919, found: 227.0912.



6b

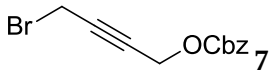
3-*O*-Acetyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (6c): ^{1}H-NMR (400 MHz; $CDCl_3$): δ=5.26 (dt, ^3J = 6.1 Hz, ^5J = 1.8 Hz, 1H; CH), 5.05–4.92 (m, 2H; CH_2) 4.36-4.28 (m, 1H; CH) 4.22-4.12 (m, 2H; CH_2) 4.08 (dd, ^2J = 8.66 Hz, ^3J = 6.50 Hz, 1H; CH_2) 3.89 (dd, ^2J = 8.64 Hz, ^3J = 5.7 Hz, 1H; CH_2) 2.52-2.42 (m, 1H; OH) 2.09 (s, 3H; CH_3) 1.42 (s, 3H; CH_3) 1.35 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.7 (C), 170.6 (C), 110.0 (C), 102.5 (C), 79.5 (CH_2), 76.5 (CH), 72.0 (CH), 66.3 (CH_2), 62.0 (CH_2), 26.6 (CH_3), 25.3 (CH_3), 21.1 (CH_3); HRMS (EI): *m/z*: $[M\text{-}CH_3]^+$ calcd for $C_{11}H_{15}O_5$: 227.0919, found: 227.0913.



6c

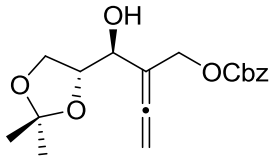
1-*O*-Benzyloxycarbonyl-4-bromo-but-2-yn-1-ol (7): To a solution of 2-butyne-1,4-diol (5 g, 0.058 mol) in anhydrous CH_2Cl_2 (100 mL) and pyridine (14.2 mL), dimethylaminopyridine (DMAP) (0.35 g, 2.9 mmol) was added and the mixture was cooled to 0°C. Benzyl chloroformate (Cbz-Cl) (13.8 g, 0.082 mol) was added slowly under string and after 2 hours the reaction was quenched by addition of a saturated aqueous NH_4Cl solution (100 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 100 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was separated by silica gel

column chromatography to yield 4-*O*-benzyloxycarbonyl-but-2-yne-1,4-diol (7.55 g, 59%). Analogous to method E, to a solution of 4-O-benzyloxycarbonyl-but-2-yne-1,4-diol (7.55 g, 0.034 mol) in anhydrous CH_2Cl_2 (50 mL), CBr_4 (13.65 g, 0.041 mol) and PPh_3 (10.79 g, 0.0419) were added. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 8/1) to yield 1-*O*-benzyloxycarbonyl-4-bromo-2-butyn-1-ol **7** (9.3 g, 95 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.42-7.30 (m, 5H; ArH) 5.19 (s, 2H; CH_2) 4.79 (t, ^5J = 2.0 Hz, 2H; CH_2) 3.92 (t, ^5J = 2.0 Hz, 2H; CH_2); ^{13}C NMR (400 MHz; $CDCl_3$): δ=154.6 (C), 135.0 (C), 128.8 (CH), 128.7 (2xCH), 128.5 (2xCH), 82.6 (C), 80.0 (C), 70.2 (CH_2), 55.7 (CH_2), 13.8 (CH_2); IR (film) 3034, 1751, 1262, 616 cm^{-1}; HRMS (EI): *m/z*: $[M]^+$ calcd for $C_{12}H_{11}O_3Br$: 281.9882, found: 281.9885.



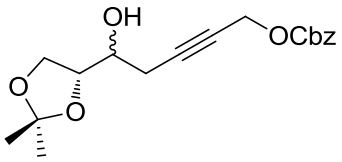
7

1-*O*-Benzyloxycarbonyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (7a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 1-O-benzyloxycarbonyl-4-bromo-but-2-yn-1-ol **7** (142 mg, 0.5 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 5/1) to yield compounds **7a** (53 mg, 32%) and **7b** (55 mg, 33 %). **(7a):** $[\alpha]_D^{20}$ = +9.0° (*c* 0.74, EtOAc); ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.40-7.28 (m, 5H; ArH) 5.16 (s, 2H; CH_2) 5.00-4.97 (m, 2H; CH_2) 4.80 (dt, ^2J = 12.1 Hz, ^5J = 2.2 Hz, 1H; CH_2) 4.72 (dt, ^2J = 11.9 Hz, ^5J = 2.1 Hz, 1H; CH_2) 4.38-4.27 (m, 1H; CH) 4.22-4.15 (m, 1H; CH) 4.03 (dd, ^2J = 8.4 Hz, ^3J = 6.2 Hz, 1H; CH_2) 3.96 (dd, ^2J = 8.4 Hz, ^3J = 6.2 Hz, 1H; CH_2) 2.43 (s, 1H; OH) 1.42 (s, 3H; CH_3), 1.33 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.9 (C), 155.0 (C), 135.2 (C), 128.7 (3xCH), 128.4 (2xCH), 109.4 (C), 100.1 (C), 79.6 (CH_2), 77.7 (CH), 69.9 (CH), 69.8 (CH_2), 66.1 (CH_2), 65.4 (CH_2), 26.6 (CH_3), 25.1 (CH_3); IR (film) 3445, 2986, 1957, 1743, 1240, 753, 696 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{22}O_6Na$: , 357.1314 found: 357.1299.



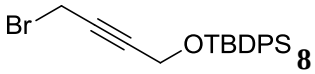
7a

(2*R*)-7-*O*-Benzyloxycarbonyl-1,2-*O*-isopropylidene-5-heptyne-1,2,3,7-tetrol (7b): Mixture of diastereomeres: major: minor = 4.8:1; ^{1}H NMR (400 MHz; $CDCl_3$): δ^{major}=7.38-7.26 (m, 5H; ArH) 4.59 (s, 2H; CH_2) 4.19-4.15 (m, 2H; CH_2) 4.10-4.04 (m, 2H; CH_2) 4.01-3.93 (m, 1H; CH) 3.81-3.73 (m, 1H; CH) 2.63-2.47 (m, 2H; CH_2) 2.15 (d, ^3J = 4.7 Hz, 1H; OH) 1.42 (s, 3H; CH_3), 1.36 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ^{major}=155.3 (C), 137.1 (C), 128.6 (2xCH), 128.2 (2xCH), 128.1 (CH), 109.2 (C), 82.1 (C), 79.1 (C), 77.5 (CH), 71.9 (CH_2), 70.4 (CH), 66.1 (CH_2), 57.8 (CH_2), 26.8 (CH_3), 25.3 (CH_3), 24.1 (CH_2); IR (film) 3317, 2924, 1747, 1241, 756, 697 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{22}O_6Na$: , 357.1314 found: 357.1230.



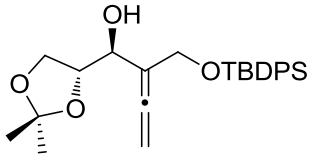
7b

1-*O*-*tert*-Butyldiphenylsilyl-4-bromobut-2-yn-1-ol (8): Analogous to method E, to a solution of 4-O-*tert*-butyldiphenylsilylbut-2-yne-1,4-diol[2] (1.45 g, 5 mmol) in anhydrous CH_2Cl_2 (20 mL), CBr_4 (1.66 g, 5 mmol) and PPh_3 (1.31 g, 5 mmol) were added. The crude product was purified by silica gel column chromatography (toluol/ethyl acetate = 20/1) to yield 1-*O*-*tert*-butyldiphenylsilyl-4-bromobut-2-yn-1-ol **8** (1.84 g, 95 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.77-7.67 (m, 4H; ArH) 7.52-7.34 (m, 6H; ArH) 4.39 (t, ^5J = 2.0 Hz, 2H; CH_2) 3.91 (t, ^5J = 2.0 Hz, 2H; CH_2) 1.09 (s, 9H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=135.8 (4xCH), 133.1 (2xC), 130.0 (2xCH), 127.9 (4xCH), 85.4 (C), 80.3 (C), 52.9 (CH_2), 26.9 (CH_3), 19.3 (C), 14.7 (CH_2); IR (film) 3072, 2932, 1112, 701, 612 cm^{-1}; HRMS (EI): *m/z*: $[M]^+$ calcd for $C_{20}H_{23}BrOSi$: 386.0702, found: 386.0707.



8

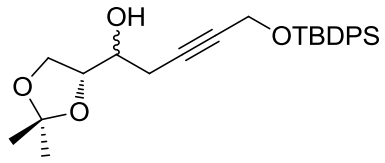
1-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (8a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 1-O-*tert*-butyldiphenylsilyl-4-bromobut-2-yn-1-ol **8** (194 mg, 0.5 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 6/1) to yield compounds **8a** (70 mg, 32 %) and **8b** (35 mg, 16 %). **(8a)**: $[\alpha]_D^{20}$ = +23.1° (*c* 0.3, CH_2Cl_2); ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.77–7.62 (m, 4H; ArH) 7.48–7.34 (m, 6H; ArH) 4.88–4.83 (m, 2H; CH_2) 4.45–4.35 (m, 2H; CH, CH_2) 4.34–4.23 (m, 2H; CH, CH_2) 4.08-3.98 (m, 2H; CH_2) 2.82 (d, ^3J = 4.0 Hz, 1H; OH) 1.43 (s, 3H; CH_3) 1.35 (s, 3H; CH_3) 1.07 (s, 9H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.1 (C), 135.7 (4xCH), 133.0 (2xC), 130.0 (CH), 129.9 (CH), 127.9 (2xCH), 127.8 (2xCH), 109.3 (C), 102.6 (C), 78.2 (CH_2), 77.5 (CH), 71.1 (CH), 65.7 (CH_2), 64.1 (CH_2), 26.9 (3xCH_3), 26.7 (CH_3), 25.3 (CH_3), 19.3 (C); IR (film) 3452, 3051, 2932, 1961, 1112, 702, 611 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{26}H_{34}O_4SiNa$: 461.2124, found: 461.212.



8a

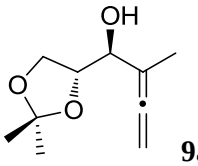
(2*R*)-7-*O*-*tert*-Butyldiphenylsilyl-1,2-*O*-isopropylidenehept-5-yne-1,2,3,7-tetrol (8b): Mixture of diastereomeres: major: minor = 5:1: ^{1}H NMR (400 MHz; $CDCl_3$): δ^{major}=7.76–7.64 (m, 4H; ArH) 7.48–7.33 (m, 6H; ArH) 4.35 (s, 2H; CH_2) 4.06–3.87 (m, 3H; CH_2, CH) 3.69–3.52 (m, 1H; CH) 2.52-2.34 (m, 2H; CH_2) 1.98 (d, ^3J = 5.1 Hz, 1H; OH) 1.41 (s, 3H; CH_3) 1.35 (s, 3H; CH_3) 1.06 (s, 9H; 3xCH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ^{major}=135.7 (4xCH), 133.4 (2xC), 130.0 (2xCH), 127.8 (4xCH), 109.5 (C), 81.7 (C), 80.9 (C), 77.4 (CH), 70.4

(CH), 66.1 (CH_2), 53.0 (CH_2), 26.83 (3xCH_3), 26.82 (CH_3), 25.3 (CH_3), 24.1 (CH_2), 19.3 (C); HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{26}H_{34}O_4SiNa$: 461.2124, found: 461.211.



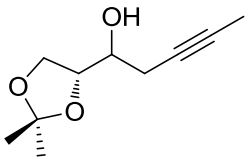
8b

(2*R*,3*S*)-1,2-*O*-Isopropylidene-4-methylhex-4,5-dien-1,2,3-triol (9a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 1-bromobut-2-yne **9** (67 mg, 0.5 mmol) in THF (4 mL). The crude product was purified by silica gel column chromatography (hexane/ ethyl acetate = 4:1) to yield compound **9a** (68 mg, 74 %) and **9b** (1 mg, 1 %). $[\alpha]_D^{20}$ = +26.8 (c=1.0, CH_2Cl_2);^{1}H NMR (400 MHz; $CDCl_3$): δ=4.79-4.69 (m, 2H; 2xCH) 4.17-4.08 (m, 2H; CH_2) 3.98 (dd, ^2J = 7.28 Hz, ^3J = 7.28 Hz, 1H; CH_2) 3.92 (dd, ^2J = 7.14 Hz, ^3J = 7.14 Hz, 1H; CH_2), 2.42 (s, 1H; OH) 1.72 (t, ^5J = 3.2 Hz, 3H; CH_3) 1.39 (s, 3H; CH_3) 1.31 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=205.8 (C), 109.2 (C), 99.1 (C), 77.4 (CH), 76.8 (CH_2), 72.2 (CH), 65.4 (CH_2), 26.6 (CH_3), 25.2 (CH_3), 15.3 (CH_3); IR 3457, 2931, 1060, 700, 611 (film) cm^{-1}; HRMS (EI): *m/z*:$[M-CH_3]^+$ calcd for $C_9H_{13}O_3$: 169.0865, found: 169.0864.



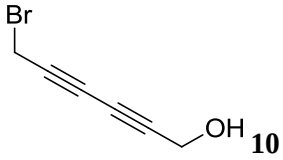
9a

(2*R*)-1,2-*O*-Isopropylidenehept-5-yne-1,2,3-triol (9b): Spectroscopical data: A. McCluskey, I. W. Muderawan, Muntari, D. J. Young; *J. Org. Chem.* **2001**, *66*, 7811-7817.



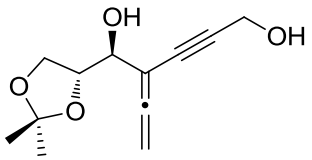
9b

6-Bromohexa-2,4-diyn-1-ol (10): To a solution of hexa-2,4-diyne-1,6-diol[3] (2.2 g, 0.02 mol) in anhydrous CH_2Cl_2 (40 mL), CBr_4 (7.3 g, 0.022 mol) was added and cooled to 0°C. In small portions PPh_3 (5.73 g, 0.022 mol) was added over a period of 30 minutes and the reaction mixture was further stirred at room temperature until TLC monitoring showed conversion of PPh_3. MeOH (5mL) was added and the solvent were evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 5/1) to yield 6-bromohexa-2,4-diyn-1-ol **10** (1.9 g, 56 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=4.34 (bs, 2H; CH_2) 3.96 (t, ^7J = 1.0 Hz, 2H; CH_2) 2.55 (bs, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=79.0 (C), 74.1 (C), 70.6 (C), 69.6 (C), 51.4 (CH_2), 14.1 (CH_2); IR (film) 3317, 2917, 2048, 1198, 1021 cm^{-1}; HRMS (EI): *m/z*: $[M]^+$ calcd for C_6H_5OBr: 171.9524, found: 171.9519.



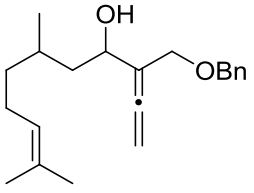
10

(2*R*,3*S*)-4-Ethenylidene-1,2-*O*-isopropylidene-hept-5-yne-1,2,3,7-tetrol (10a): Analogously to method A, 2,3-*O*-isopropylidene-D-glyceraldehyde **1** (65 mg, 0.5 mmol) was added to a suspension of LiI (67 mg, 0.5 mmol), indium (32 mg, 0.275 mmol) and 6-bromohexa-2,4-diyn-1-ol **10** (87 mg, 0.5 mmol) in THF (4 mL). The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 4/1) to yield compound **10a** (45 mg, 40 %). $[\alpha]_D^{20}$ = +18.0° (*c* 0.2, CH_2Cl_2);^{1}H NMR (400 MHz; $CDCl_3$): δ=5.17-5.13 (m, 2H; CH_2) 4.40 (t, *J* = 1.1 Hz, 2H; CH_2) 4.31-4.22 (m, 2H; 2xCH) 4.07-4.02 (m, 2H; CH_2) 1.44 (s, 3H; CH_3) 1.37 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=213.6 (C), 109.8 (C), 92.2 (C), 90.3 (C), 86.3 (C) 79.6 (CH_2), 77.2 (CH), 72.0 (CH), 65.2 (CH_2), 51.7 (CH_2), 26.8 (CH_3), 25.3 (CH_3); IR (film) 3385, 2926, 1936, 1065 cm^{-1}; HRMS (ESI): *m/z:* $[M+Na]^+$ calcd for $C_{12}H_{16}O_4Na$: 247.0946, found: 247.0950.



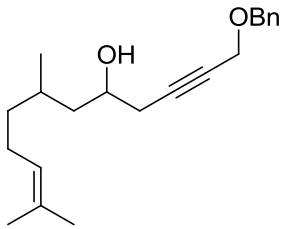
10a

3-((Benzyloxy)methyl)-6,10-dimethylundeca-1,2,9-trien-4-ol (11a): Analogously to method A, (+/-)-citronellal (72 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (8 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 7/1) to yield compounds **11a** (142 mg, 45 %) and **11b** (40 mg, 13 %) in an diasteromeric mixture. ^{1}H NMR (400 MHz; $CDCl_3$): δ^{major}=7.40-7.26 (m, 5H; ArH) 5.09 (tt, ^3J = 3.5 Hz, ^5J = 1.4 Hz, 1H; CH) 4.92-4.87 (m, 2H; CH_2) 4.55 (s, 2H; CH_2) 4.35–4.28 (m, 1H; CH) 4.22 (dt, ^2J = 12.2 Hz, ^5J = 1.5 Hz, 1H; CH_2) 4.14 (dt, ^2J = 11.2 Hz, ^5J = 2.1 Hz, 1H; CH_2) 2.60-2.19 (m, 1H; OH) 2.13-1.85 (m, 2H; CH_2) 1.73-1.56 (m, 1H; CH) 1.68 (s, 3H; CH_3) 1.60 (s, 3H; CH_3) 1.53-1.28 (m, 3H; CH_2) 1.25-1.09 (m, 1H; CH_2) 0.918 (d, ^3J = 6.6 Hz, 3H; CH_3); ^{1}H NMR (400 MHz; $CDCl_3$): δ^{minor}=7.40-7.26 (m, 5H; ArH) 5.09 (tt, ^3J = 3.5 Hz, ^5J = 1.4 Hz, 1H; CH) 4.92-4.87 (m, 2H; CH_2) 4.55 (s, 2H; CH_2) 4.35–4.28 (m, 1H; CH) 4.13 (dt, ^2J = 11.2 Hz, ^5J = 2.2 Hz, 1H; CH_2) 4.14 (dt, ^2J = 11.2 Hz, ^5J = 2.1 Hz, 1H; CH_2) 2.60-2.19 (m, 1H; OH) 2.13-1.85 (m, 2H; CH_2) 1.73-1.56 (m, 1H; CH) 1.68 (s, 3H; CH_3) 1.60 (s, 3H; CH_3) 1.53-1.28 (m, 3H; CH_2) 1.25-1.09 (m, 1H; CH_2) 0.92 (d, ^3J = 6.7 Hz, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.4, 206.2, 137.9, 137.8, 131.3, 131.2, 128.6 (4x), 128.1 (4x), 128.0 (2x), 125.0, 124.9, 103.8, 103.1, 77.6, 77.4, 72.5, 72.4, 69.9, 69.8, 69.0, 43.2, 43.1, 37.8, 37.0, 29.5, 29.0, 25.9 (2x), 25.6, 25.5, 20.1, 19.2, 17.8 (2x); IR (film) 3400, 2917, 1959, 1069, 737, 697 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{21}H_{30}O_2Na$: 337.2144, found: 337.2152.



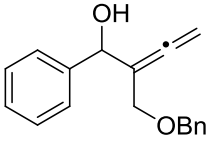
11a

1-Benzyloxy-7,11-dimethyldodeca-10-en-2-yn-5-ol (11b): δ^{major}=7.41-7.26 (m, 5H; ArH) 5.16-5.02 (m, 1H; CH) 4.59 (s, 2H; CH_2) 4.18 (t, ^5J = 2.1 Hz, 2H; CH_2) 3.92-3.76 (m, 1H; CH) 2.57-2.20 (m, 2H; CH_2) 2.13-1.79 (m, 3H; CH, CH_2) 1.68 (s, 3H; CH_3) 1.60 (s, 3H; CH_3) 1.54-1.35 (m, 2H; CH_2) 1.33-1.06 (m, 2H; CH_2) 0.93 (d, ^3J = 6.6 Hz, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=137.6, 131.5, 128.6 (2x), 128.2 (2x), 128.0, 124.8, 83.5, 78.8, 71.2, 68.3, 57.8, 44.0, 36.8, 29.5, 28.2, 25.8, 25.5, 20.3, 19.3; IR (film) 3430, 2922, 2342, 1070, 746, 697 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{21}H_{30}O_2Na$: 337.2144, found: 337.2150.



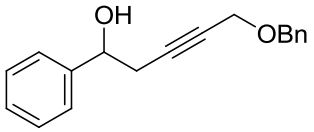
11b

2-((Benzyloxy)methyl)-1-phenylbuta-2,3-dien-1-ol (12a): Analogously to method A, benzaldehyde (106 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (8 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 6/1) to yield compounds **12a** (182 mg, 68 %) and **12b** (58 mg, 22 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.42–7.26 (m, 10H; ArH) 5.38 (dt, ^3J = 5.0 Hz, ^5J = 2.5 Hz, 1H; CH) 4.96–4.93 (m, 2H; CH_2) 4.49 (s, 2H; CH_2) 4.08 (dt, ^2J = 11.1 Hz, ^5J = 2.0 Hz, 1H; CH_2) 3.99 (dt, ^2J = 11.1 Hz, ^5J = 1.9 Hz, 1H; CH_2) 3.09 (d, ^3J = 5.5 Hz, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$) δ=206.6 (C), 142.2 (C), 137.7 (C), 128.6 (2xCH), 128.4 (2xCH), 128.1 (2xCH), 128.0 (CH), 127.7 (CH), 126.4 (2xCH_2), 103.8 (C), 78.1 (CH_2), 73.8 (CH), 72.5 (CH_2), 69.4 (CH_2); IR (film) 3424, 3062, 2864, 1068, 740, 699 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{18}O_2Na$: 289.1204, found: 289.1201.



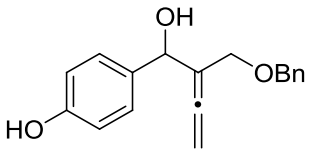
12a

5-(Benzyloxy)-1-phenylpent-3-yn-1-ol (12b): (400 MHz; $CDCl_3$): δ=7.45–7.26 (m, 10H; ArH) 4.87 (t, ^3J = 6.2 Hz, 1H; CH) 4.53 (s, 2H; CH_2) 4.16 (t, ^5J = 2.1 Hz, 2H; CH_2) 2.71 (dt, ^3J = 6.34 Hz, ^5J = 2.0 Hz, 2H; CH_2) 2.38 (bs, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$) δ=142.6 (C), 137.5 (C), 128.5 (4xCH), 128.4 (2xCH), 127.9 (2xCH), 127.8 (CH), 125.8 (CH), 83.1 (C), 78.7 (C), 72.5 (CH), 71.5 (CH_2), 57.6 (CH_2), 29.8 (CH_2); IR (film) 3411, 3031, 2926, 2327, 1058, 748, 698 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{18}O_2Na$: 289.1204, found: 289.1200.



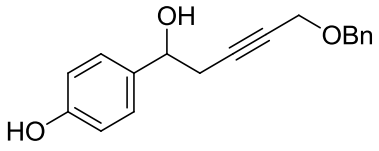
12b

2-((Benzyloxy)methyl)-1-(4-hydroxyphenyl)buta-2,3-dien-1-ol (13a): Analogously to method A, 4-hydroxybenzaldehyde (122 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (8 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 2/1) to yield compounds **13a** (129 mg, 46 %) and **13b** (41 mg, 14 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.38 (m, 7H; ArH) 6.79-6.74 (m, 2H; ArH) 5.34-5.22 (m, 1H; CH_2) 4.96-4.93 (m, 2H; CH_2) 4.53 (m, 2H; CH_2) 4.07 (dt, ^{2}J = 11.3 Hz, ^{5}J = 1.9 Hz, 1H; CH_2) 3.97 (dt, ^{2}J = 11.3 Hz, ^{5}J = 2.0 Hz, 1H; CH_2) 3.05 (d, ^{3}J = 3.9 Hz, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.4 (C), 155.4 (C), 137.7 (C), 134.2 (C), 128.6 (2xCH), 128.1 (2xCH), 128.0 (CH), 127.9 (2xCH), 115.3 (2xCH), 104.0 (C), 78.3 (CH), 73.2 (CH_2), 72.4 (CH_2), 69.4 (CH_2); IR (film) 3318, 3030, 2923, 1056, 743, 698 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{18}O_3Na$: 305.1154, found: 305.1160.



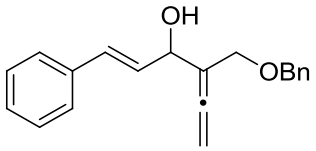
13a

1-Benzyloxy-5-(4-hydroxyphenyl)-pent-2-yn-5-ol (13b): (400 MHz; $CDCl_3$): δ=7.39–7.24 (m, 7H; ArH) 6.83-6.76 (m, 2H; ArH) 4.91 (bs, 1H; OH) 4.82 (t, ^{3}J = 6.3 Hz, 1H; CH) 4.53 (s, 2H; CH_2) 4.16 (t, ^{5}J = 2.1 Hz, 2H; CH_2) 2.72-2.64 (m, 2H; CH_2) 2.29 (bs, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=155.3 (C), 137.5 (C), 134.9 (C), 128.4 (2xCH), 128.1 (2xCH), 127.8 (CH), 127.3 (2xCH), 115.3 (2xCH), 83.3 (C), 78.6 (C), 72.1 (CH), 71.5 (CH_2), 57.6 (CH_2), 29.8 (CH_2); IR (film) 3330, 3030, 2925, 2351, 1056, 748, 698 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{18}H_{18}O_3Na$: 305.1154, found: 305.1160.



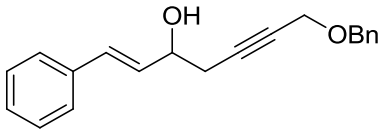
13b

(*E*)-4-((Benzyloxy)methyl)-1-phenylhexa-1,4,5-trien-3-ol (14a): Analogously to method A, cinnamaldehyde (133 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (8 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 7/1) to yield compounds **14a** (161 mg, 55 %, 69 % brsm.[4]) and **14b** (46 mg, 16 %, 19 % brsm.[4]). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.43-7.21 (m, 10H; ArH) 6.67 (d, ^{3}J = 15.8 Hz, 1H; CH) 6.30 (dd, ^{3}J = 15.9 Hz, ^{3}J = 6.3 Hz, 1H; CH) 5.01–4.91 (m, 3H; CH, CH_2) 4.56 (s, 2H; CH_2) 4.25 (dt, ^{2}J = 11.2 Hz, ^{5}J = 1.9 Hz, 1H; CH_2) 4.17 (dt, ^{2}J = 11.2 Hz, ^{5}J = 1.9 Hz, 1H; CH_2) 2.77 (d, ^{3}J = 5.1 Hz, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): 206.4 (C), 137.8 (C), 136.8 (C), 131.0 (CH), 129.9 (CH), 128.7 (2xCH), 128.6 (2xCH), 128.1 (2xCH), 128.0 (CH), 127.8 (CH), 126.7 (2xCH), 102.9 (C), 78.2 (CH_2), 72.4 (CH_2), 71.9 (CH), 69.4 (CH_2); IR (film) 3411, 3031, 2925, 1962, 1452, 1065, 747, 696 cm^{-1}; HRMS (EI): *m/z:* $[M-H_2O]^+$ calcd for $C_{20}H_{18}O$: 274.1358, found: 274.1362.



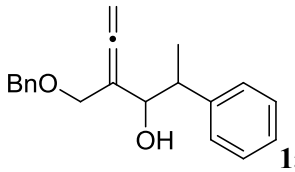
14a

(*E*)-7-(Benzyloxy)-1-phenylhept-1-en-5-yn-3-ol (14b): ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.37-7.13 (m, 10H; ArH) 6.60 (d, ^{3}J = 15.9 Hz, 1H; CH) 6.21 (dd, ^{3}J = 15.9 Hz, ^{3}J = 6.2 Hz, 1H; CH) 4.51 (s, 2H; CH_2) 4.44-4.35 (m, 1H; CH) 4.11 (t, ^{5}J = 2.1 Hz, 2H; CH_2) 2.57 (ddt, ^{2}J = 16.9 Hz, ^{3}J = 5.4 Hz, ^{5}J = 2.3 Hz, 1H; CH_2) 2.52 (ddt, ^{2}J = 16.6 Hz, ^{3}J = 6.3 Hz, ^{5}J = 2.1 Hz, 1H; CH_2) 2.77 (d, ^{3}J = 4.6 Hz, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): 137.9 (C), 136.8 (C), 131.7 (CH), 130.6 (CH), 129.0 (2xCH), 128.8 (2xCH), 128.4 (2xCH), 128.3 (CH), 128.2(CH), 127.0 (2xCH), 83.1 (C), 79.3 (C), 71.9 (CH_2), 71.3 (CH), 58.0 (CH_2), 28.6 (CH_2); IR (film) 3396, 3063, 2926, 2328, 1455, 1070, 749, 698 cm^{-1}; HRMS (EI): *m/z:* $[M-H_2O]^+$ calcd for $C_{20}H_{18}O$: 274.1358, found: 274.1360.



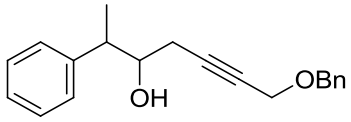
14b

4-((Benzyloxy)methyl)-2-phenylhexa-4,5-dien-3-ol (15a): Analogously to method A, 2-phenylpropanal (134 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (8 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 6/1) to yield compounds **15a** (171 mg, 58 %, 83% de) and **15b** (73 mg, 25 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.39-7.23 (m, 7H; ArH), 7.22-7.16 (m, 3H; ArH) 4.83-4.72 (m, 2H; CH_2) 4.56 (s, 2H; CH_2) 4.35 (m, 1H; CH) 4.09 (dt, ^{2}J = 11.4 Hz, ^{5}J = 2.0 Hz, 1H; CH_2) 3.93 (dt, ^{2}J = 11.4 Hz, ^{5}J = 1.9 Hz, 1H; CH_2) 3.01 (m, 1H; CH) 2.52 (s, 1H; OH) 1.37 (d, ^{3}J = 7.0 Hz, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=207.1 (C), 144.6 (C), 137.8 (C), 128.6 (2xCH), 128.3 (2xCH), 128.1 (CH), 128.0 (2xCH), 127.9 (CH), 126.4 (2xCH), 102.2 (C), 77.8 (CH_2), 75.6 (CH), 72.3 (CH_2), 70.0 (CH_2), 44.7 (CH), 17.1 (CH_3); IR (film) 3424, 2928, 1955, 1064, 749, 698 cm^{-1}; HRMS (EI): *m/z:* $[M-H_2O]^+$ calcd for $C_{20}H_{20}O$: 276.1514, found: 276.1519.



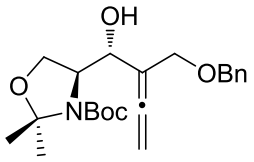
15a

1-Benzyloxy-6-phenylhept-2-yn-5-ol (15b): ^{1}H NMR (400 MHz; $CDCl_3$) δ=7.44-7.16 (m, 10H; ArH), 4.83 (s, 2H; CH_2) 4.19 (t, ^{5}J = 2.12 Hz, 2H; CH_2) 3.81 (m, 1H; CH) 2.89 (dq, ^{3}J = 7.1 Hz, ^{3}J = 7.0 Hz, 1H; CH) 2.37 (ddt, ^{2}J = 16.9 Hz, ^{3}J = 4.4 Hz, ^{5}J = 2.1 Hz ,1H; CH_2) 2.23 (ddt, ^{2}J = 16.9 Hz, ^{3}J = 4.4 Hz, ^{5}J = 2.1 Hz ,1H; CH_2) 1.97 (d, ^{3}J = 3.76 Hz, 1H; OH); 1.38 (d, ^{3}J = 6.8 Hz, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$):δ=144.0 (C), 137.7 (C), 128.8 (2xCH), 128.6 (2xCH), 128.2 (2xCH), 128.0 (CH), 127.8 (2xCH), 126.8 (CH), 83.5 (C), 79.0 (C), 74.6 (CH), 71.7 (CH_2), 57.8 (CH_2), 45.1 (CH), 25.9 (CH_2), 17.1 (CH_3); IR (film) 3432, 2927, 1068, 745, 698 cm^{-1}; HRMS (EI): *m/z:* $[M]^+$ calcd for $C_{20}H_{22}O_2$: 294.1620, found: 294.1632.



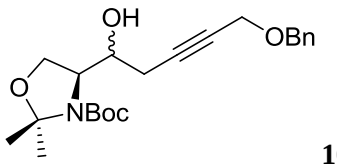
15b

1-*O*-Benzyl-4-(*tert*-butyloxycarbonyl)amino-2,4-dideoxy-2-*C*-ethenylidene-4,5-*N*,*O*-isopropylidene-L-*erythro*-pentitol (16a): Analogously to method A, garner's aldehyde[5] (230 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (115 mg, 1 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (8 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 6/1) to yield compounds **16a** (95 mg, 24%, 27 % brsm.[4]) and **16b** (177 mg, 45%, 50 % brsm.[4]). **(16a):** $[\alpha]_D^{20}$ = -37.4° (*c* 0.85, CH_2Cl_2); ^{1}H NMR (400 MHz; DMSO, 330 K) δ=7.39–7.22 (m, 5H; ArH) 5.03 (d, ^3J = 5.8 Hz, 1H; OH) 4.96-4.81 (m, 2H; CH_2) 4.48 (s, 2H; CH_2) 4.44-4.38 (m, 1H; CH) 4.15-3.90 (m, 4H; CH, CH_2) 3.84 (dd, ^3J = 8.3 Hz, ^3J = 6.8 Hz, 1H; CH_2) 1.50 (s, 3H; CH_3) 1.45-1.38 (m, 12H; CH_3); ^{13}C-NMR (400 MHz; DMSO, 330 K): δ=205.9 (C), 138.9 (C), 127.8 (2xCH), 127.2 (2xCH), 127.0 (CH), 102.2 (C), 94.1 (C), 78.8 (C), 77.0 (CH_2), 70.8 (CH_2), 67.9 (CH), 67.4 (CH_2), 63.0 (CH_2), 59.7 (CH), 27.8 (4xCH_3), 25.8 (CH_3); IR (film) 3429, 2977, 1961, 1698, 1390, 1098, 747, 698 cm^{-1}; HRMS (ESI): *m/z:*[M+Na]$^+$ calcd for $C_{22}H_{31}O_5NNa$: 412.2100, found: 412.2114.



16a

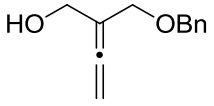
(2*S*)-7-*O*-Benzyl-2-(*tert*-butyloxycarbonyl)amino-1,2-*O*,*N*-isopropylidene-hept-5-yne-1,3,7-triol (16b): ^{1}H NMR (400 MHz; $CDCl_3$) δ=7.40–7.26 (m, 5H; ArH) 4.59 (s, 2H; CH_2) 4.26-4.05 (m, 3H; CH, CH_2) 4.05-3.79 (m, 3H; CH, CH_2) 2.58 (ddt, ^2J = 16.9 Hz, ^3J = 4.1 Hz, ^5J = 2.2 Hz, 1H; CH_2) 2.43 (ddt, ^2J = 17.1 Hz, ^3J = 6.3 Hz, ^5J = 2.1 Hz, 1H; CH_2) 1.59 (s, 3H; CH_3) 1.49 (s, 9H; CH_3) 1.48 (s, 3H; CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$): δ=137.7 (C), 128.5 (2xCH), 128.2 (2xCH), 127.9 (CH), 94.6 (C), 94.5 (C), 83.0 (C), 78.8 (C), 72.2 (CH), 71.9 (CH_2), 64.7 (CH_2), 61.4 (CH), 57.8 (CH_2), 28.5 (4xCH_3), 26.8 (CH_3) 24.4 (CH_2); IR (film) 3424, 2980, 2327, 1698, 1367, 1068, 746, 698 cm^{-1}; HRMS (ESI): *m/z:*[M+Na]$^+$ calcd for $C_{22}H_{31}O_5NNa$: 412.2100, found: 412.2117.



16b

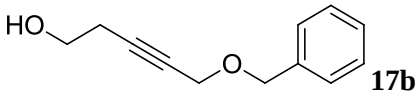
2-((Benzyloxy)methyl)buta-2,3-dien-1-ol (17a): Analogously to method A, a freshly prepared aqueous solution (2 ml) of formaldehyde (120 mg, 4 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (76 mg, 0.66 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 4/1) to yield compounds **17a** (123 mg, 65 %) and **17b** (43 mg, 22 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.39–7.26 (m, 5H; Ar-H) 4.93–4.88 (m, 2H; CH_2)

4.54 (s, 2H; CH_2) 4.23 (t, ^5J = 2.5 Hz, 2H; CH_2) 4.16 (t, ^5J = 2.1 Hz, 2H; CH_2) 2.4 (s, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.4 (C), 137.9 (C), 128.6 (2xCH), 128.0 (2xCH), 127.9 (CH), 100.5 (C), 77.3 (CH_2), 72.3 (CH_2), 69.5 (CH_2), 62.2 (CH_2); IR (film) 3386, 3064, 2927, 1961, 1069, 747, 698 cm^{-1}; HRMS (EI): *m/z:* calcd for $C_{12}H_{14}O_2$: 190.0994, found: 190.0987.



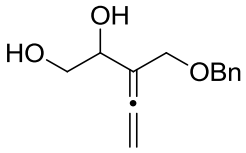
17a

5-(Benzyloxy)pent-3-yn-1-ol (17b): ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.40–7.26 (m, 5H; Ar-H) 4.59 (s, 2H; CH_2) 4.17 (t, ^5J = 2.2 Hz, 2H; CH_2) 3.72 (t, ^3J = 6.3 Hz, 2H; CH_2) 2.55-2.47 (m, 2H; CH_2) 1.85 (bs, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$) δ=137.6 (C), 128.6 (2xCH), 128.1 (2xCH), 128.0 (CH), 83.7 (C), 78.1 (C), 71.8 (CH_2), 61.1 (CH_2), 57.8 (CH_2), 23.3 (CH_2); IR (film) 3316, 3064, 2857, 2239, 1043, 747, 698 cm^{-1}; HRMS (EI): *m/z:* calcd for $C_{12}H_{14}O_2$: 190.0994, found: 190.0989.



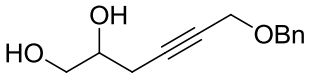
17b

3-((Benzyloxy)methyl)-penta-3,4-dien-1,2-diol (18a): Analogously to method A, an aqueous solution (2ml) of glycolaldehyde (60 mg, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 3/1) to yield compounds **18a** (116 mg, 53 %) and **18b** (74 mg, 34 %). ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.40–7.26 (m, 5H; ArH) 4.97–4.91 (m, 2H; CH_2) 4.54 (s, 2H; CH_2) 4.37–4.28 (m, 1H; CH) 4.17 (dt, ^3J = 11.1 Hz, ^5J = 1.9 Hz, 1H; CH_2) 4.13 (dt, ^2J = 11.2 Hz, ^5J = 1.8 Hz, 1H; CH_2) 3.73 (dd, ^2J = 11.4 Hz, ^3J = 4.1 Hz, 1H; CH_2) 3.67 (dd, ^2J = 14.4 Hz, ^3J = 6.0 Hz, 1H; CH_2) 2.90 (s, 1H ; OH) 2.60 (s, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.7 (C), 137.5 (C), 128.7 (2xCH), 128.6 (CH), 128.1 (2xCH), 100.8 (C), 78.0 (CH_2), 72.5 (CH), 71.0 (CH_2), 69.2 (CH_2), 65.6 (CH_2); IR (film) 3351, 3064, 2924, 1955, 1058, 737, 697 cm^{-1}; HRMS (ESI): *m/z:* $[M+Na]^+$ calcd for $C_{13}H_{16}O_3Na$: 243.0997, found: 243.0998.



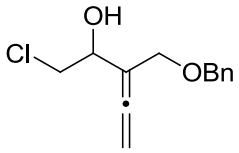
18a

6-(Benzyloxy)-hex-4-yne-1,2-diol (18b): ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.4–7.26 (m, 5H; ArH) 4.58 (s, 2H; CH_2) 4.17 (t, ^5J = 2.2 Hz, 2H; CH_2) 3.92-3.83 (m, 1H; CH) 3.74 (dd, ^2J = 11.2 Hz, ^3J = 3.5 Hz, 1H; CH_2) 3.59 (dd, ^2J = 11.2 Hz, ^3J = 6.4 Hz, 1H; CH_2) 2.56-2.37 (m, 2H; CH_2) 1.84-1.53 (bs, 2H; 2xOH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=137.5 (C), 128.6 (2xCH), 128.2 (2xCH), 128.1 (CH), 82.7 (C), 78.8 (C), 71.9 (CH_2), 70.4 (CH), 65.7 (CH_2), 57.8 (CH_2), 24.0 (CH_2); IR (film) 3411, 3063, 2925, 2237, 1069, 747, 698 cm^{-1}; HRMS (ESI): *m/z:* $[M+Na]^+$ calcd for $C_{13}H_{16}O_3Na$: 243.0997, found: 243.0996.



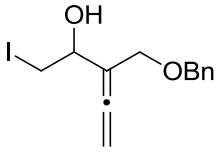
18b

3-((Benzyloxy)methyl)-1-chloropenta-3,4-dien-2-ol (19a-chloride): Analogously to method A, an aqueous solution (2ml) of chloroacetaldehyde (~50% solution in water, 127µl, 1 mmol) was added to a suspension of LiI (134 mg, 1 mmol), indium (63 mg, 0.55 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (hexane/ethyl acetate = 7/1) to yield compounds **19a** (chloride) (29 mg, 12 %) and **19a** (iodide) (119 mg, 36 %) and **19b** (24 mg, 10 %). ^{1}H NMR (400 MHz; $CDCl_3$) δ=7.39-7.27 (m, 5H; ArH), 5.03-4.98 (m, 2H; CH_2) 4.56-4.52 (s, 2H, CH_2) 4.51-4.41 (m, 1H; CH) 4.25-4.10 (m, 2H; CH_2) 3.73 (dd, ^{2}J = 11.2 Hz, ^{3}J = 4.6 Hz, 1H; CH_2) 3.67 (dd, ^{2}J = 11.2Hz, ^{3}J = 6.7Hz, 1H; CH_2) 2.84 (s, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.6 (C), 137.7 (C), 128.7 (2xCH), 128.06 (CH), 128.06 (2xCH), 100.6 (C), 78.8 (CH_2), 72.4 (CH_2), 70.5 (CH), 69.4 (CH_2), 48.2 (CH_2); HRMS (EI): *m/z:*$[M]^+$ calcd for $C_{13}H_{15}O_2Cl$: 238.0761, found: 238.0756.



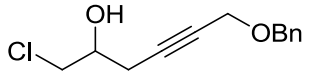
19a (chloride)

3-((Benzyloxy)methyl)-1-iodopenta-3,4-dien-2-ol (19a-iodide): ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.39-7.27 (m, 5H; ArH) 5.03-4.98 (m, 2H; CH_2) 4.56-4.52 (s, 2H; CH_2) 4.51-4.41 (m, 1H; CH) 4.25-4.10 (m, 2H; CH_2) 3.46 (dd, ^{2}J = 10.2 Hz, ^{3}J = 5.0 Hz, 1H; CH_2) 3.40 (dd, ^{2}J = 10.2 Hz, ^{3}J = 6.3 Hz, 1H; CH_2) 2.84 (s, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.5 (C), 137.6 (C), 128.7 (CH), 128.1 (4xCH), 101.8 (C), 79.2 (CH_2), 72.5 (CH_2), 70.2 (CH), 69.4 (CH_2), 12.7 (CH_2); IR (film) 3411, 3063, 2925, 1069, 748, 699 cm^{-1}; HRMS (EI): *m/z*: $[M]^+$ calcd for $C_{13}H_{15}O_2INa$: 353.0014, found: 353.0017.



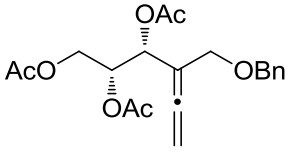
19a (iodide)

6-(Benzyloxy)-1-chlorohex-4-yn-2-ol (19b): ^{1}H NMR (400 MHz; $CDCl_3$) δ=7.40-7.26 (m, 5H; ArH), 4.58 (s, 2H; CH_2) 4.17 (t, ^{5}J = 2.2 Hz, 2H; CH_2) 4.05-3.92 (m, 1H; CH) 3.71 (dd, ^{2}J = 11.2 Hz, ^{3}J = 4.4 Hz, 1H; CH_2) 3.64 (dd, ^{2}J = 11.2 Hz, ^{3}J = 6.1 Hz, 1H; CH_2) 2.73-2.51 (m, 2H; CH_2) 2.38 (bs, 1H; OH); ^{13}C NMR (400 MHz; $CDCl_3$): δ=137.5 (C), 128.6 (2xCH), 128.2 (2xCH), 128.0 (CH), 81.7 (C), 79.3 (C), 71.9 (CH_2), 69.9 (CH), 57.7 (CH_2), 48.5 (CH_2), 24.8 (CH_2); IR (film) 3411, 3031, 2924, 1071, 748, 698 cm^{-1}; HRMS (EI): *m/z:*$[M]^+$ calcd for $C_{13}H_{15}O_2Cl$: 238.0761, found: 238.0756.



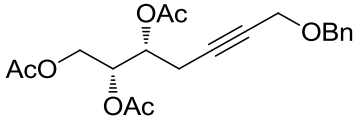
19b

3,4,5-Tri-*O*-acetyl-1-*O*-benzyl-2-deoxy-2-*C*-ethenylidene-D-*erythro*-pentitol (20a): To a suspension of 2,3-O-isopropylidine-D-glyceraldehyde (156 mg, 1.2 mmol) in 2,5 mL of a mixture of water/THF (4:1), 0.75 g of DOWEX 50 W (H^+) were added and the mixture was stirred at room temperature. After TLC monitoring showed conversion of the starting material, the pH was adjusted to ~6.5 by addition of a 0.5 N NaOH solution. Analogously to method A, this solution was added a suspension of LiI (134 mg, 1 mmol), indium (115 mg, 1 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (4 mL). After sonication of the reaction mixture for 12 hours, the pH was readjusted to ~6.5 by addition of a 0.5 N NaOH solution. The reaction mixture was filtered over a pad of zelite and the solvent was evaporated. To the dried reaction mixture, pyridine (1 mL), acetic anhydride (550 µL) and 4-dimethylaminopyridine (DMAP) were added. After 12 hours the solvents were removed by codestillation with toluene at reduced pressure. The crude product was carefully separated by silica gel column chromatography (hexane/ethyl acetate = 8:1) to yield compounds **20a** (76 mg, 20 %) and **20b** (124 mg, 33 %). **(20a):** $[\alpha]_D^{20}$ = -1.8° (*c* 0.6, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.38-7.26 (m, 5H; Ar-H) 5.57 (dt, ^3J = 6.3 Hz, ^5J = 1.7 Hz, 1H; CH) 5.43 (ddd, ^3J = 6.2 Hz, ^3J = 6.2 Hz, ^3J = 3.9 Hz, 1H; CH) 4.98-4.94 (m, 2H; CH_2) 4.53 (d, ^2J = 11.9 Hz, 1H; CH_2) 4.55 (d, ^2J = 11.7 Hz, 1H; CH_2) 4.48 (d, ^2J = 11.7 Hz, 1H; CH_2) 4.33 (dd, ^2J = 12.0 Hz, ^3J = 3.8 Hz, 1H; CH_2) 4.15 (dd, ^2J = 11.9, ^3J = 6.0 Hz, 1H; CH_2) 4.14 (dt, ^2J = 11.6 Hz, ^5J = 2.2 Hz, 1H; CH_2) 4.04 (dt, ^2J = 11.6 Hz, ^5J = 1.9 Hz, 1H; CH_2) 2.06 (s, 3H; CH_3), 2.05 (s, 3H; CH_3), 2.02 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=208.0 (C), 170.7 (C), 170.2 (C), 170.0 (C), 138.0 (C), 128.5 (2xCH), 128.1 (2xCH), 127.8 (CH), 97.9 (C), 78.5 (CH_2), 72.3 (CH_2), 70.9 (CH), 70.1 (CH), 68.5 (CH_2), 62.7 (CH_2), 21.0 (CH_3), 20.9 (CH_3), 20.8 (CH_3); IR (film) 3063, 2926, 1962, 1742, 1216, 746, 699 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{20}H_{24}O_7Na$: 399, 1420, found: 399,1413.



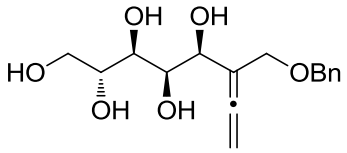
20a

(2*R*,3*R*)-7-(Benzyloxy)-1,2,3-tri-*O*-acetyl-5-heptyne-1,2,3-triol (20b): $[\alpha]_D^{20}$ = -7.2° (*c* 0.25, CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.40-7.26 (m, 5H; Ar-H) 5.40 (ddd, ^3J = 6.1 Hz, ^3J = 4.6 Hz, ^3J = 4.6 Hz, 1H; CH) 5.21 (ddd, ^3J = 6.4 Hz, ^3J = 6.4 Hz, ^3J = 4.4 Hz, 1H; CH) 4.57 (s, 2H; CH_2) 4.32 (dd, ^2J = 11.9 Hz, ^3J = 4.8 Hz, 1H; CH_2) 4.14 (t, ^5J = 2.1 Hz, 2H; CH_2) 4.08 (dd, ^2J = 11.9 Hz, ^3J = 6.2 Hz, 1H; CH_2) 2.67-2.52 (m, 2H; CH_2) 2.11 (s, 3H; CH_3), 2.10 (s, 3H; CH_3), 2.05 (s, 3H; CH_3); ^{13}C NMR (400 MHz; $CDCl_3$): δ=170.6 (C), 170.1 (C), 170.0 (C), 137.6 (C), 128.5 (2xCH), 128.2 (2xCH), 128.0 (CH), 80.7 (C), 79.1 (C), 71.6 (CH_2), 70.5 (CH), 69.7 (CH), 62.1 (CH_2), 57.6 (CH_2), 21.2 (CH_2), 20.9 (CH_3), 20.87 (CH_3), 20.83 (CH_3); IR (film) 2932, 1747, 1219, 748, 699 cm^{-1}; HRMS (ESI): *m/z*: $[M+Na]^+$ calcd for $C_{20}H_{24}O_7Na$: 399, 1420, found: 399,1418.

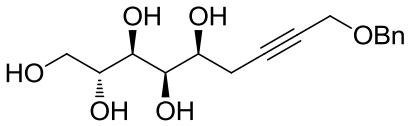


20b

1-*O*-Benzyl-2-deoxy-2-*C*-ethenylidene-D-gluco-heptitol (21a) and (2R,3R,4R,5S)-9-(Benzyloxy)non-7-yne-1,2,3,4,5-pentaol (21b): Analogously to method A, an aqueous solution (2 ml) of arabinose (150 mg, 1 mmol) was added to a suspension of LiCl (85 mg, 2 mmol), indium (115 mg, 1 mmol) and 1-(benzyloxy)-4-bromo-2-butyne **2** (240 mg, 1 mmol) in THF (4 mL). The crude product was separated by silica gel column chromatography (ethyl acetate/methanol = 8/1) to yield an inseperable mixture of compound **21a** and **21b** (16 mg, 5 %, **21a**:**21b**=50:50) and the sideproducts **30a** (21 mg, 16 %), **30b** (13 mg, 12 %), and **31a** (19 mg, 11 %) together with **31b** (15 mg, 7 %). **(21a):** ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.26–7.17 (m, 5H; ArH) 4.87–4.84 (m, 2H; CH_2) 4.44 (s, 2H; CH_2) 4.24 (dt, ^{2}J = 5.5 Hz, ^{5}J = 1.7 Hz, 1H; CH) 4.10–4.04 (m, 2H; CH_2) 3.86–3.79 (m, 1H; CH) 3.71 (s, 5H; OH) 3.68–3.52 (m, 4H; CH, CH_2) **(21b)**: ^{1}H NMR (400 MHz; $CDCl_3$): δ=7.27–7.17 (m, 5H; ArH) 4.47 (s, 2H; CH_2) 4.10–4.04 (m, 2H; CH_2) 3.86–3.79 (m, 1H; CH) 3.79-3.75 (m, 1H; CH) 3.71 (s, 5H; OH) 3.68-3.52 (m, 4H; CH, CH_2) 2.50 (ddt, ^{2}J = 16.7 Hz, ^{3}J = 6.4 Hz, ^{5}J = 2.1 Hz, 1H; CH_2), 2.43 (ddt, ^{2}J = 16.6 Hz, ^{3}J = 6.6 Hz, ^{5}J = 2.0 Hz, 1H; CH_2); ^{13}C NMR (400 MHz; $CDCl_3$): **21a/21b**: δ=207.4 ($C_{\text{-}23a}$), 137.3 (C), 137.1 (C), 128.4 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.94 (CH), 127.90 (CH), 100.5 ($C_{\text{-}23a}$), 83.5 ($C_{\text{-}23b}$), 77.6 ($CH_{2\text{-}23a}$), 77.5 ($C_{\text{-}23b}$), 73.6 (CH), 72.34 (CH), 72.32 (CH), 72.3 (CH_2), 71.8 (CH), 71.7 (CH), 71.69 (CH_2), 71.4 (CH), 71.1 (CH), 70.1 (CH), 68.7($CH_{2\text{-}23a}$), 63.57 (CH_2), 63.55 (CH_2), 57.7 ($CH_{2\text{-}23b}$), 23.7 ($CH_{2\text{-}23b}$); IR (film) 3339, 2926, 2351, 1955, 1064, 743, 698 cm^{-1}; HRMS (ESI): *m/z:* $[M+Na]^+$ calcd for $C_{16}H_{22}O_6Na$: 333.1314, found: 333.1312.

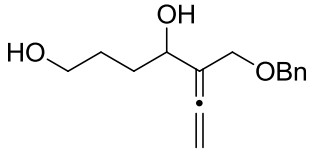


21a



21b

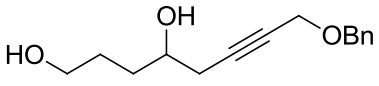
5-((Benzyloxy)methyl)hepta-5,6-dien-1,4-diol (30a): ^{1}H NMR (600 MHz; $CDCl_3$): δ=7.39-7.27 (m, 5H; Ar-H) 4.93-4.90 (m, 2H; CH_2) 4.55 (d, ^{2}J = 11.8 Hz, 1H; CH_2) 4.53 (d, ^{2}J = 11.8 Hz, 1H; CH_2) 4.29-4.24 (m, 1H; CH) 4.20 (dt, ^{2}J = 11.2 Hz, ^{5}J = 1.9 Hz, 1H; CH_2) 4.14 (dt,^{2} J = 11.2 Hz, ^{5}J = 2.0 Hz, 1H; CH_2) 3.71-3.61 (m, 2H; CH_2) 2.75 (s, 2H; 2xOH) 1.83-1.75 (m, 1H; CH_2) 1.73-1.64 (m, 3H; CH_2); ^{13}C NMR (400 MHz; $CDCl_3$): δ=206.2 (C), 137.7 (C), 128.6 (2xCH), 128.1 (2xCH), 128.0 (CH), 103.1 (C), 77.9 (CH), 72.4 (CH_2), 70.9 (CH_2), 69.9 (CH), 63.0 (CH_2), 32.7 (CH_2), 29.3 (CH_2); IR (film) 3385, 2927, 1956, 1066, 748, 699 cm^{-1}; HRMS (ESI): *m/z:* $[M+Na]^+$ calcd for $C_{15}H_{20}O_3Na$: 271.1310, found: 271.1308.



30a

8-(Benzyloxy)oct-6-yne-1,4-diol (30b): ^{1}H NMR (600 MHz; $CDCl_3$): δ=7.37-7.32 (m, 4H; Ar-H) 7.32-7.27 (m, 1H; Ar-H) 4.59 (s, 2H; CH_2) 4.18 (t, ^{5}J = 2.13, 2H; CH_2) 3.83-3.76 (m, 1H; CH) 3.75-3.62 (m, 2H; CH_2) 2.48 (ddt, ^{2}J = 16.6 Hz, ^{3}J = 6.8 Hz, ^{5}J = 2.1 Hz, 1H; CH_2) 2.42 (ddt, ^{2}J = 16.6 Hz, ^{3}J = 5.1 Hz, ^{5}J = 2.2 Hz, 1H; CH_2) 1.78-1.65 (m, 3H; CH_2) 1.65-1.51 (m, 1H; CH_2); ^{13}C-NMR (600 MHz; $CDCl_3$): δ=137.6 (C), 128.6 (2xCH), 128.2 (2xCH), 128.0 (CH), 83.5 (C), 78.7 (C), 71.8 (CH_2), 70.2 (CH), 63.0 (CH_2), 57.8 (CH_2), 33.5 (CH_2), 29.2 (CH_2),

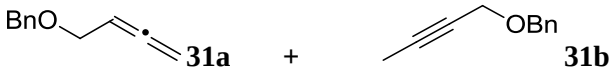
28.1 (CH_2); IR (film) 3342, 2924, 2237, 1066, 748, 699 cm^{-1}; HRMS (ESI): *m/z:* $[M+Na]^+$ calcd for $C1_5H_{20}O_3Na$: 271.1310, found: 271.1308.



30b

1-*O*-Benzyl-2,3-butadien-1-ol (31a): ^1H NMR (600 MHz; $CDCl_3$): δ=7.38-7.26 (m, 5H; Ar-H) 5.28 (m, 1H; CH) 4.81 (dt, ^4J = 6.6 Hz; ^5J = 2.5 Hz, 2H; CH_2) 4.54 (s, 2H; CH_2) 4.07 (dt, ^4J = 6.9 Hz, ^5J = 2.4 Hz, 2H; CH_2); ^{13}C NMR (600 MHz; $CDCl_3$): δ=209.5 (C), 137.7 (C), 128.6 (2xCH), 127.9 (CH), 127.8 (2xCH), 87.8 (CH), 75.8 (CH_2), 71.6 (CH_2), 68.0 (CH_2);

1-*O*-Benzyl-but-2-yn-1-ol (31b): ^1H NMR (600 MHz; $CDCl_3$): δ=7.38-7.26 (m, 5H; Ar-H) 4.59 (s, 2H; CH_2) 4.14 (q, ^5J = 2.3Hz, 2H; CH_2) 1.88 (t, ^5J = 2.3Hz, 3H; CH_3);^{13}C NMR (600 MHz; $CDCl_3$): δ=138.2 (C), 128.5 (2xCH), 128.2 (CH), 128.0 (2xCH), 82.8 (C), 75.2 (C), 71.9 (CH_2), 57.9 (CH_2), 3.8 (CH_3); (**33a** + **33b**): IR (film) 3064, 2922, 1956, 747, 698 cm^{-1}; HRMS (EI): *m/z:* $[M-H]^-$ calcd for $C_{11}H_{11}O$: 159.0810 found: 159.0809.



31a + **31b**

References:

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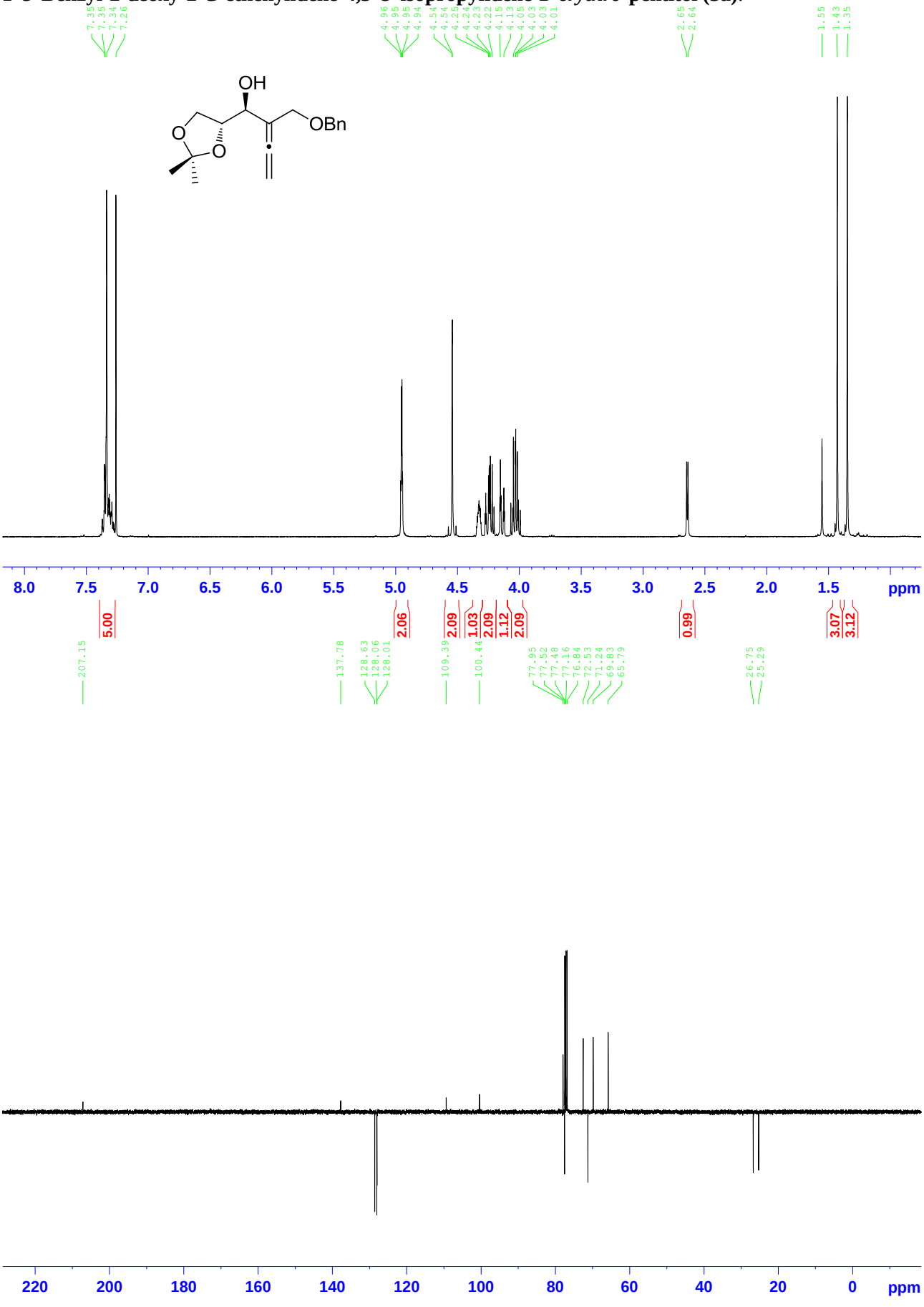
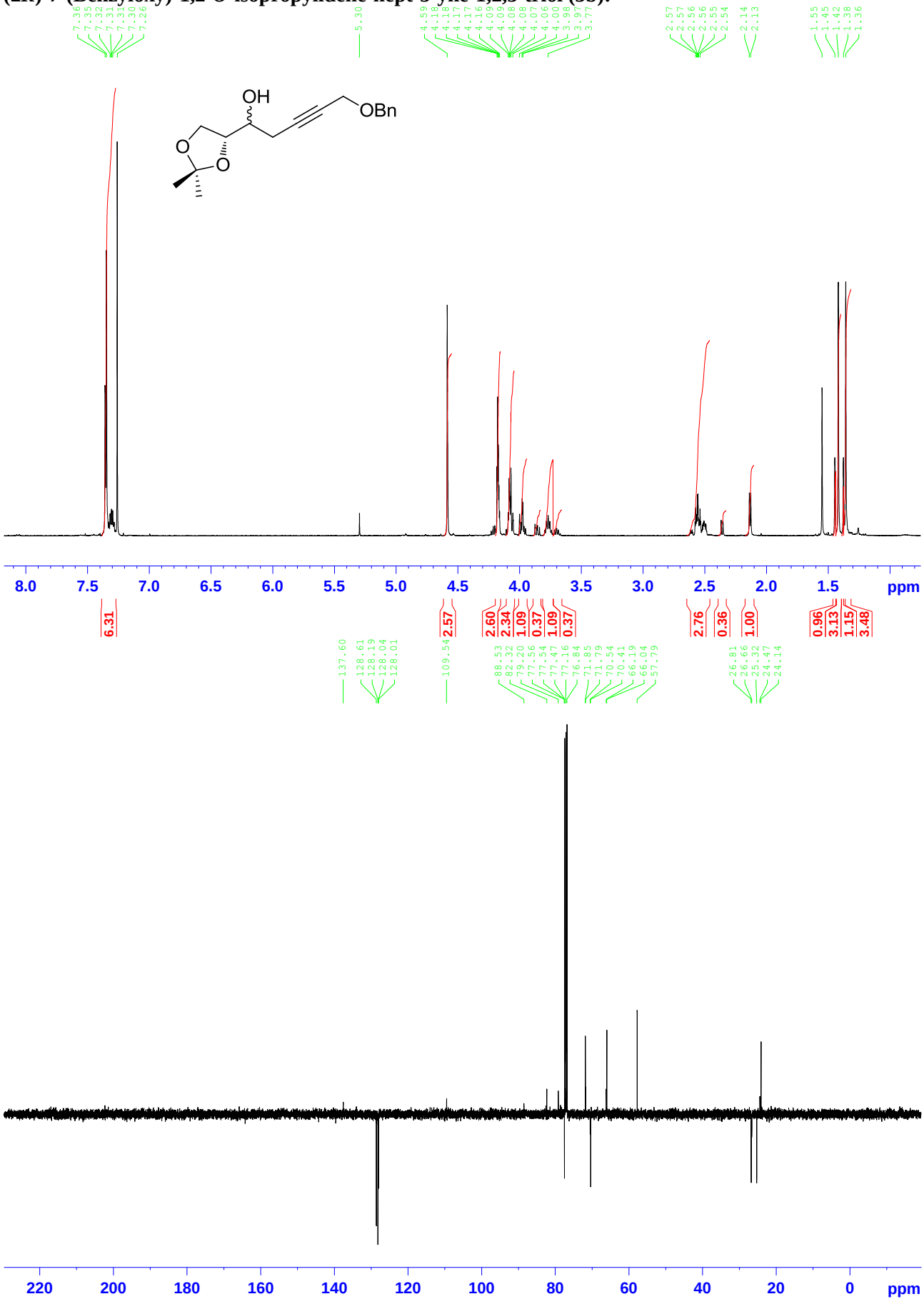
[2] T. Luu, W. Shi; T. L. Lowary, R. R. Tykwinski, *Synthesis* **2005**, *18*, 3167-3178.
[3] D. E. Bierer, J. M. Dener, L. G. Dubenko, R. E. Gerber, J. Litvak, S. Peterli, P. Peterli-Roth, T. V. Truong, G. Mao, B. E. Bauert *J. Med. Chem.* **1995**, *38*, 2628-2648.
[4] brsm. = based on recovered starting material
[5] P. Garner, J. M. Park, *J. Org. Chem.* **1987**, *52*, 2361-2314.

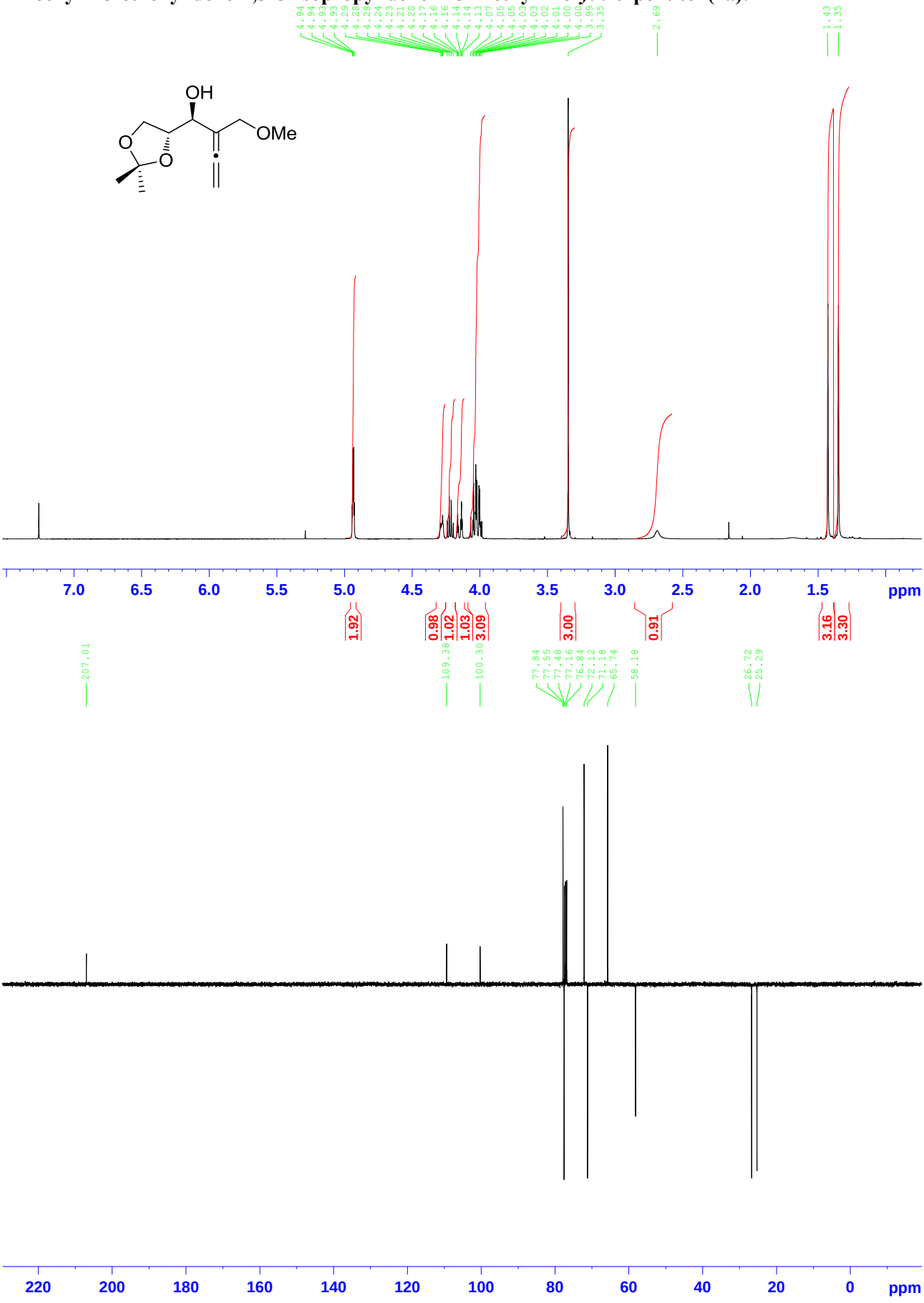
1-*O*-Benzyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (3a):



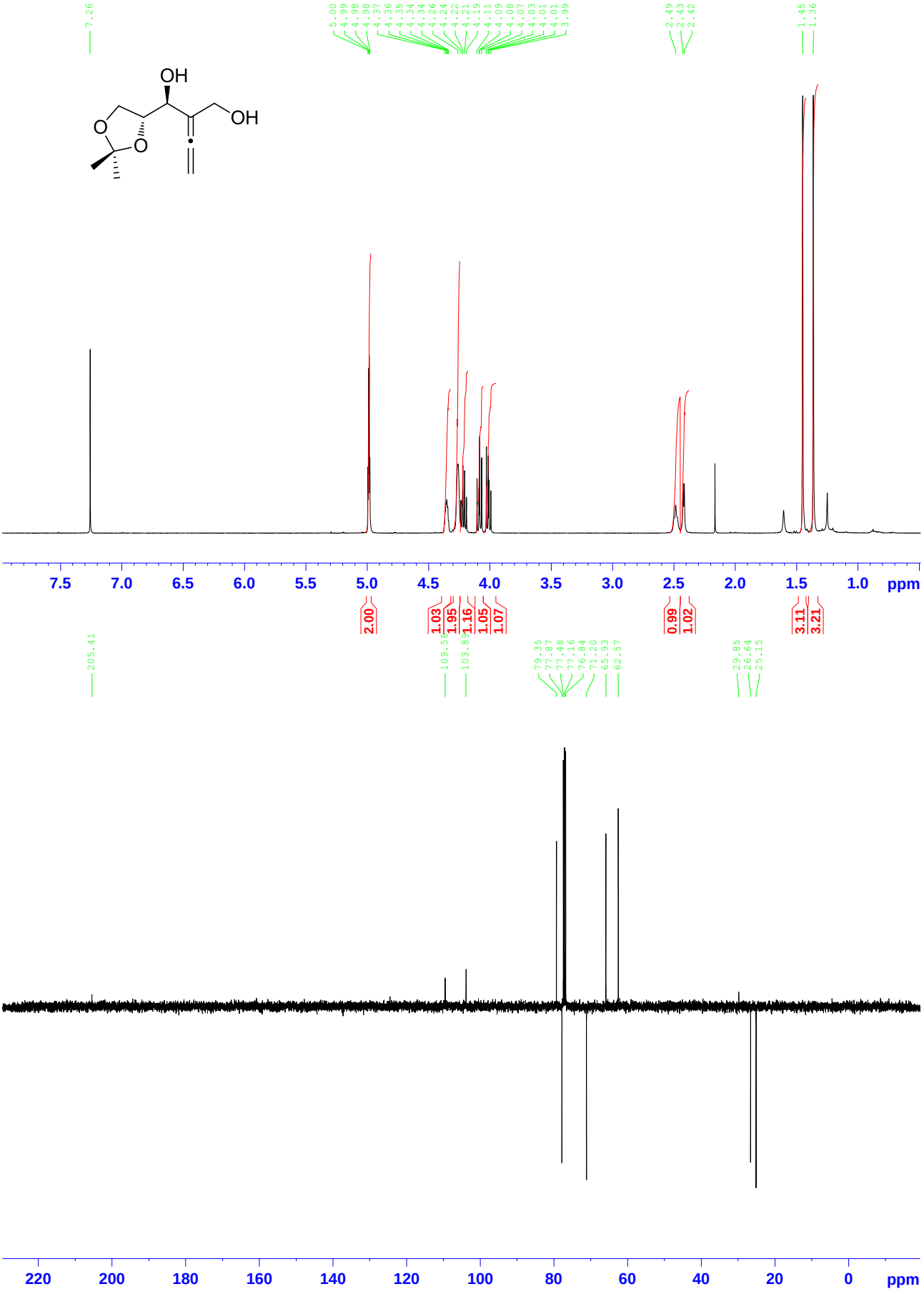
(2*R*)-7-(Benzyloxy)-1,2-*O*-isopropylidene-hept-5-yne-1,2,3-triol (3b):



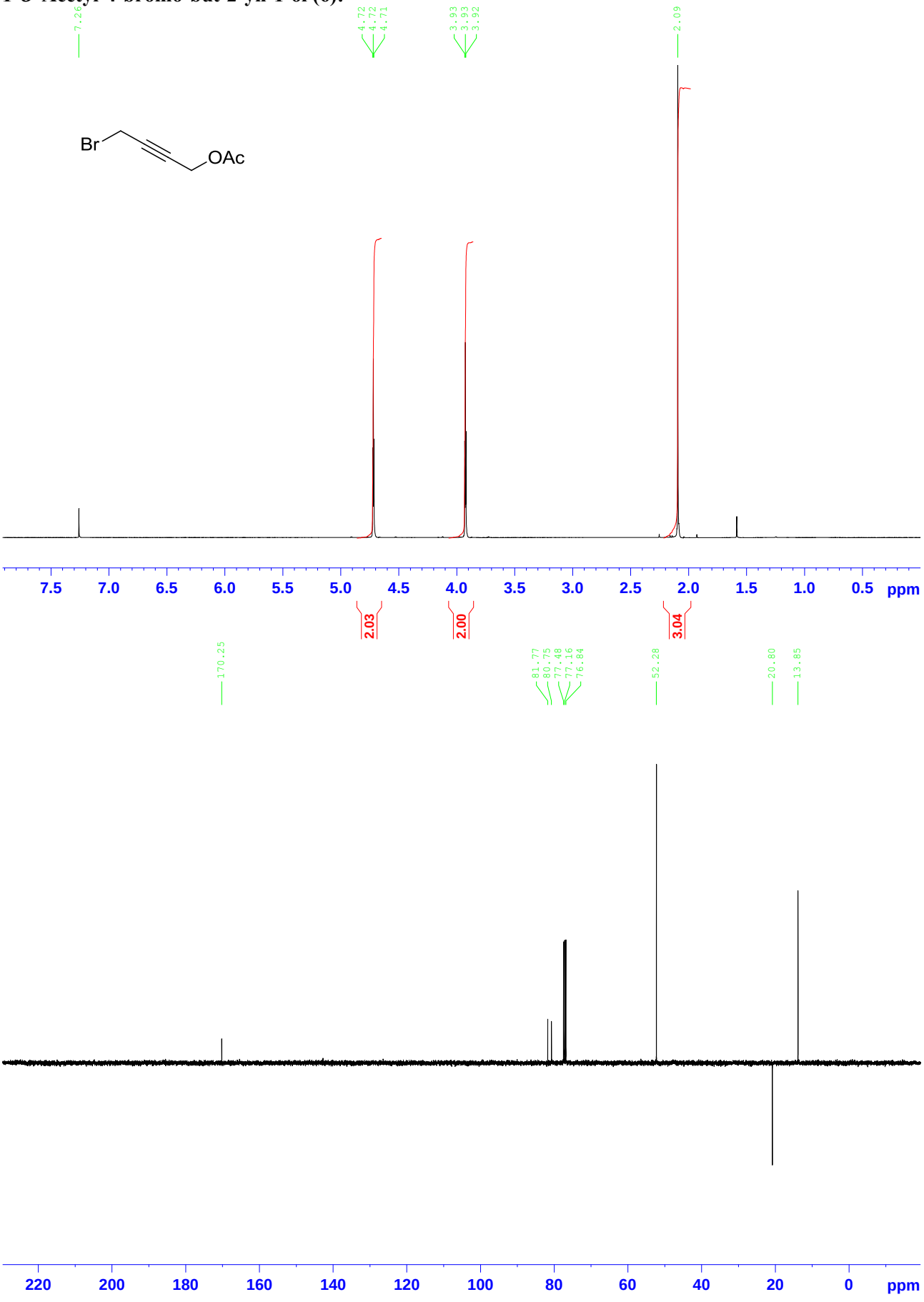
2-Deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-1-*O*-methyl-D-*erythro*-pentitol (4a):



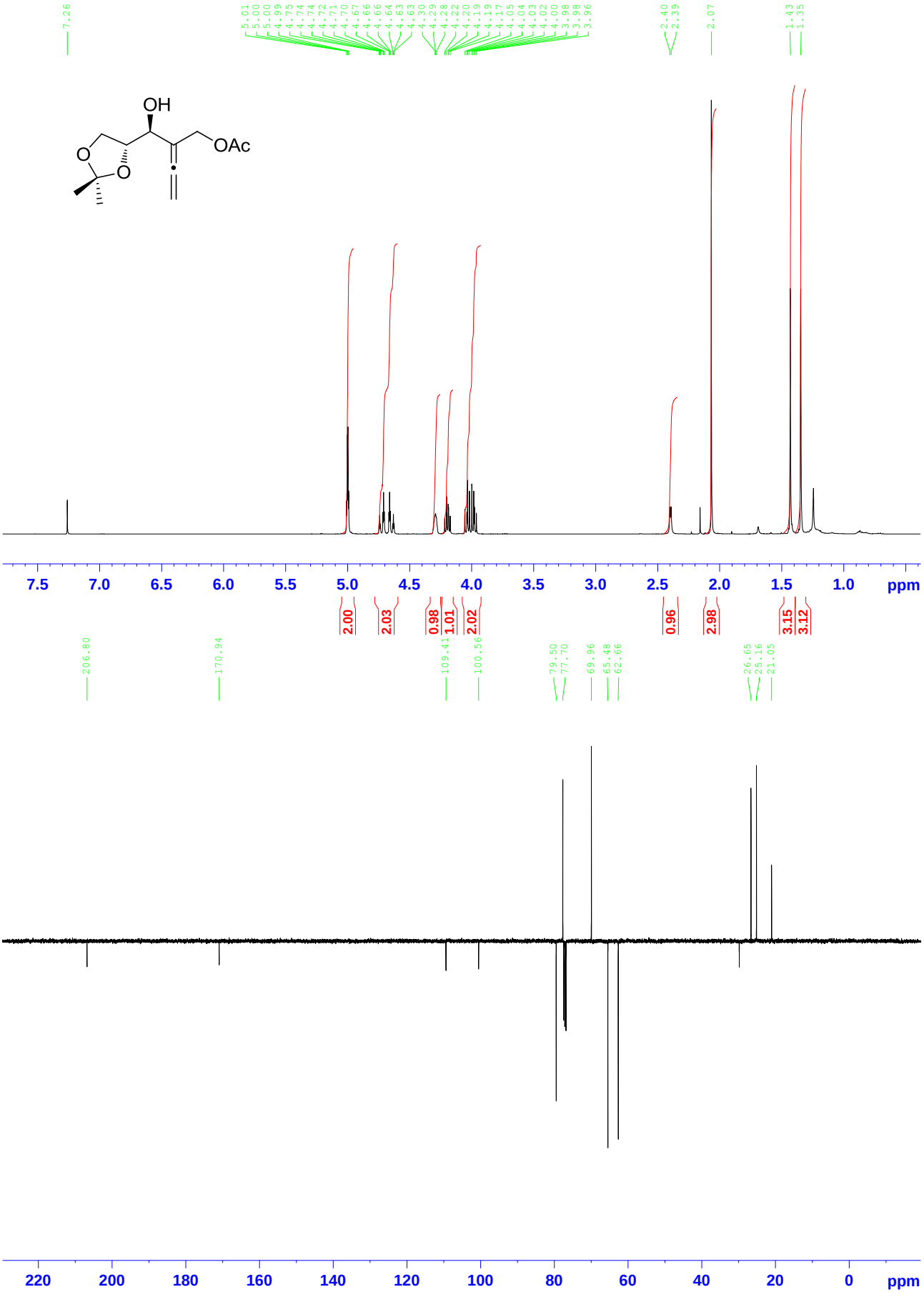
2-Deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (5a):



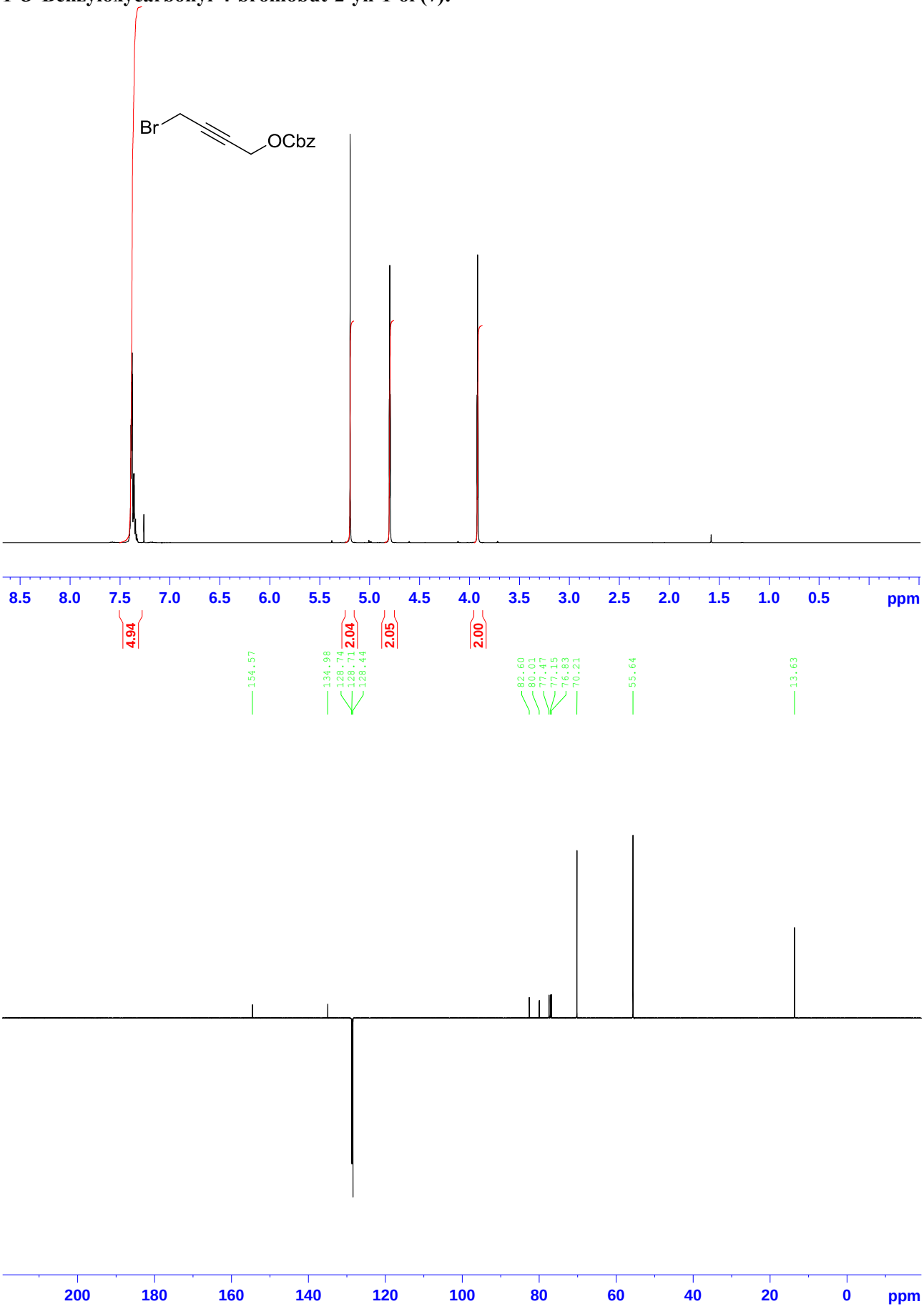
1-*O*-Acetyl-4-bromo-but-2-yn-1-ol (6):



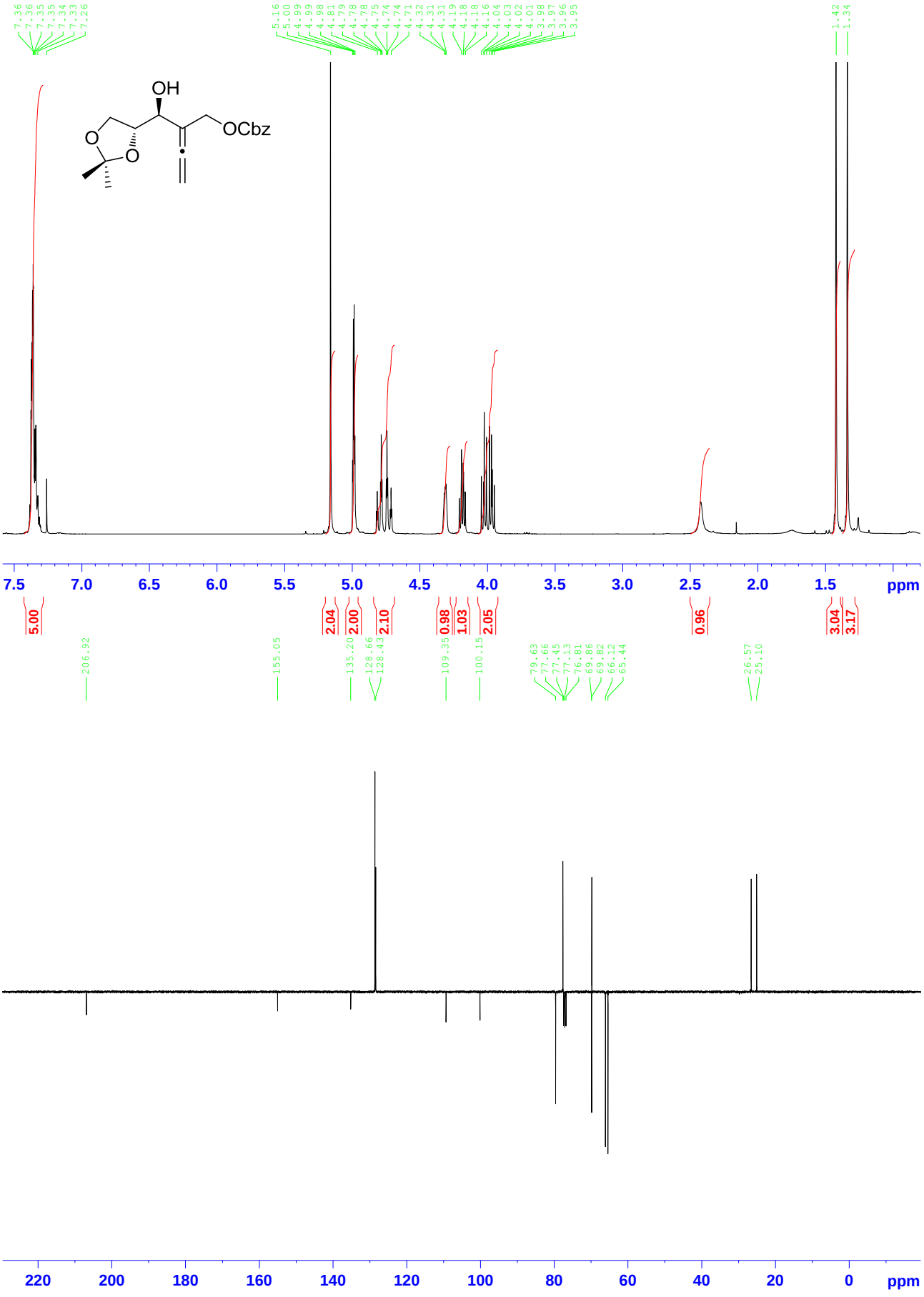
1-*O*-Acetyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (6a):



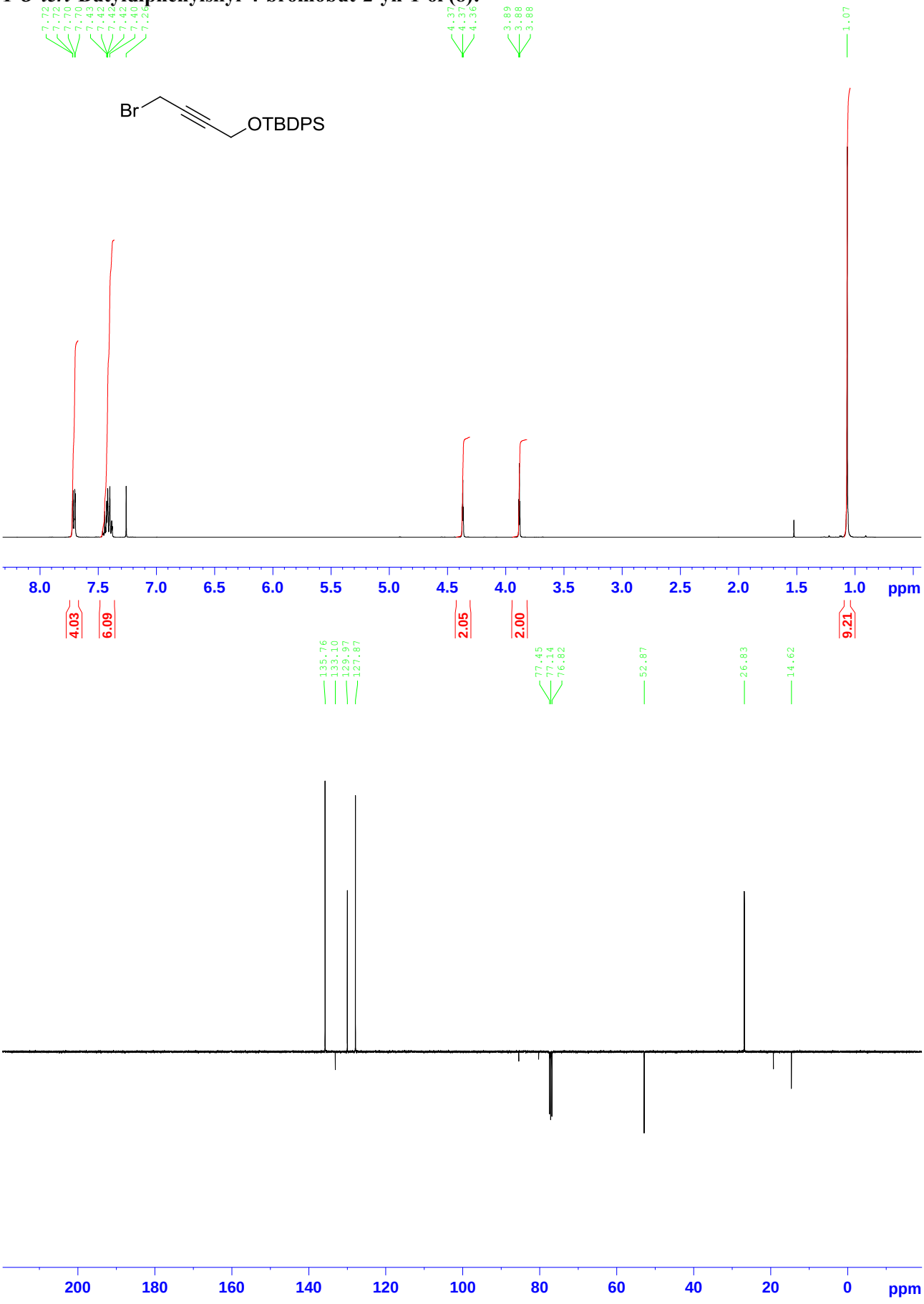
1-*O*-Benzyloxycarbonyl-4-bromobut-2-yn-1-ol (7):



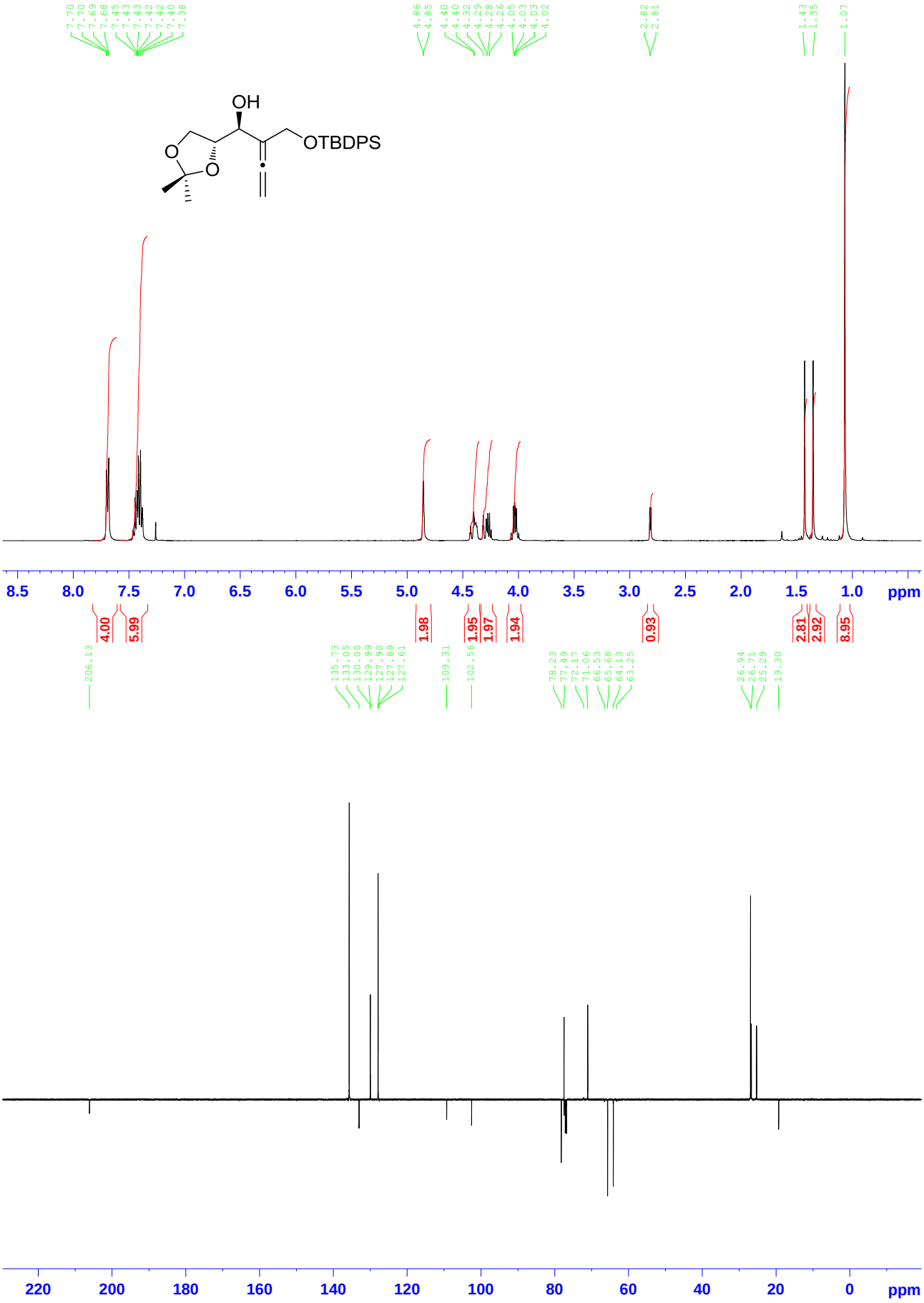
1-*O*-Carbonyloxybenzyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (7a):



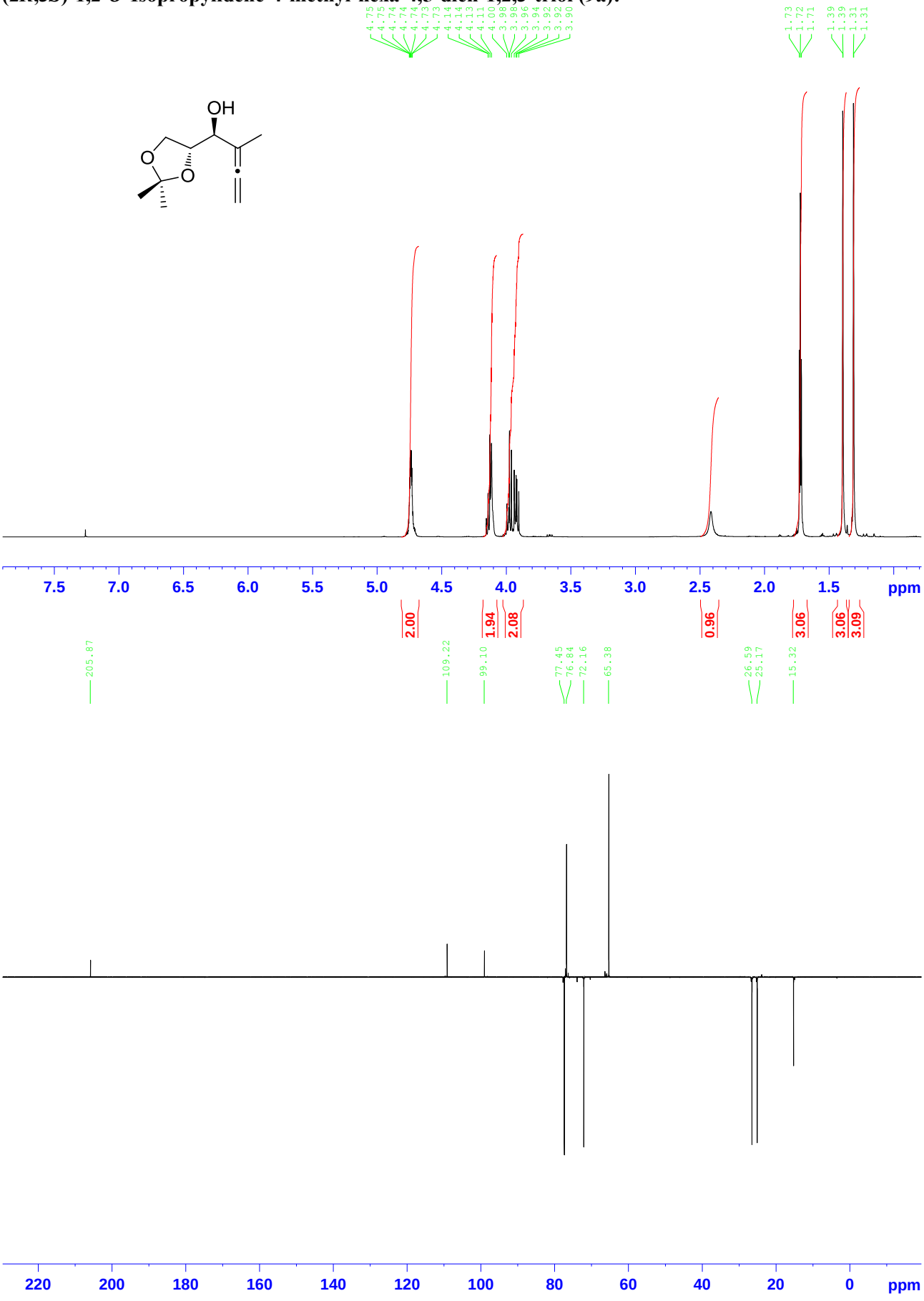
1-*O*-*tert*-Butyldiphenylsilyl-4-bromobut-2-yn-1-ol (8):



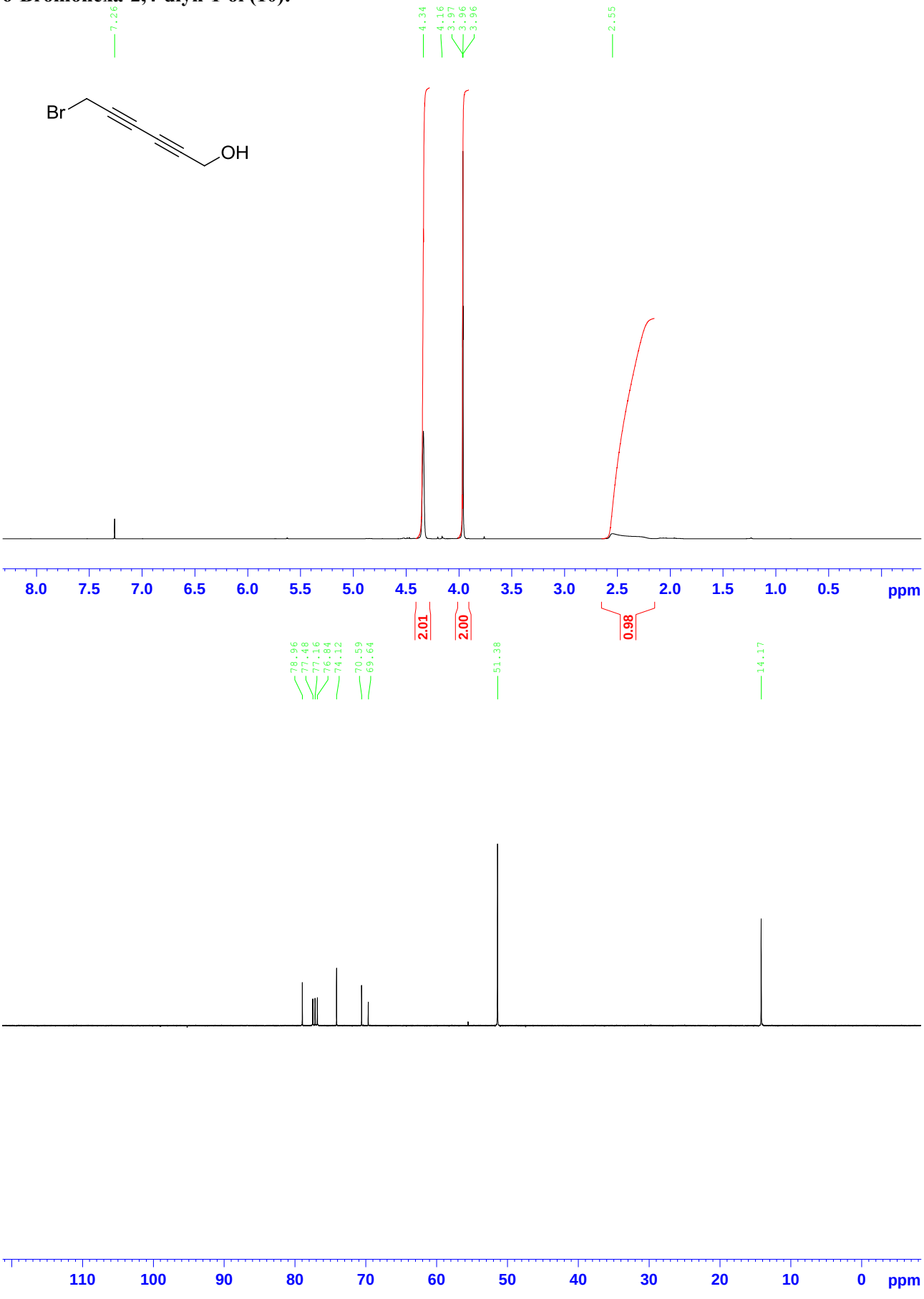
1-*O*-*tert*-Butyldiphenylsilyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (8a):



(2*R*,3*S*)-1,2-*O*-Isopropylidene-4-methyl-hexa-4,5-dien-1,2,3-triol (9a):

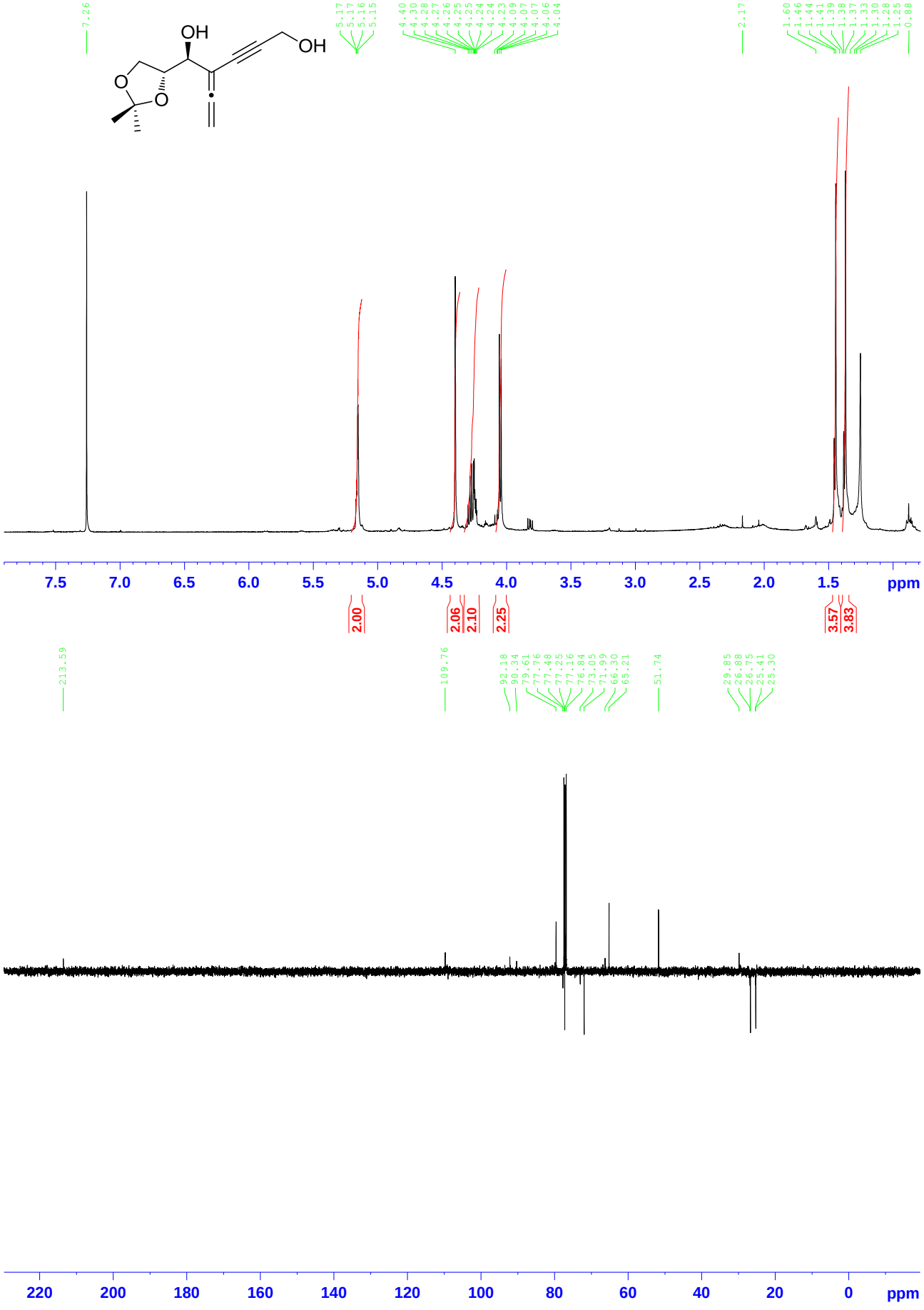


6-Bromohexa-2,4-diyn-1-ol (10):

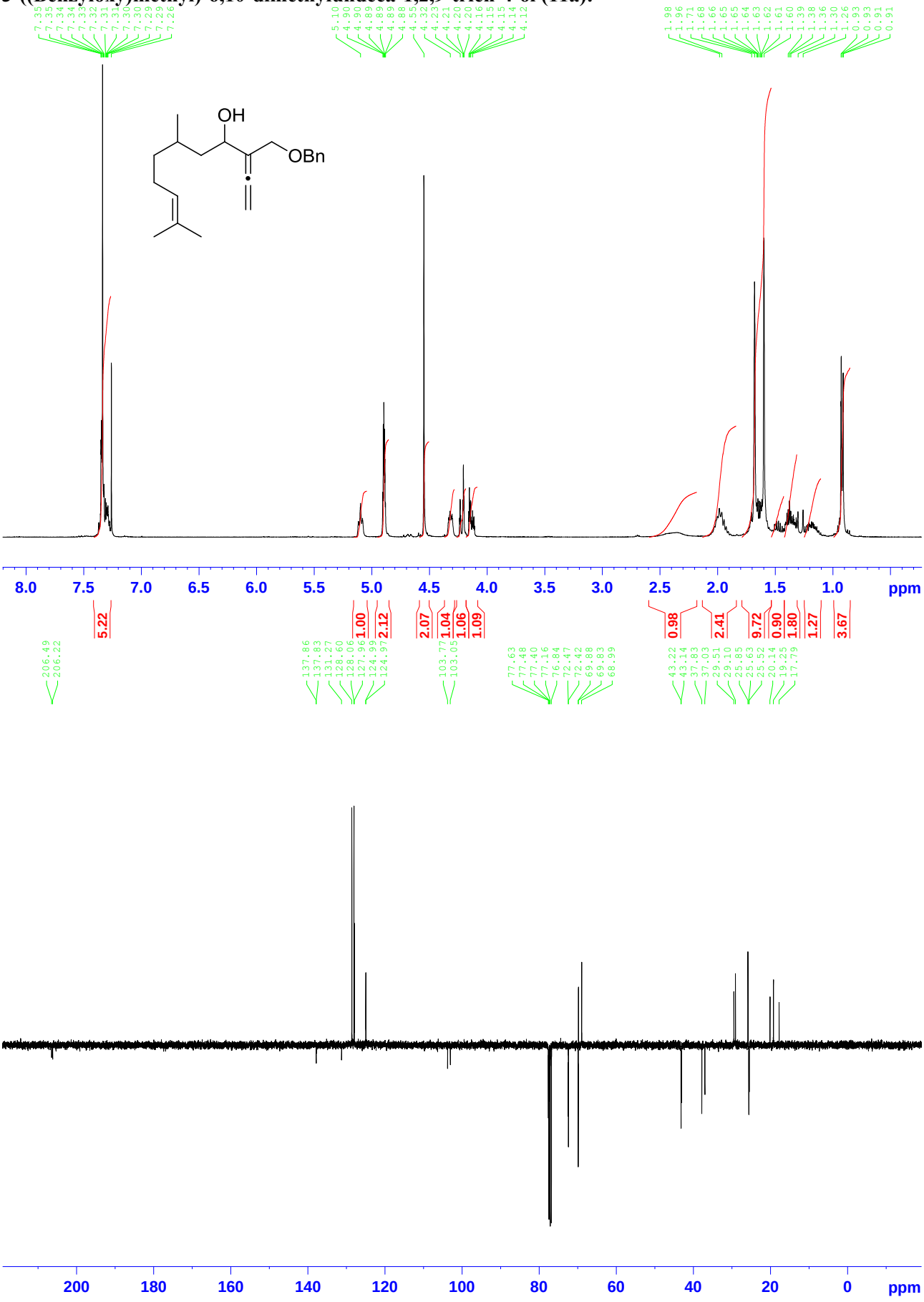


(2*R*,3*S*)-4-Ethenylidene-1,2-O-isopropylidene-hept-5-yne-1,2,3,7-tetrol

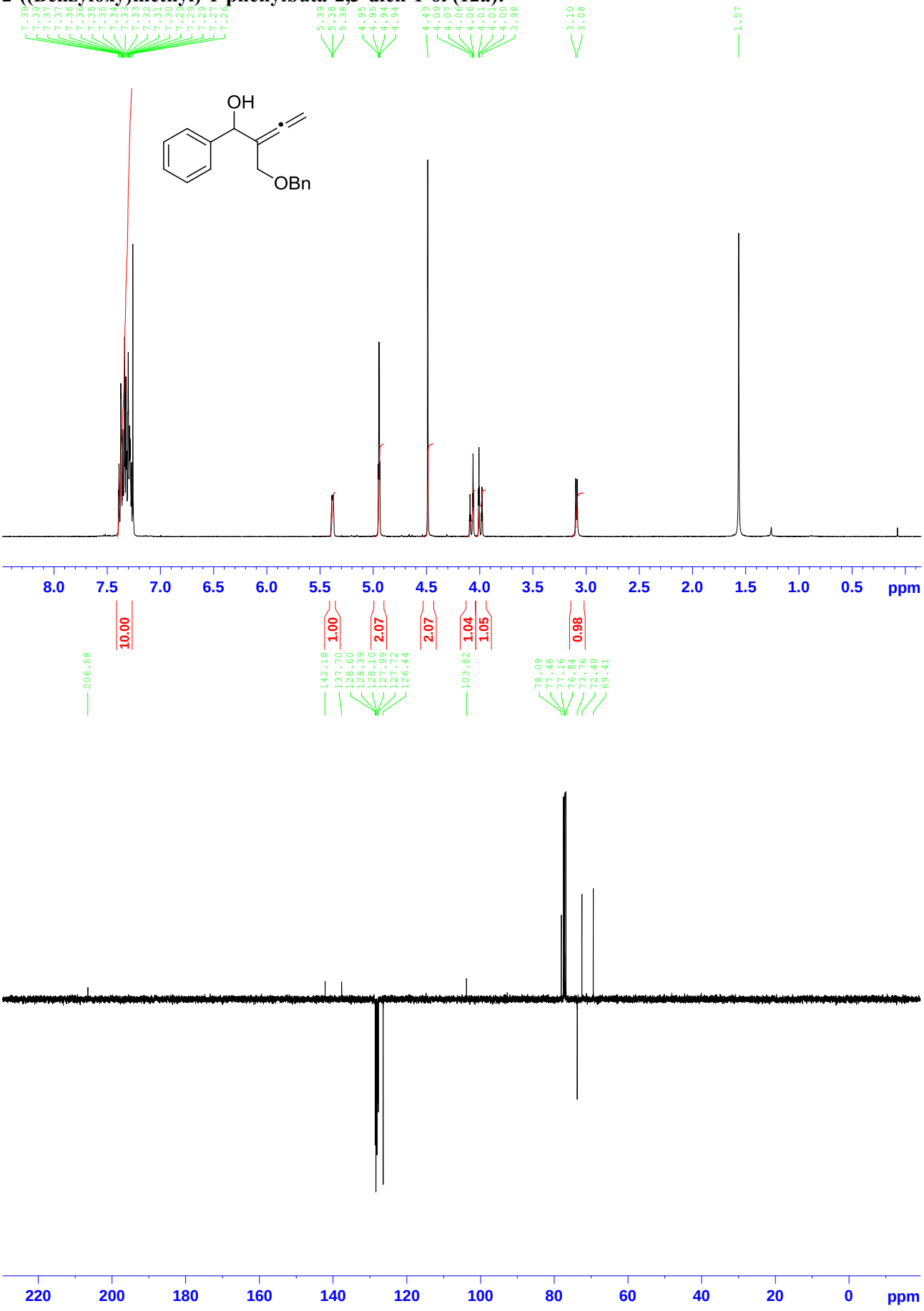
(10a)



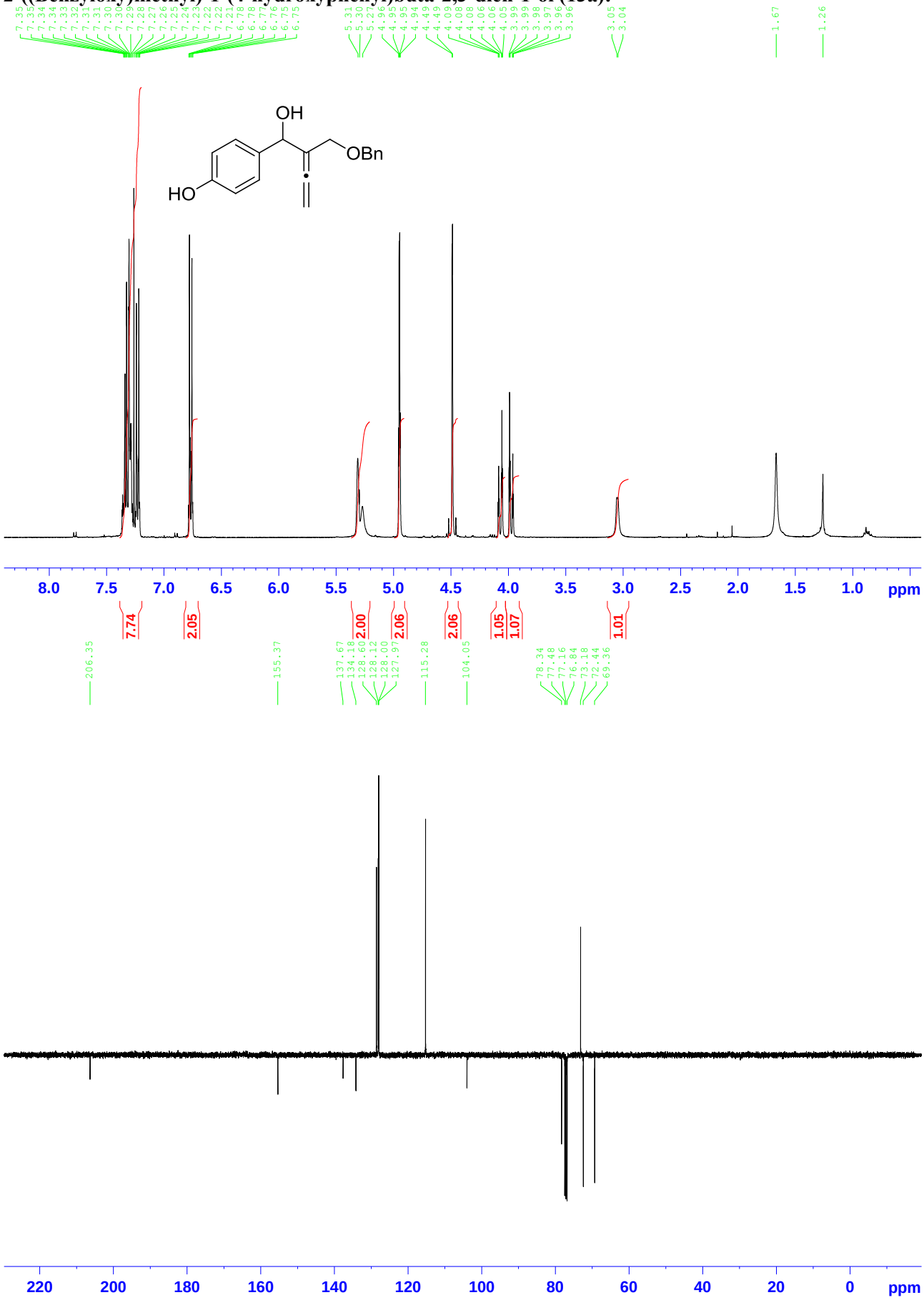
3-((Benzyloxy)methyl)-6,10-dimethylundeca-1,2,9-trien-4-ol (11a):



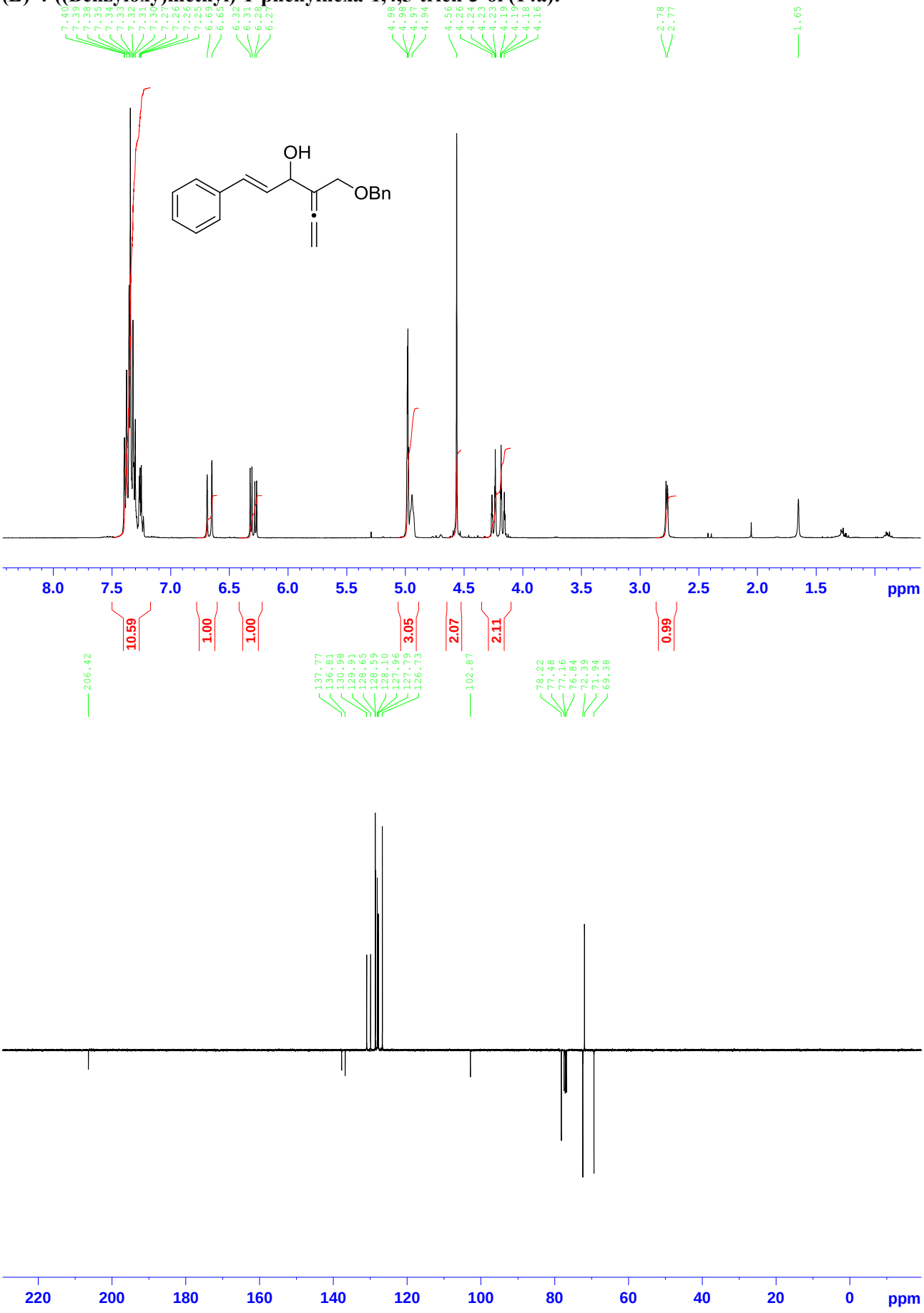
2-((Benzyloxy)methyl)-1-phenylbuta-2,3-dien-1-ol (12a):



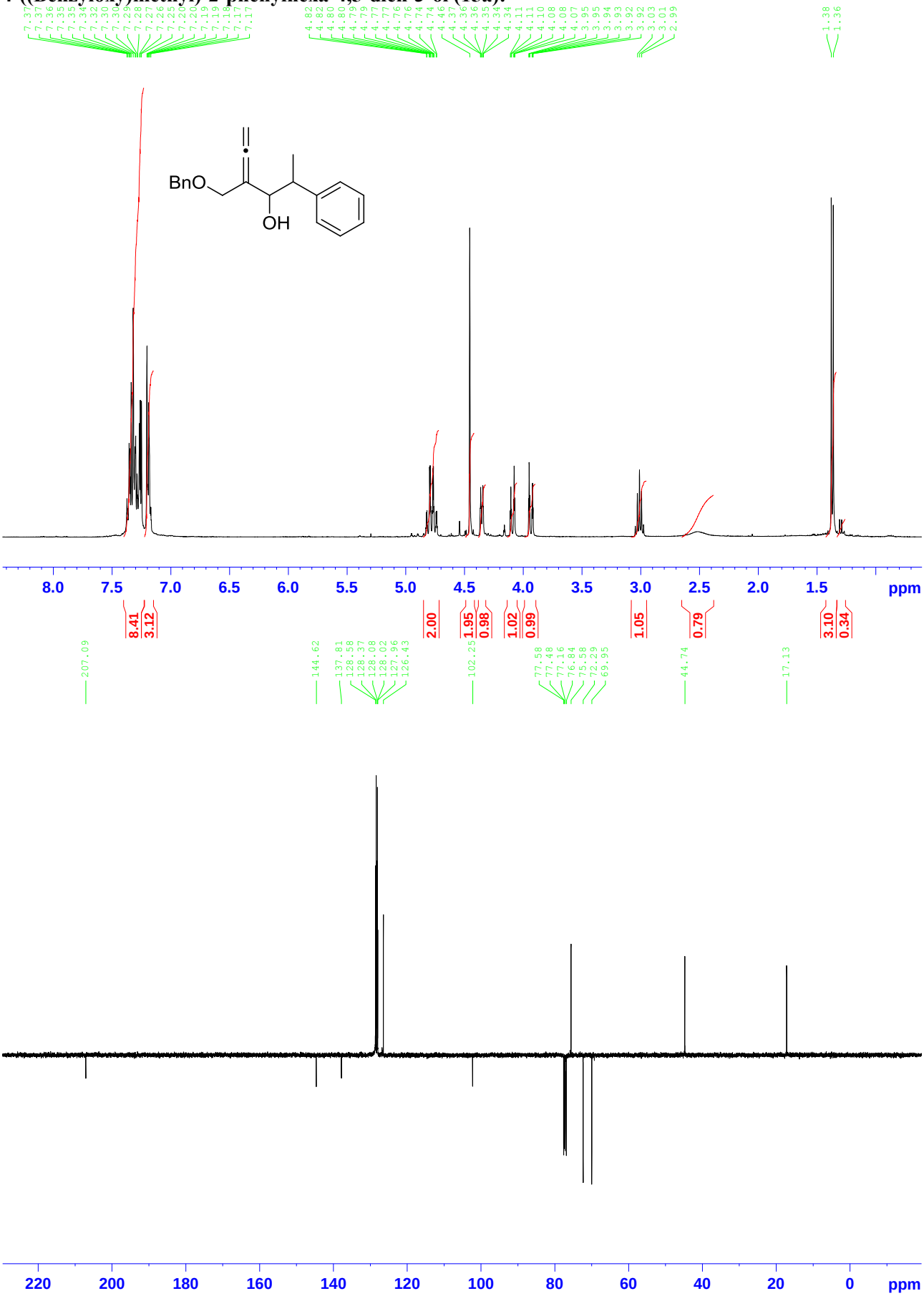
2-((Benzyloxy)methyl)-1-(4-hydroxyphenyl)buta-2,3-dien-1-ol (13a):



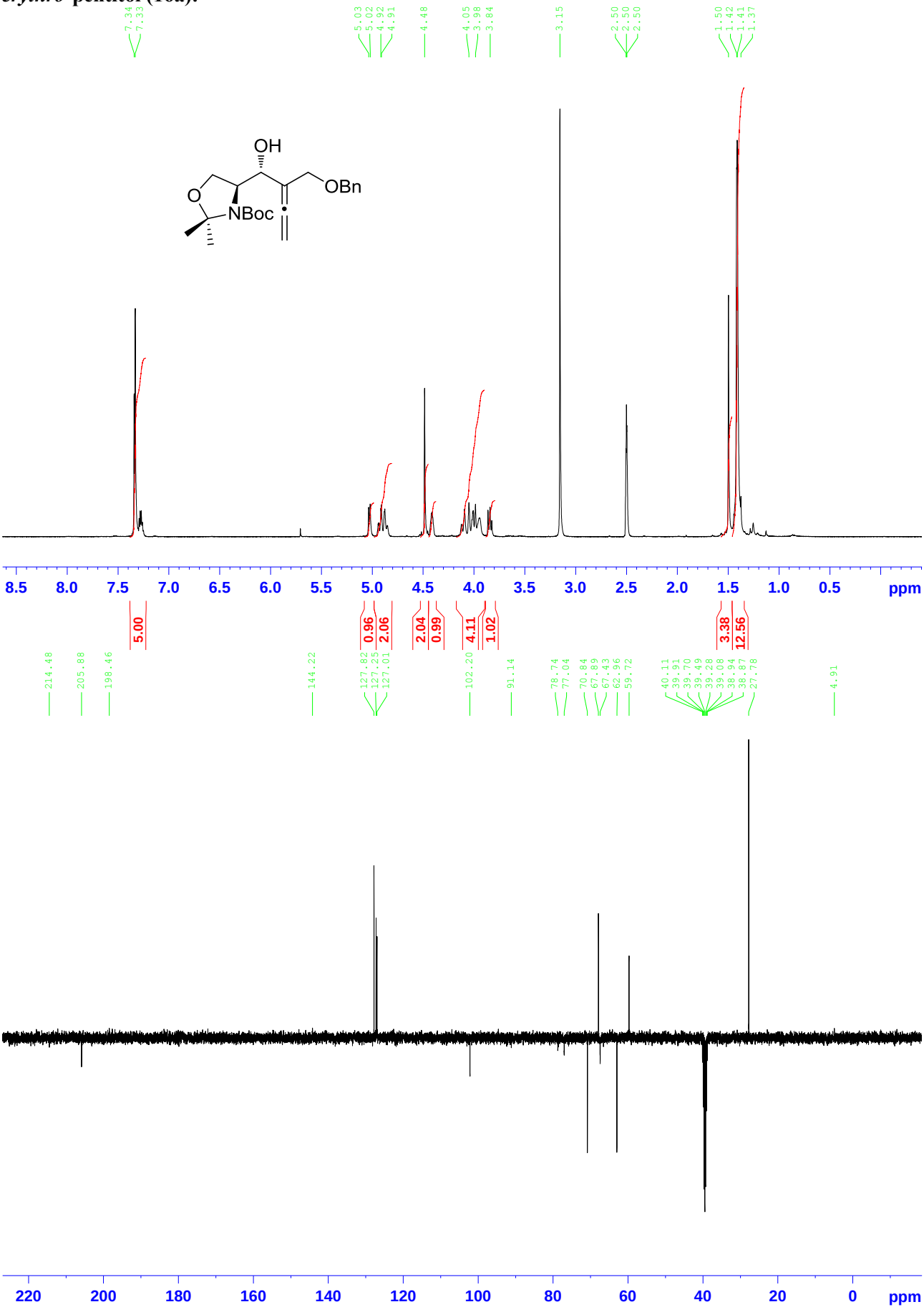
(*E*)-4-((Benzyloxy)methyl)-1-phenylhexa-1,4,5-trien-3-ol (14a):



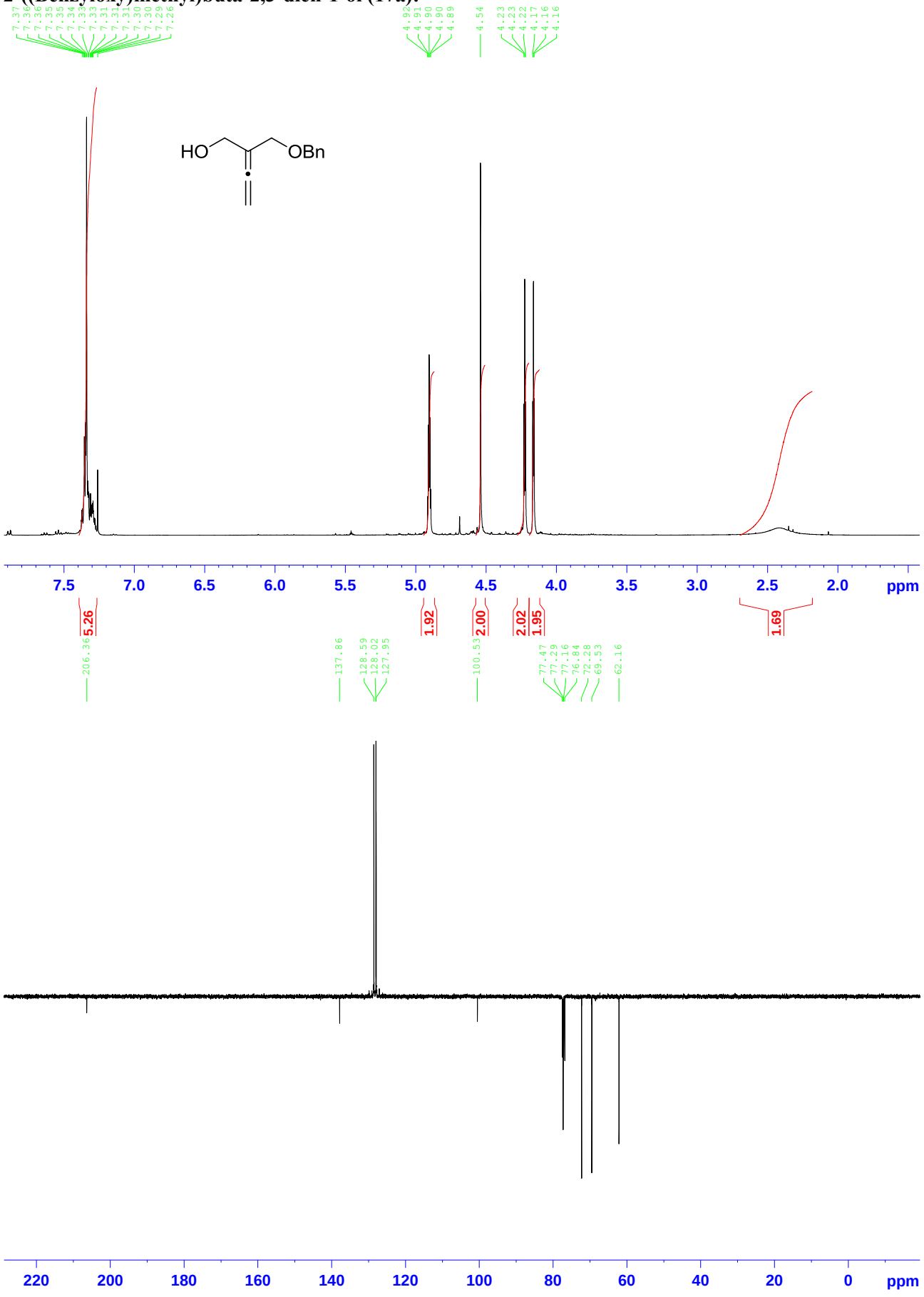
4-((Benzyloxy)methyl)-2-phenylhexa-4,5-dien-3-ol (15a):



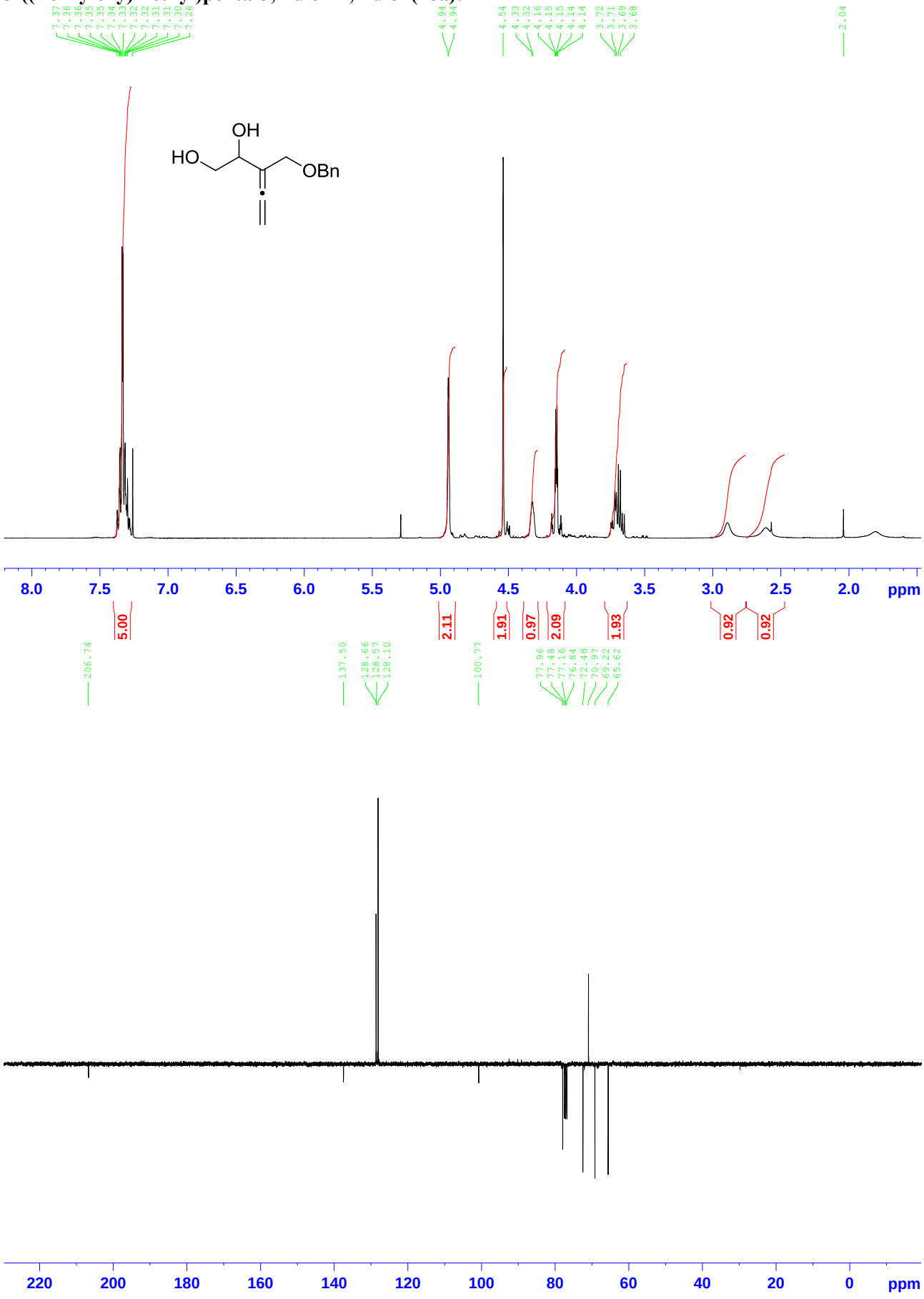
1-*O*-Benzyl-4-(*tert*-butyloxycarbonyl)amino-2,4-dideoxy-2-*C*-ethenylidene-4,5-*N*,*O*-isopropylidene-L-*erythro*-pentitol (16a):



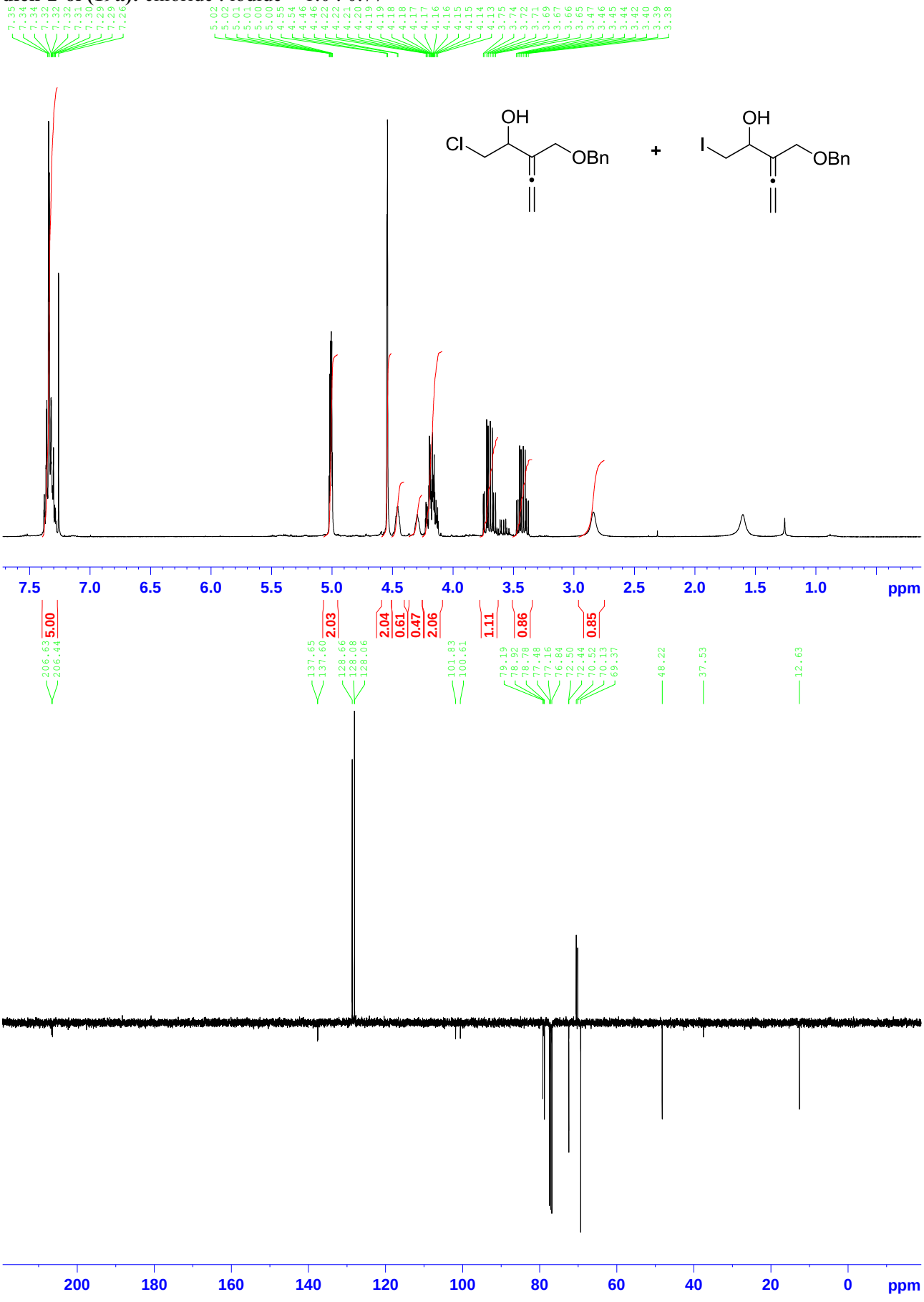
2-((Benzyloxy)methyl)buta-2,3-dien-1-ol (17a):



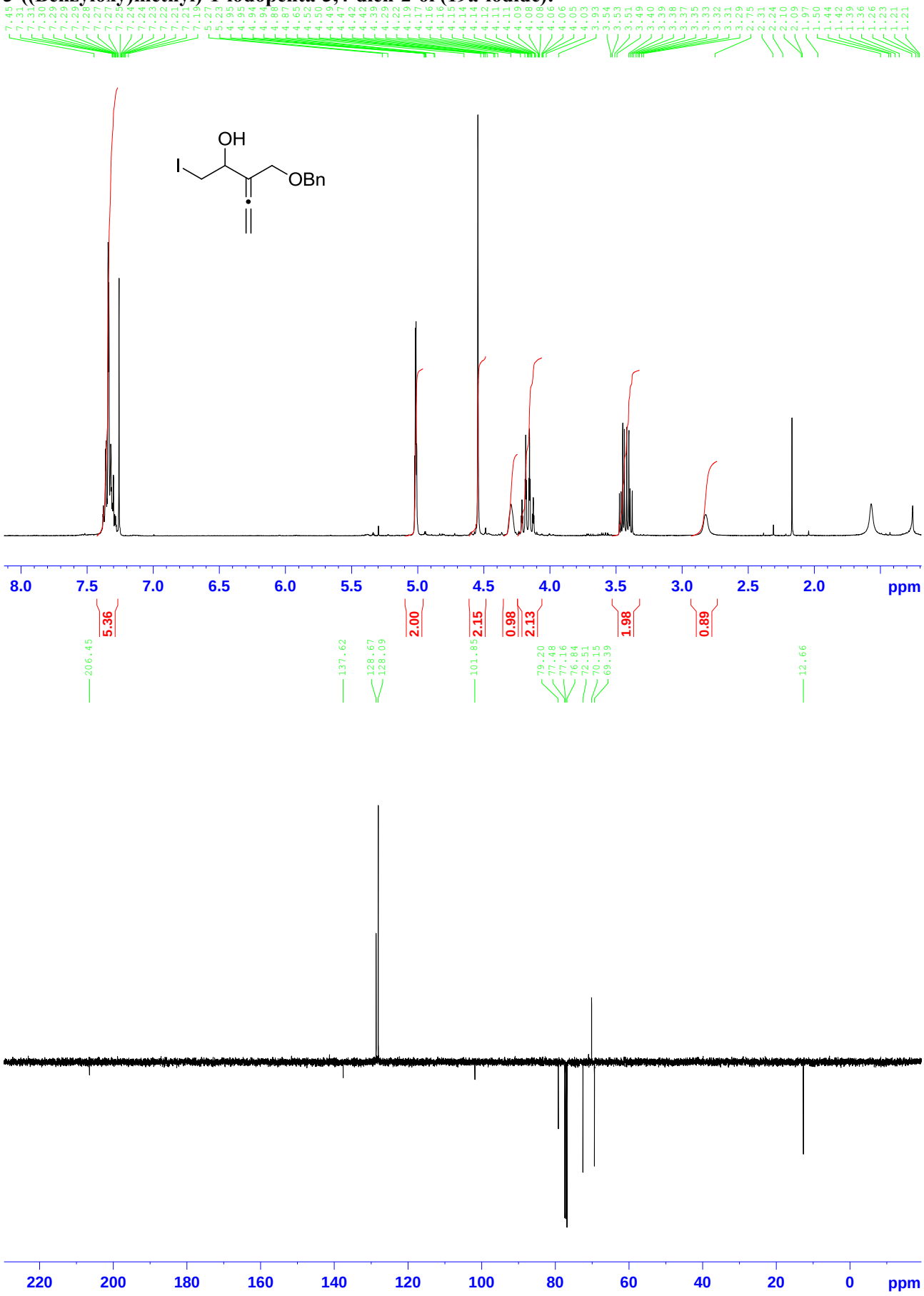
3-((Benzyloxy)methyl)penta-3,4-dien-1,2-diol (18a):



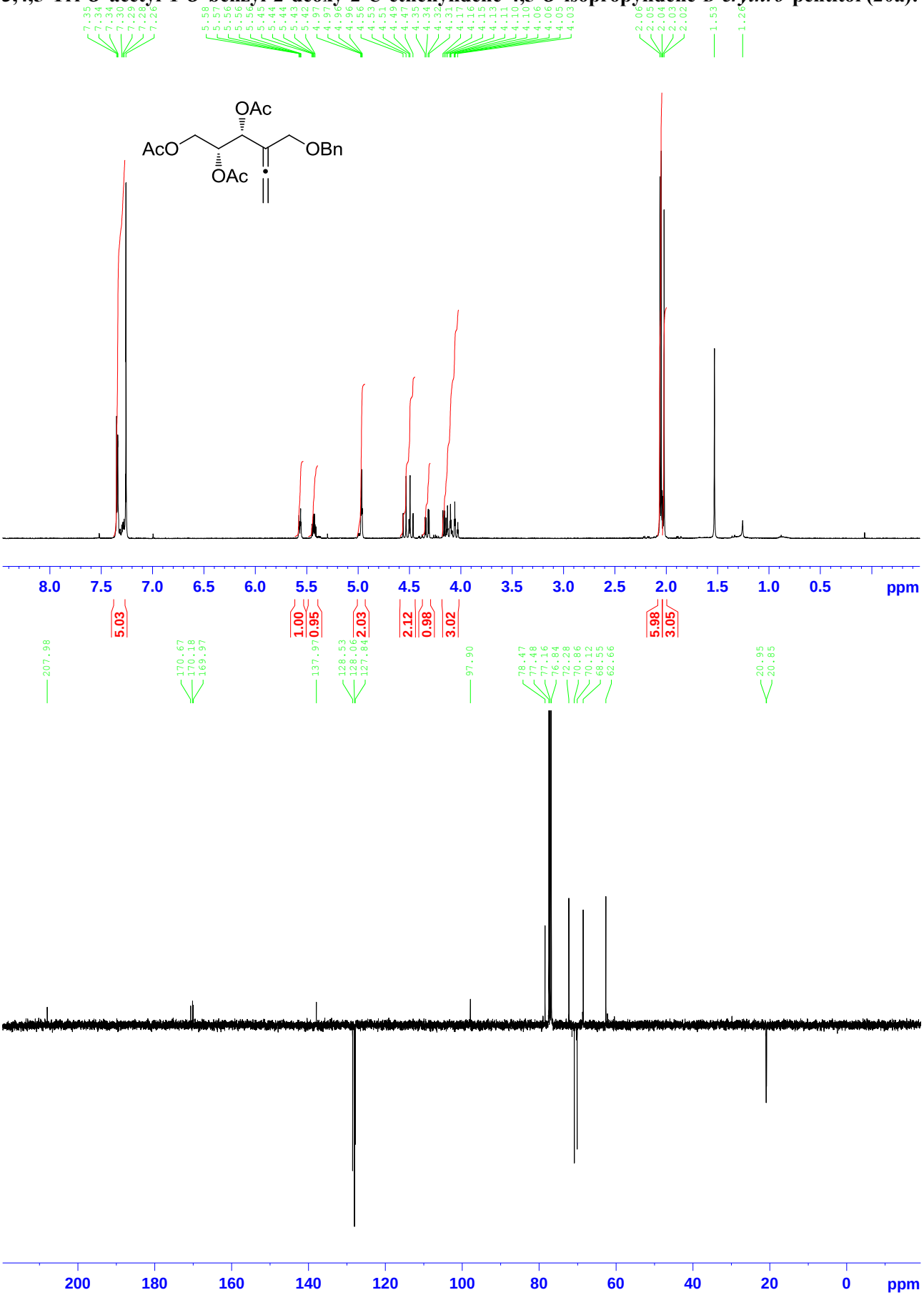
Mixture of 3-((Benzyloxy)methyl)-1-chloropenta-3,4-dien-2-ol and 3-((benzyloxy) methyl)-1-iodopenta-3,4-dien-2-ol (19a): chloride : iodide = 1.0 : 0.77



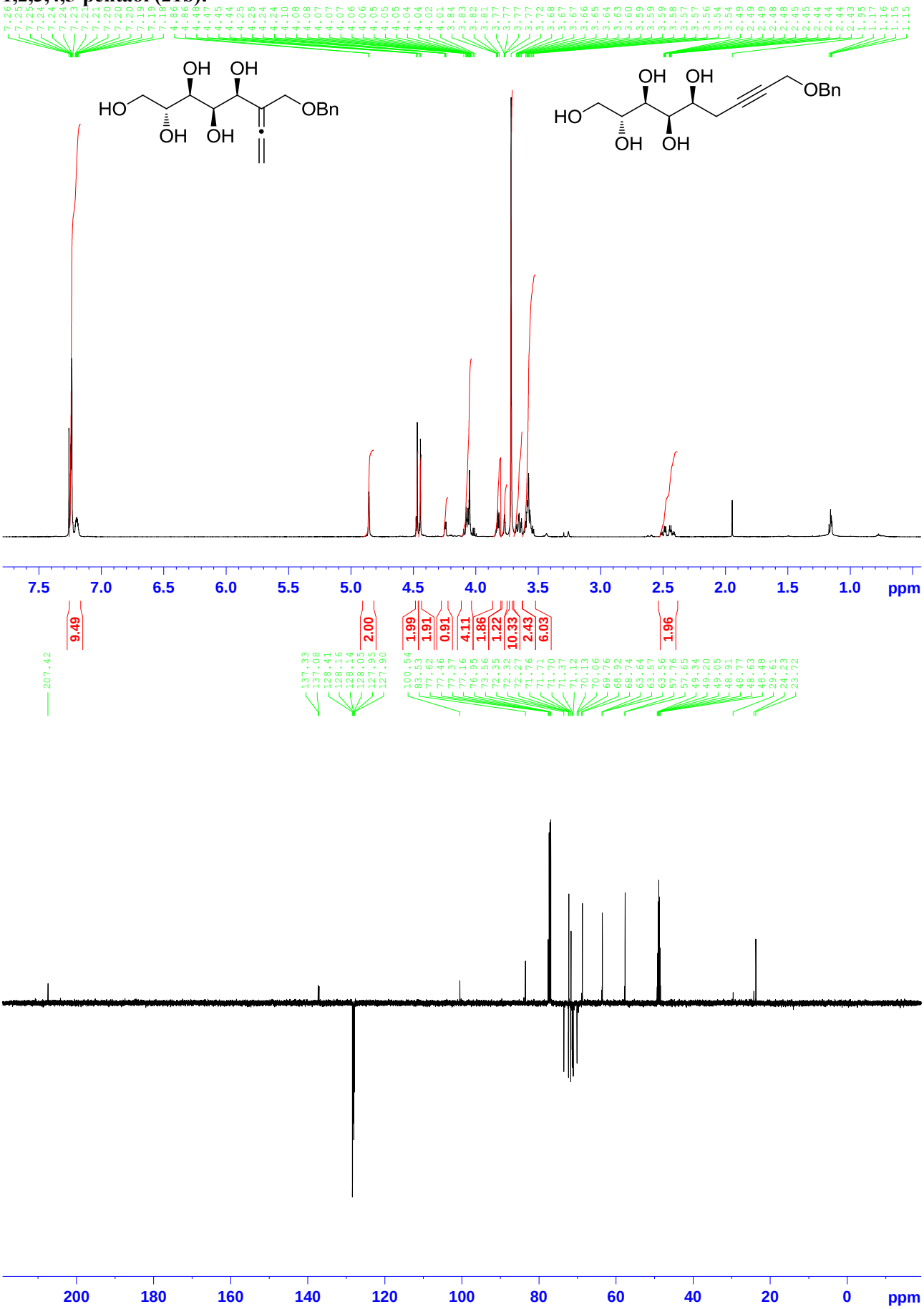
3-((Benzyloxy)methyl)-1-iodopenta-3,4-dien-2-ol (19a-iodide):



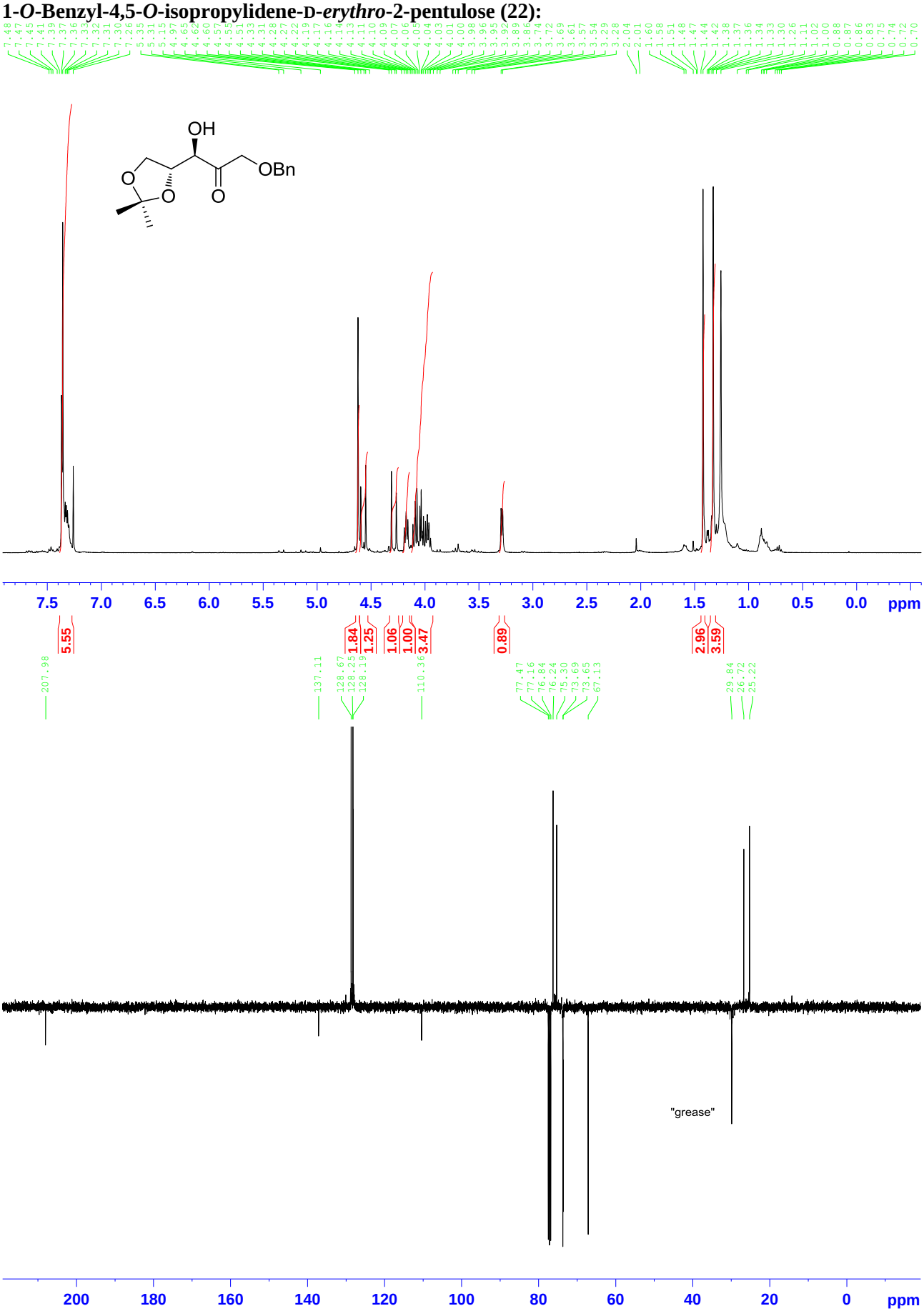
3,4,5-Tri-*O*-acetyl-1-*O*-benzyl-2-deoxy-2-*C*-ethenylidene-4,5-*O*-isopropylidene-D-*erythro*-pentitol (20a):



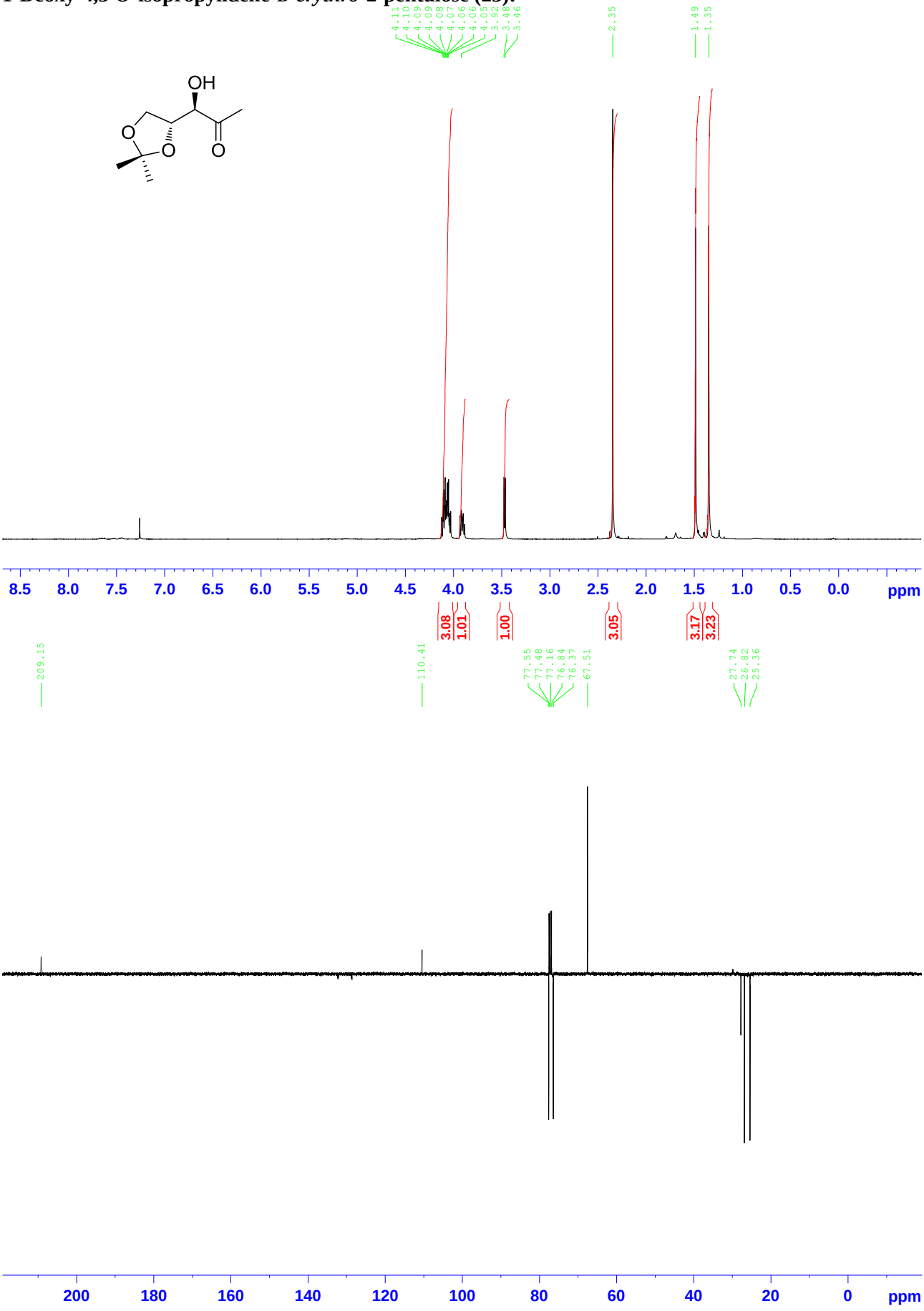
1-*O*-Benzyl-2-deoxy-2-*C*-ethenylidene-D-gluco-heptitol (21a) and (2R,3R,4R)-9-(benzyloxy)non-7-yne-1,2,3,4,5-pentaol (21b):



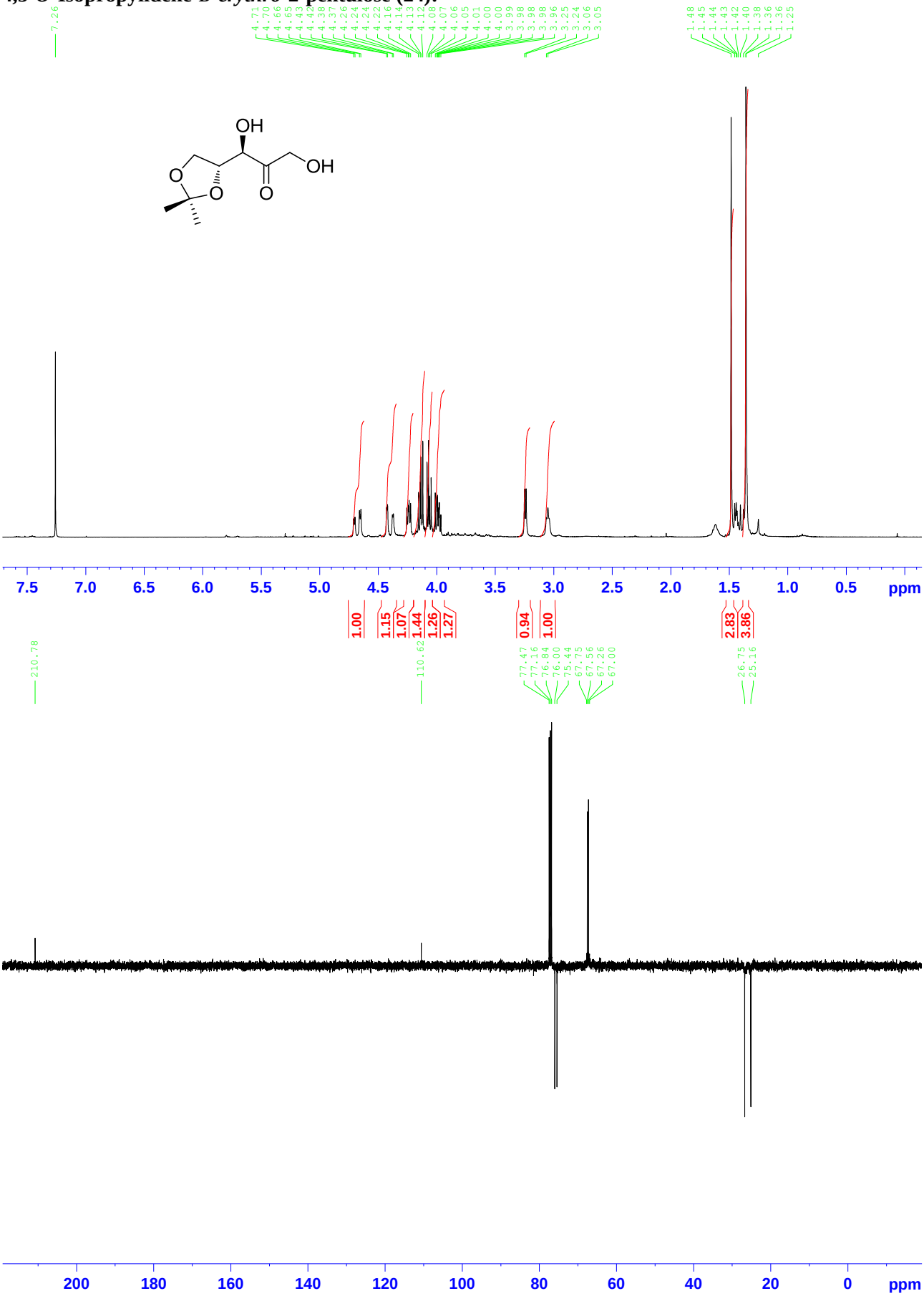
1-*O*-Benzyl-4,5-*O*-isopropylidene-D-*erythro*-2-pentulose (22):



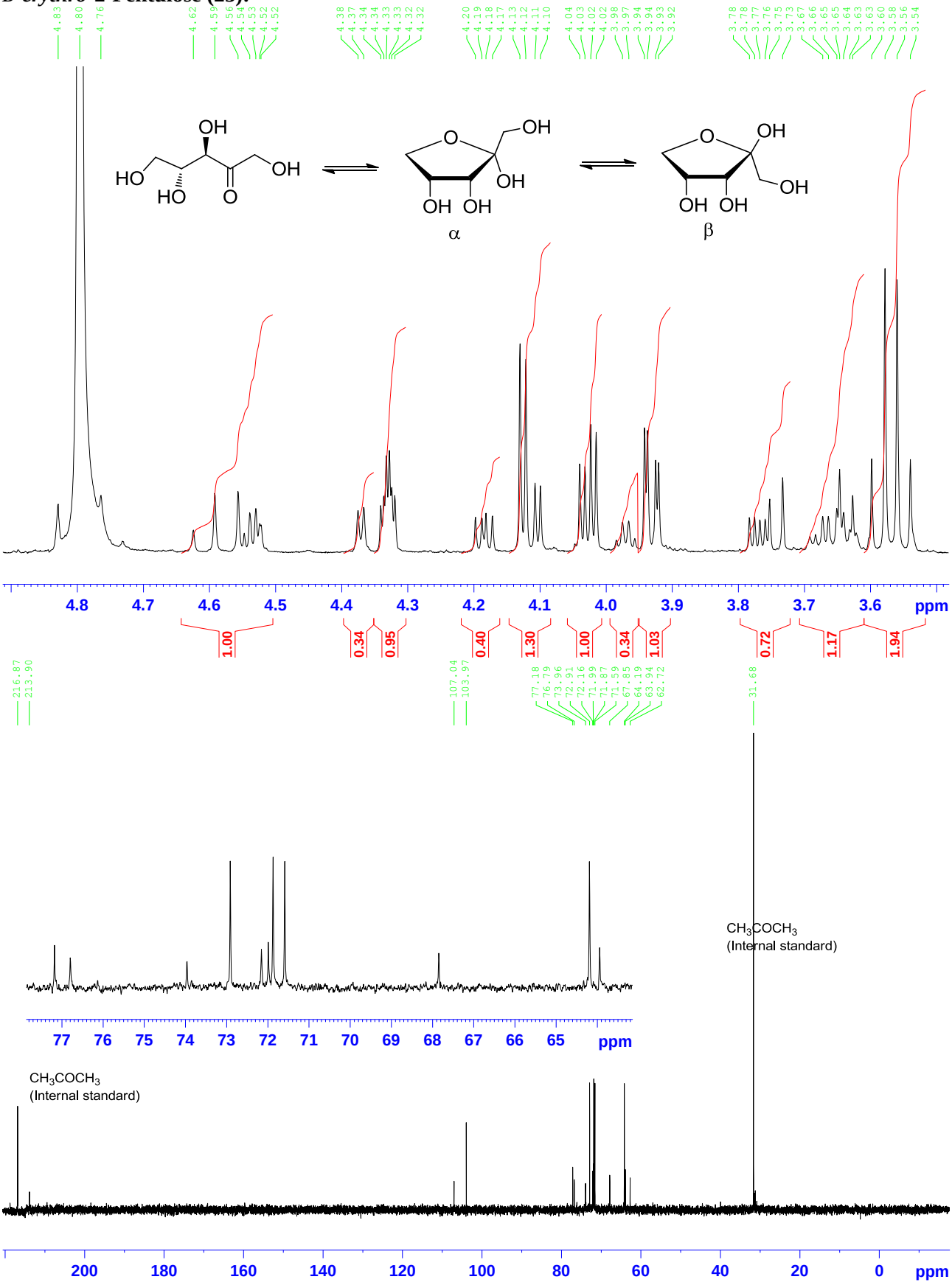
1-Deoxy-4,5-*O*-isopropylidene-D-*erythro*-2-pentulose (23):



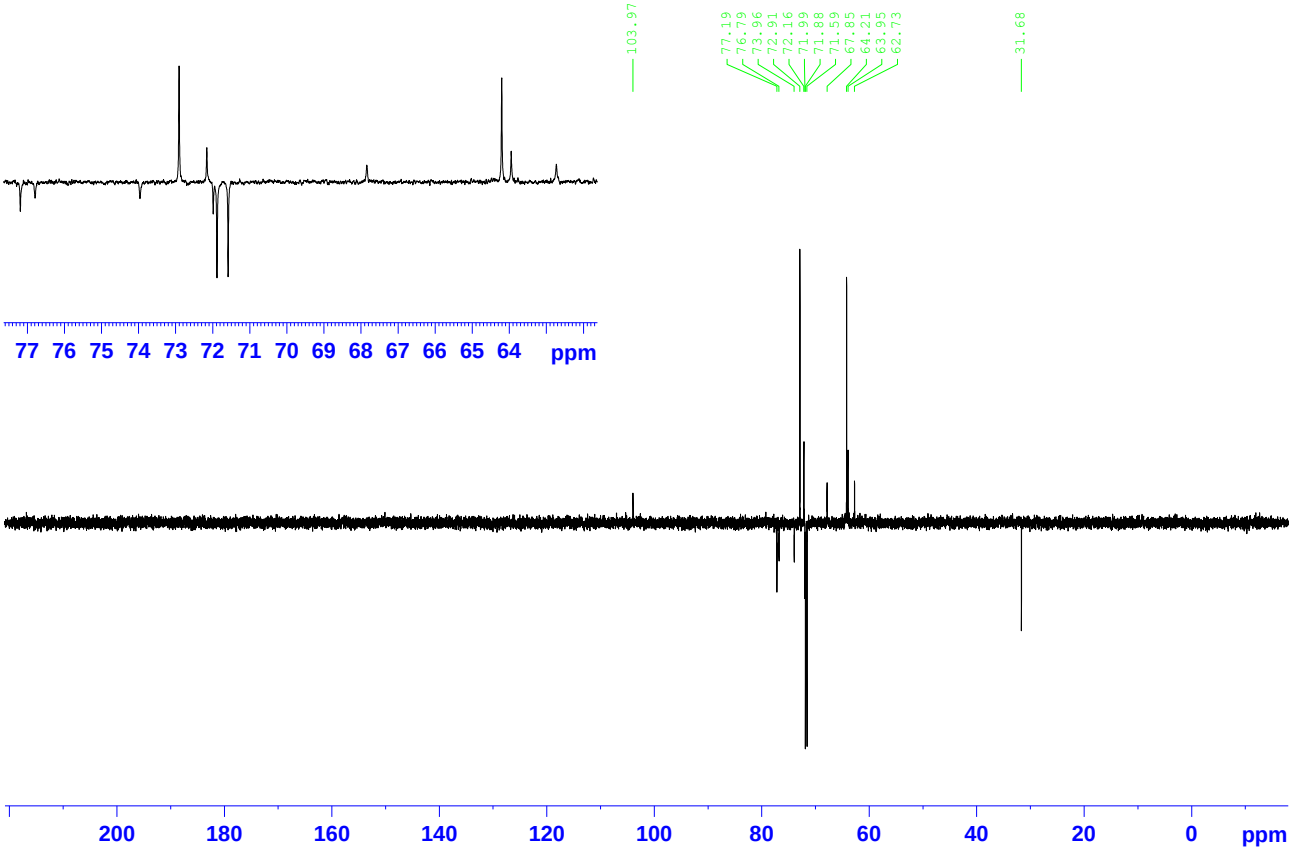
4,5-*O*-Isopropylidene-D-*erythro*-2-pentulose (24):



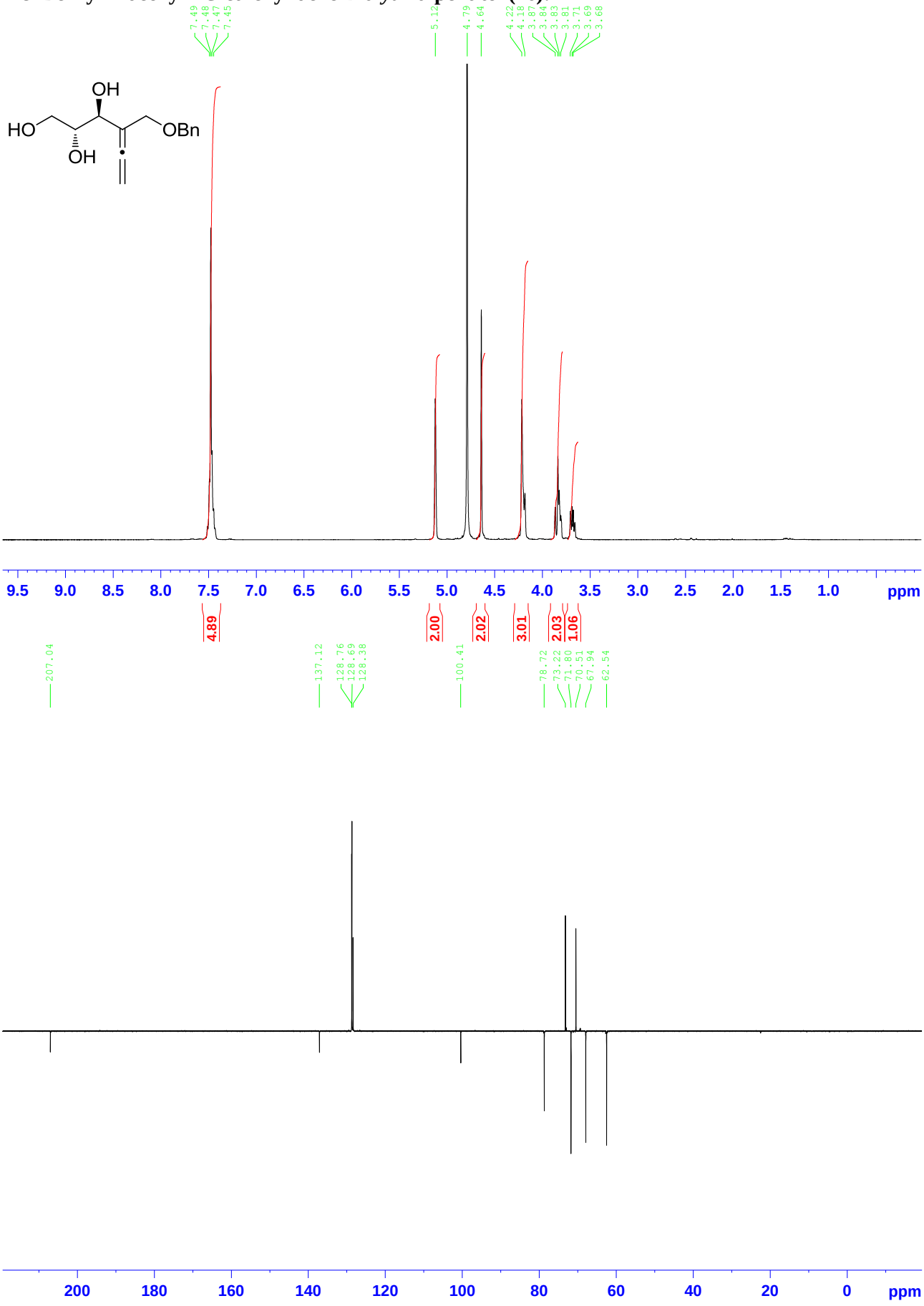
D-*erythro*-2-Pentulose (25):



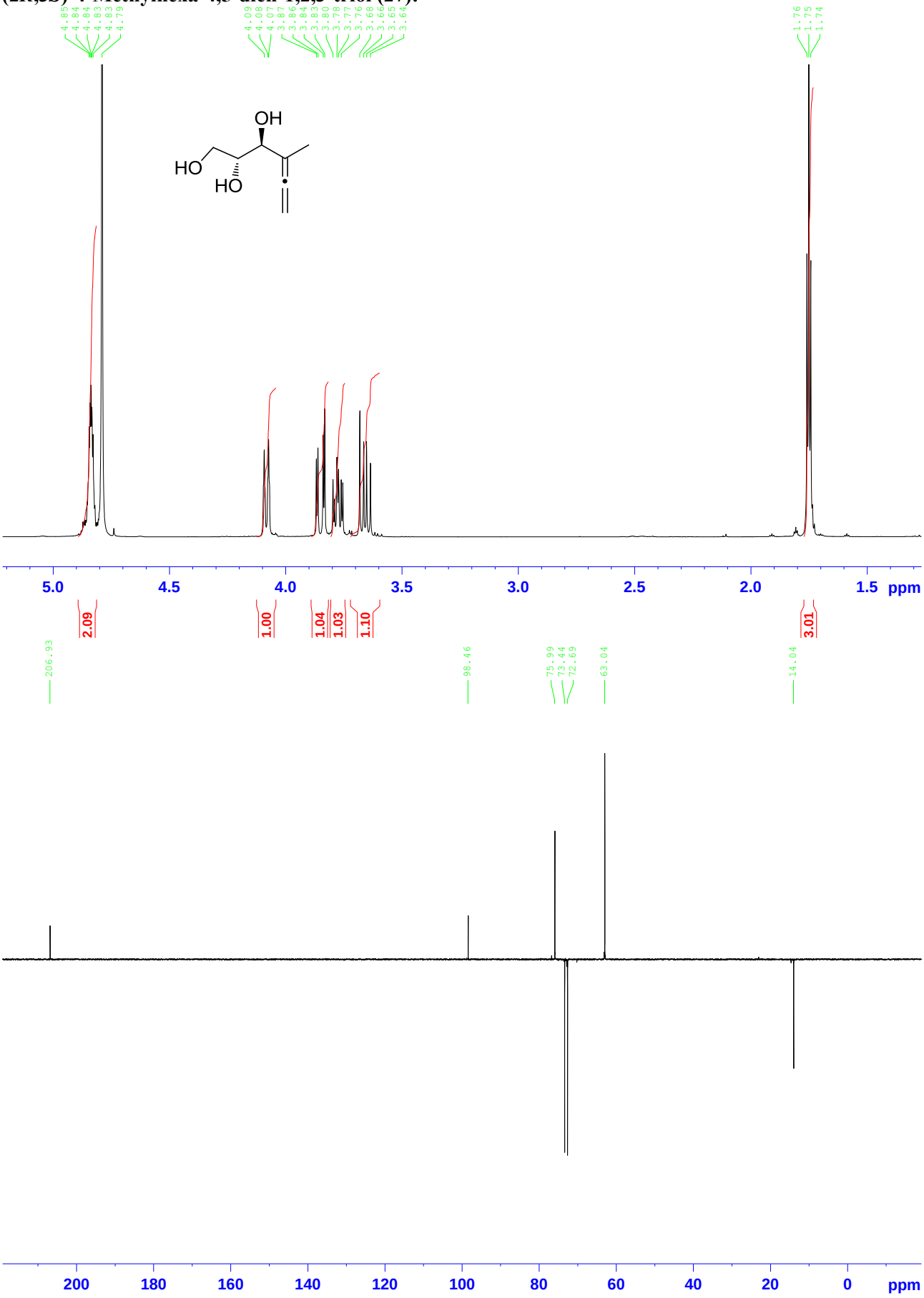
^{13}C NMR(JMOD): For the carbonyl carbon of D-*erythro*-2-pentulose and the quaternary carbon of β-D-*erythro*-2-pentulose see above.



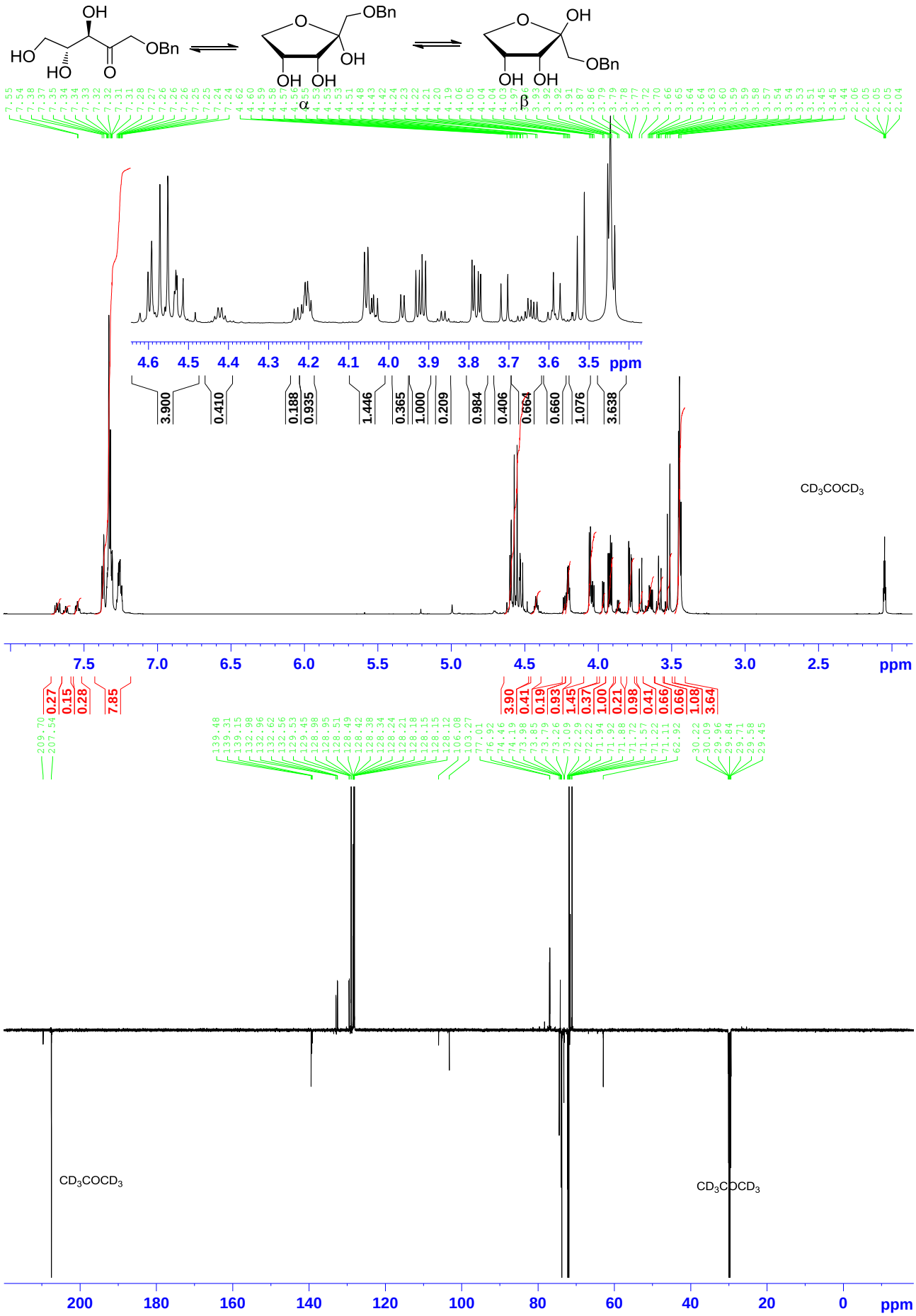
1-*O*-Benzyl-2-deoxy-2-*C*-ethenylidene-D-*erythro*-pentitol (26):



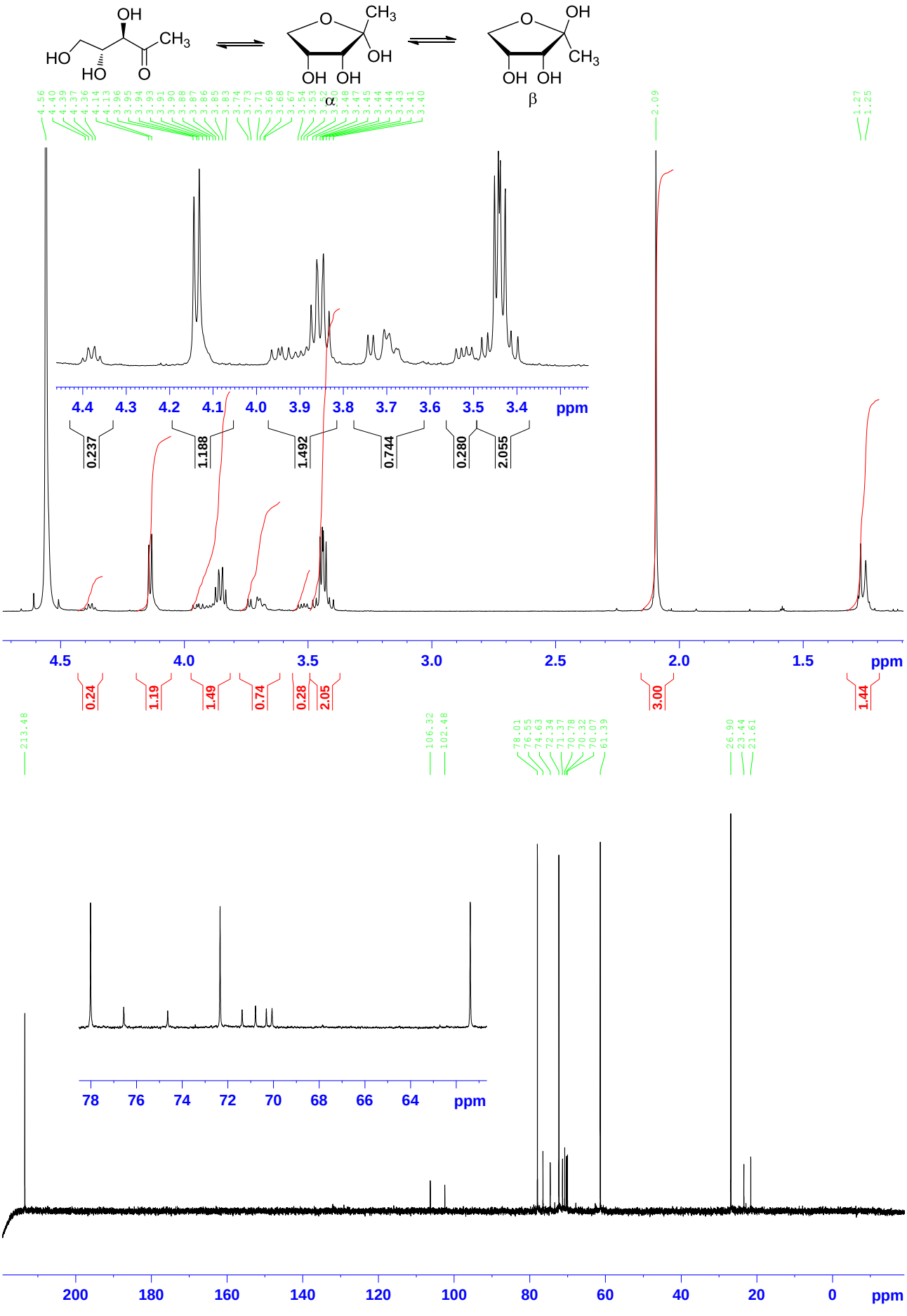
(2*R*,3*S*)-4-Methylhexa-4,5-dien-1,2,3-triol (27):



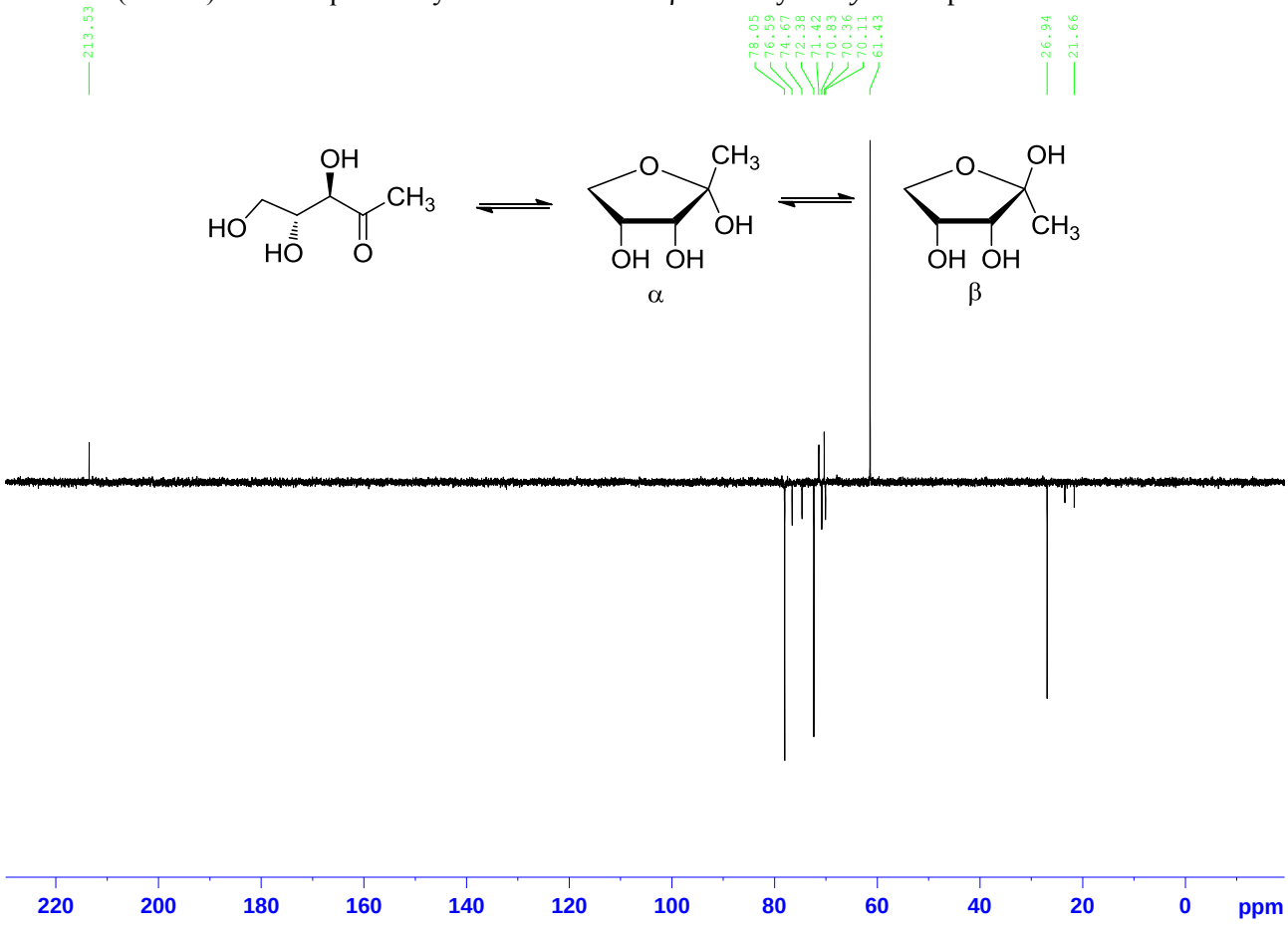
1-*O*-Benzyl-D-*erythro*-2-pentulose (28):



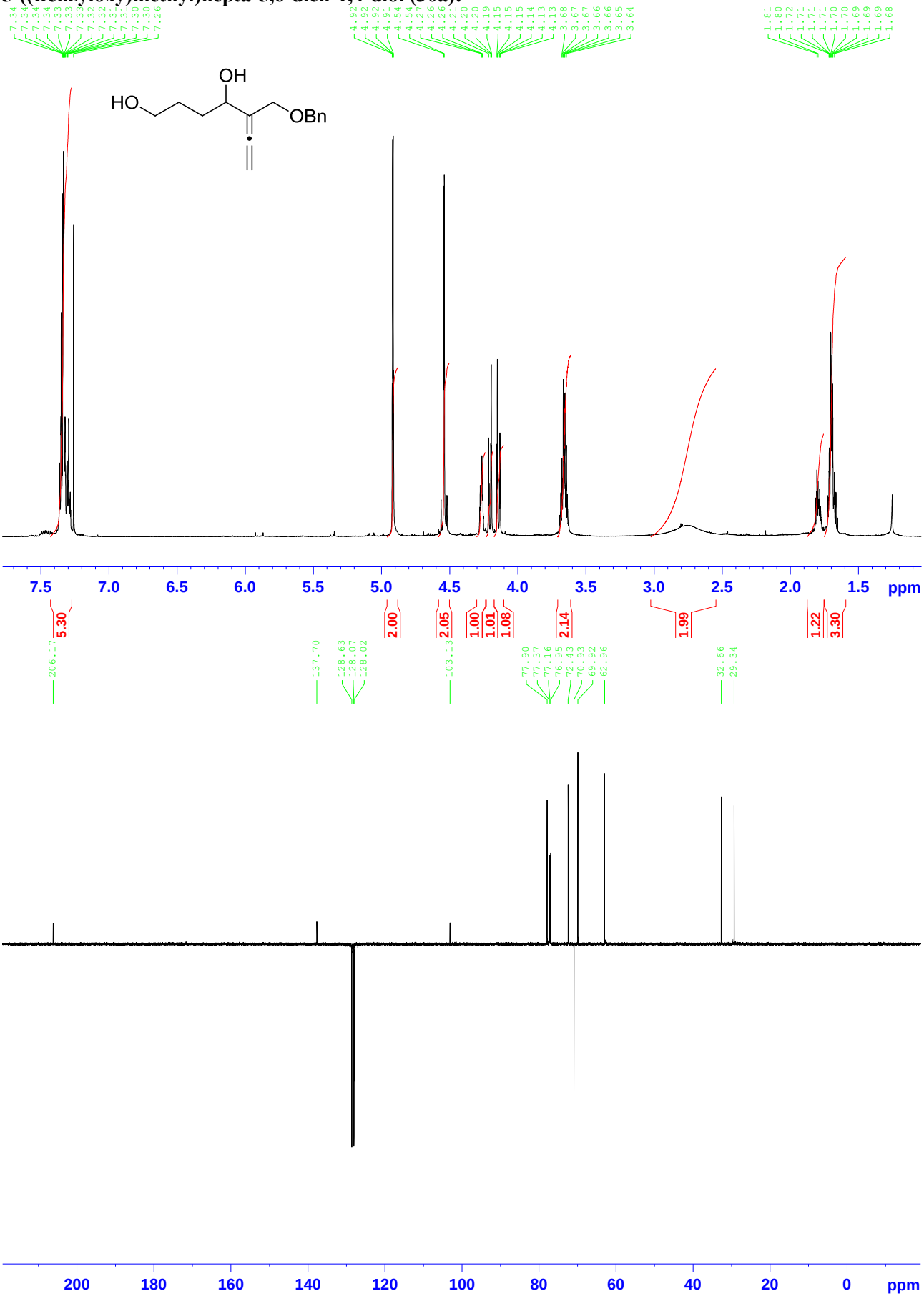
1-Deoxy-D-*erythro*-2-pentulose (29):



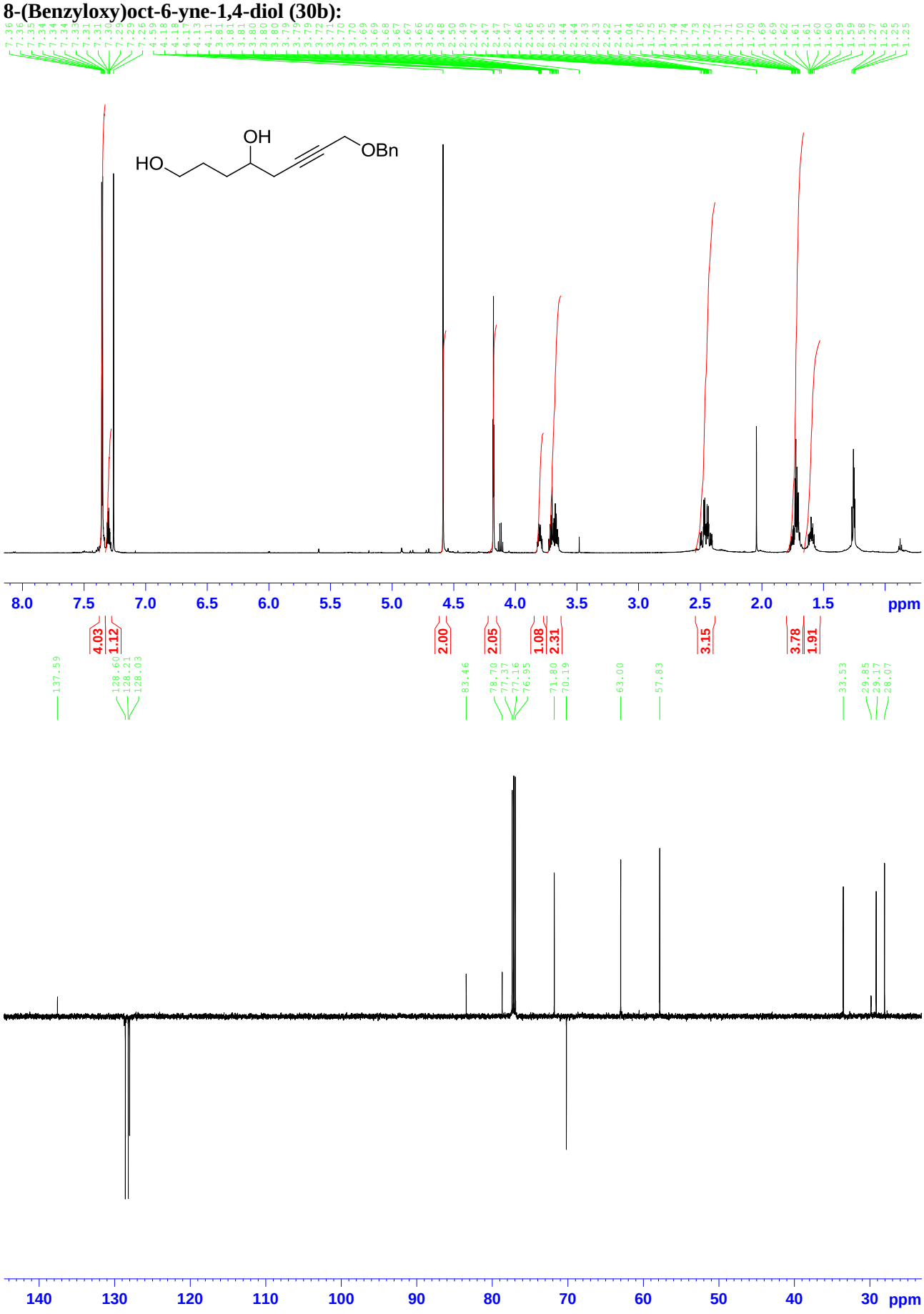
^{13}C NMR(JMOD): For the quaternary carbons of α- and β-1-deoxy-D-*erythro*-2-pentulose see above.



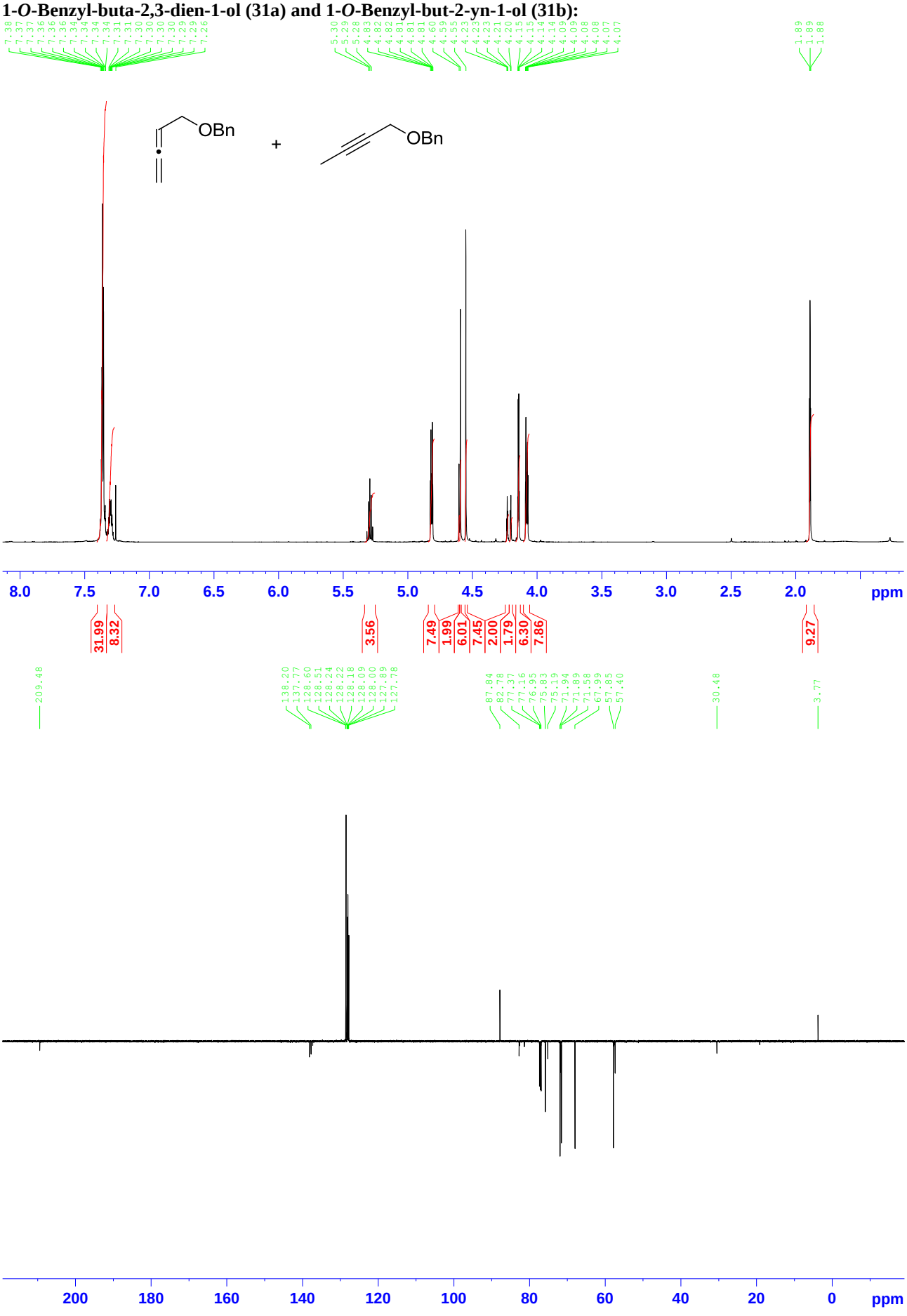
5-((Benzyloxy)methyl)hepta-5,6-dien-1,4-diol (30a):



8-(Benzyloxy)oct-6-yne-1,4-diol (30b):

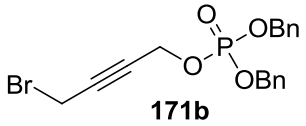


1-*O*-Benzyl-buta-2,3-dien-1-ol (31a) and 1-*O*-Benzyl-but-2-yn-1-ol (31b):



Unpublished Work:

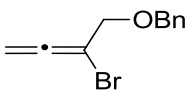
Dibenzyl (4-bromobut-2-yn-1-yl) phosphate (171b):



171b

To a solution of 2-butyne-1,4-diol (86 mg, 1 mmol) in dry pyridine (2 mL), freshly prepared dibenzyl phosphorylchloride (0.356 g, 1.2 mmol) was added at 0°C. After 1 houre the reaction was quenched by addition of a water (1 mL) and the solvent was removed under reduced pressure. The product was re-dispended in ethyl acetate (5 mL) and water (1 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The crude product was separated twice by silica gel column chromatography (hexane/ethyl acetate = 2:1 and hexane/ethyl acetate = 1:2) to yield dibenzyl (4-hydroxybut-2-yn-1-yl) phosphate (132 mg, 38%). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 7.68-7.12 (m, 10H, CH), 5.18-5.00 (m, 4H, CH_2), 4.73-4.56 (m, 2H, CH_2), 4.31-4.19 (m, 2H, CH_2), 3.82-3.71 (m, 1H, OH). ^{13}C-NMR (400 MHz; $CDCl_3$) δ = 135.5 (d, *J* = 7.1 Hz), 128.63, 128.61, 128.0, 87.1, 78.8 (d, *J* = 7.0 Hz), 69.6 (d, *J* = 5.8 Hz), 55.6 (d, *J* = 5.0 Hz), 50.4. The phosphate was re-dissolved in anhydrous CH_2Cl_2 (1 mL), CBr_4 (149 mg, 0.45 mmol) and PPh_3 (118 mg, 0.45) were added at 0°. The reaction was quenched by addition of methanol (1 mL) after 2 hours and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 2/1) to yield dibenzyl (4-bromobut-2-yn-1-yl) phosphate (**171b**) (103 mg, 67%). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 7.47-7.29 (m, 10H, CH), 5.16-5.01 (m, 4H, CH_2), 4.66 (dt, *J* = 10.4, *J* = 2.1 Hz, 2H, CH_2), 3.83 (t, *J* = 2.0 Hz, 2H, CH_2).

1-Benzyloxy-2-bromo-but-2,3-diene (176):

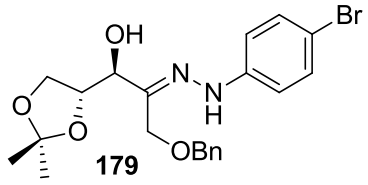


176

To a solution of 2-butyne-1,4-diol (21.5 g, 0.25 mol) in anhydrous DMF (250 mL), TBAI (0.46 g, 0.0125 mol) and sodium hydride (60%, 3.6 g, 0.125 mol) were added at 0°C. Benzyl

bromide (25.7 g, 0.15 mol) was added slowly under stirring and after 12 hours the reaction was quenched by addition of ice-cold water (75 mL) and 3N HCl-solution (75 mL). The aqueous phase was extracted with diethyl ether (3 x 150 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. A part of the crude product (10 g) was dissolved in anhydrous CH_2Cl_2 (65 mL), CBr_4 (16.1 g, 0.048 mol) and PPh_3 (12.7 g, 0.048) were added at 0°C. The reaction was quenched by addition of methanol (20 mL) after 5 hours and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 50/1) to yield 1-*O*-benzyl-4-bromo-2-butyn-1-ol (4.12 g) and 1-benzyloxy-2-bromo-but-2,3-diene (**176**) (1.89 g). (**176**): ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 7.41-7.28 (m, 5H, CH), 4.99 (t, *J* = 2.4 Hz, 2H, CH_2), 4.59 (s, *J* = 2H, CH_2), 4.22 (t, *J* = 2.4 Hz, 2H, CH_2); ^{13}C-NMR (400 MHz; $CDCl_3$) δ = 205.1, 137.6, 128.6, 128.1, 128.0, 89.0, 82.6, 71.8, 71.7.

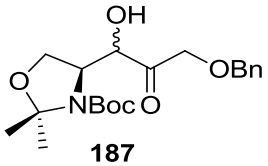
1-*O*-Benzyl-4,5-*O*-isoproylidene-D-erythro-pent-2-ulose *p*-bromophenylhydrazone (179):



179

To a solution of pentulose **184** (10 mg, 36 µmol) in dry methanol (0.8 mL) were added molecular sieves (3Å) and 4-bromophenylhydrazine hydrochloride (9 mg, 39 µmol). The reaction was quenched by addition of water (1 mL), after TLC monitoring showed conversion of the starting materials (~5 min). The aqueous phase was separated and extracted with ethyl acetate (2 x 3 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel chromatography (hexane/ethyl acetate = 4/1) to give hydrazone **179** (16 mg, 99 %). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 9.19 (s, 1H, NH), 7.41-7.28 (m, 7H, CH), 6.82-6.77 (m, 2H, CH), 4.56 (d, *J* = 11.3 Hz, 1H, CH_2), 4.52 (d, *J* = 11.4 Hz, 1H, CH_2), 4.50 (d, *J* = 14.0 Hz, 1H, CH_2), 4.39 (d, *J* = 14.0 Hz, 1H, CH_2), 4.19-413 (m, 2H, CH), 4.12-4.02 (m, 2H, CH), 3.23 (d, *J* = 4.9 Hz, 1H, OH), 1.42 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C-NMR (400 MHz; $CHCl_3$): δ = 143.9, 147.2, 136.9, 132.2, 132.1, 128.9, 128.6, 128.4, 114.8, 114.4, 112.3, 109.7, 78.7, 73.2, 73.1, 67.3, 67.1, 26.7, 25.2.

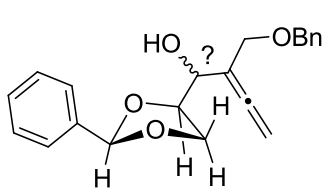
1-*O*-Benzyl-4-*N*-*tert*-butyloxycarbonyl-4,5-*N*,*O*-isoproylidene-L-erythro-pent-2-ulose (187):



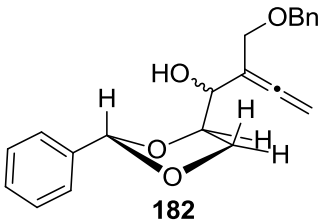
187

Pentulose (**187**) was synthesized according to method B as published the European Journal of Organic Chemistry. Method B was applied to compound **186** (20 mg, 0.051 mmol) to yield pentulose (**187**) (17 mg, 87 %). $[\alpha]_D^{20}$= +14.9 (c = 0.85, CH_2Cl_2); ^{1}H NMR (400 MHz; $CHCl_3$) δ = 7.41–7.26 (m, 5H; ArH), 4.63(d, *J* = 12.0 Hz, 1H; CH_2), 4.58 (d, *J* = 12.0 Hz, 1H; CH_2), 4.53-4.45 (m, 1H; CH) 4.40-4.13 (m, 3H) 4.10-3.84 (m, 2H) 1.46 (bs, 15H; CH_3); ^{13}C-NMR (400 MHz; $CHCl_3$): δ = 208.3, 153.7, 132.3, 128.7, 128.6, 128.2, 94.6, 81.4, 75.6, 75.1, 73.9, 73.7, 64.5, 60.8, 28.4, 26.4, 24.8.

Procedure A: Typical procedure for benzylidene protection – Synthesis of 2-((benzyloxy)methyl)-1-((2R,4R)-2-phenyl-1,3-dioxolan-4-yl)buta-2,3-dien-1-ol (181) and 2-((benzyloxy)methyl)-1-((2S,4R)-2-phenyl-1,3-dioxolan-4-yl)buta-2,3-dien-1-ol (182):



181

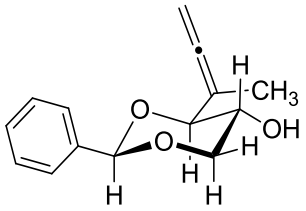


182

To a solution of 1-*O*-benzyl-2-deoxy-2-*C*-ethenyliden-D-erythro-pentitol (16 mg, 63.9 µmol) in dry DMF (1 mL), benzaldehyde dimethyl acetal (24 mg, 143 µmol) and *p*-toluenesulfonic acid (2.7 mg, 16 µmol) were added. After heating the reaction mixture at 48°C under reduced pressure (39 mbar) for 4 ½ hours, H_2O (1 mL) and ethyl acetate (5 mL) were added. The aqueous phase was separated and extracted with ethyl acetate (3 x 3 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography (hexane/ethyl acetate = 4/1) to yield five membered dioxolane ring (**181**) and (**182**) (combined yield: 8 mg, 37 %) in two conformations. ^{1}H-NMR (600 MHz; $CDCl_3$) δ = 7.52-7.42 (m, 4H, CH), 7.41-7.27 (m, 16H, CH), 5.99 (s, 1H, CH)[182], 5.79 (s, 1H, CH)[181], 4.99-4.92 (m, 4H, CH_2), 4.57-4.51 (m, 5H, 2 x CH_2, CH), 4.46-4.41 (m, 1H, CH)[181], 4.39-4.34 (m, 2H, CH), 4.30-

4.25 (m, 3H, CH_2), 4.24 (dd, *J* = 8.4 Hz, *J* = 6.6 Hz, 1H, CH_2)[182], 4.17 (dt, *J* = 11.1 Hz, *J* = 1.9 Hz, 1H, CH_2)[182], 4.14 (dt, *J* = 11.2 Hz, *J* = 1.8 Hz, 1H, CH_2)[181], 4.11 (dd, *J* = 8.5 Hz, *J* = 6.8 Hz, 1H, CH_2)[182], 4.09 (dd, *J* = 8.4 Hz, *J* = 7.1 Hz, 1H, CH_2)[181], 2.77 (s, 1H, OH)[182], 2.72 (s, 1H, OH)[181]; ^{13}C-NMR (600 MHz; $CDCl_3$) δ =207.2, 206.8, 138.9, 137.7, 137.6, 137.2, 129.6, 129.3, 128.7, 128.6, 128.5, 128.4, 128.1, 128.05, 128.02, 126.9, 126.5, 104.4, 104.2, 100.4, 100.3, 78.3, 78.0, 77.98, 77.6, 72.6, 72.5, 71.5, 71.4, 70.0, 69.8, 67.1, 66.9, 29.8.

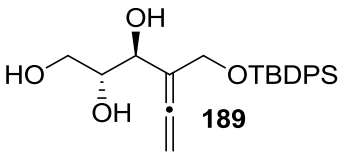
(*2R,4S,5R*)-4-(buta-2,3-dien-2-yl)-2-phenyl-1,3-dioxan-5-ol (183):



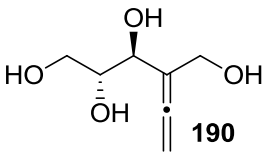
183

Procedure A was applied to allene **173e** (24 mg, 0.166 mmol) for benzylidene protection to yield 1,3-dioxane **183** (14 mg, 34%). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 7.53-7.44 (m, 2H, CH), 7.42-7.30 (m, 3H, CH), 5.55 (s, 1H, CH), 4.95-4.78 (m, 2H, CH_2), 4.36 (dd, *J* = 10.8 Hz, *J* = 5.3 Hz, 1H, CH_2), 4.04 (dt, *J* = 9.0 Hz, *J* = 1.7 Hz, 1H, CH_2), 3.93-3.81 (m, 1H, CH), 3.67 (dd, *J* = 10.8 Hz, *J* = 10.2 Hz, 1H, CH_2), 1.88 (bs, 1H, OH), 1.84 (t, *J* = 3.3 Hz, 1H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$) δ = 201.5, 138.0, 129.1, 128.3, 126.2, 109.5, 101.2, 94.7, 78.9, 76.2, 76.1, 68.9, 65.2, 26.5, 25.9.

Synthesis of (*2R,3S*)-4-(((tert-butyldiphenylsilyl)oxy)methyl)hexa-4,5-diene-1,2,3-triol (189) and (*2R,3S*)-4-vinylidenepentane-1,2,3,5-tetraol (190):



189

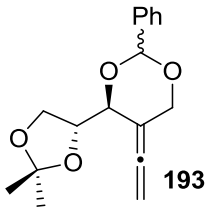


190

(*2R, 3S*)-4-(((tert-butyldiphenylsilyl)oxy)methyl)hexa-4,5-diene-1,2,3-triol (**189**) was synthesized according to method C as published the European Journal of Organic Chemistry. Method C was applied to compound **173f** (50 mg, 0.114 mmol) to yield allene (**189**) (9 mg, 20%) along with unreacted starting material (11mg) and tetrol **190** (9mg, 49 %). **(189)**: ^{1}H-

NMR (400 MHz; $CDCl_3$) δ = 7.73-7.64 (m, 4H, CH), 7.50-7.35 (m, 6H, CH), 4.88-4.84 (m, 2H, CH_2), 4.39-4.26 (m, 3H, CH, CH_2), 3.85-3.78 (m, 3H, CH, CH_2), 1.06 (s, 9H, CH_3). (**190**): 5.11 (m, 2H, CH_2), 4.27-4.14 (m, 3H, CH, CH_2), 3.91-3.78 (m, 2H, CH, CH_2), 3.69 (dd, *J* = 11.7 Hz, *J* = 6.6 Hz, 1H, CH_2).

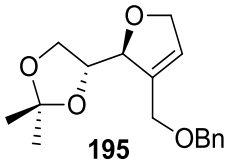
4-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-phenyl-5-vinylidene-1,3-dioxane (193):



193

Procedure A was applied to allene **192** (10 mg, 0.05 mmol) for benzylidene protection to yield 1,3-dioxane **193** (5 mg, 34%). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 7.55-7.45 (m, 2H, CH), 7.41-7.30 (m, 3H, CH), 5.66 (s, 1H, CH), 5.04-4.85 (m, 2H, CH_2), 4.67-4.61 (m, 1H, CH), 4.59-4.54 (m, 2H, CH_2), 4.41 (ddd, *J* = 6.6 Hz, *J* = 4.4 Hz, *J* = 0.1 Hz, 1H, CH), 4.16 (dd, *J* = 8.3 Hz, *J* = 6.6 Hz, 1H, CH_2), 4.11 (dd, *J* = 8.3 Hz, *J* = 6.6 Hz, 1H, CH_2), 1.43 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$) δ = 201.5, 138.0, 129.1, 128.3, 126.2, 109.5, 101.2, 94.7, 78.9, 76.2, 76.1, 68.9, 65.2, 26.5, 25.9.

(R)-4-((S)-3-((benzyloxy)methyl)-2,5-dihydrofuran-2-yl)-2,2-dimethyl-1,3-dioxolane (195):

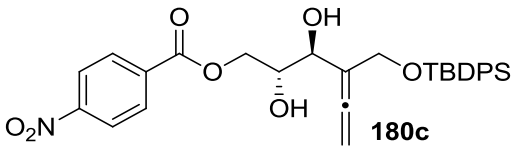


195

To a solution of allene **173d** (30 mg, 0.104 mmol) in dry THF (2 mL) was added $AuCl_3$ (1.5 mg, 5 µmol). The solvent was removed under reduced pressure, after TLC monitoring showed consumption of the starting material. The crude product was purified by silica gel chromatography (hexane/ethyl acetate = 1/1) to yield furan **195** (9 mg, 30 %). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 7.40-7.27 (m, 5H, CH), 5.94-5.89 (s, 1H, CH), 4.78-4.70 (m, 1H, CH), 4.68-4.61 (m, 2H, CH_2), 4.59-4.50 (m, 2H, CH_2), 4.25-4.11 (m, 2H, CH_2), 4.11-4.06 (m, 1H, CH), 4.03 (dd, *J* = 8.2 Hz, *J* = 6.4, 1H, CH_2), 3.92 (dd, *J* = 8.2 Hz, *J* = 5.6, 1H, CH_2), 1.42 (s, 3H, CH_3), 1.34 (s, 3H,

CH_3); ^{13}C-NMR (600 MHz; $CDCl_3$) δ = 139.0, 128.6, 127.85, 127.80, 124.5, 109.6, 85.9, 78.2, 75.3, 72.9, 66.6, 66.1, 26.7, 25.5.

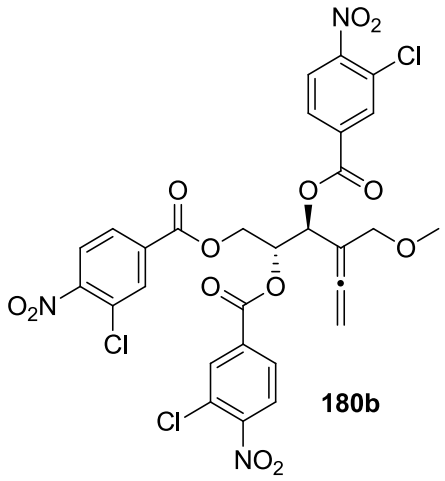
(*2R,3S*)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-1-*O*-(p-nitrobenzoyl)hexa-4,5-diene-1,2,3-triol:



180c

To a solution of allene **189** (7 mg, 17.6 µmol) in dry pyridine (1 mL) and dry CH_2Cl_2 (0.9 mL), *p*-nitrobenzoylchloride (20 mg, 105 µmol) and a catalytic amount of DMAP were added. The reaction was stirred for 18 hours at room temperature and quenched by addition of a saturated solution of ammonium chloride (1 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (3 x 3 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated to give mono benzoyl protected allene **180c** (9 mg, 93%). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 8.14 (bs, 3H, CH), 7.70-7.60 (m, 4H, CH), 7.49-7.29 (m, 7H, CH), 4.85-4.84 (m, 2H, CH_2), 4.53-4.38 (m, 1H, CH), 4.38-4.23 (m, 2H, CH_2), 3.90 (m, 3H, CH, CH_2), 3.69 (bs, 2H, OH) 1.04 (s, 9H, CH_3).

(*2R,3S*)-4-(Methyloxy)methyl)-1,2,3-tri-*O*-(*m*-chloro-*p*-nitrobenzoyl)hexa-4,5-diene-1,2,3-triol:



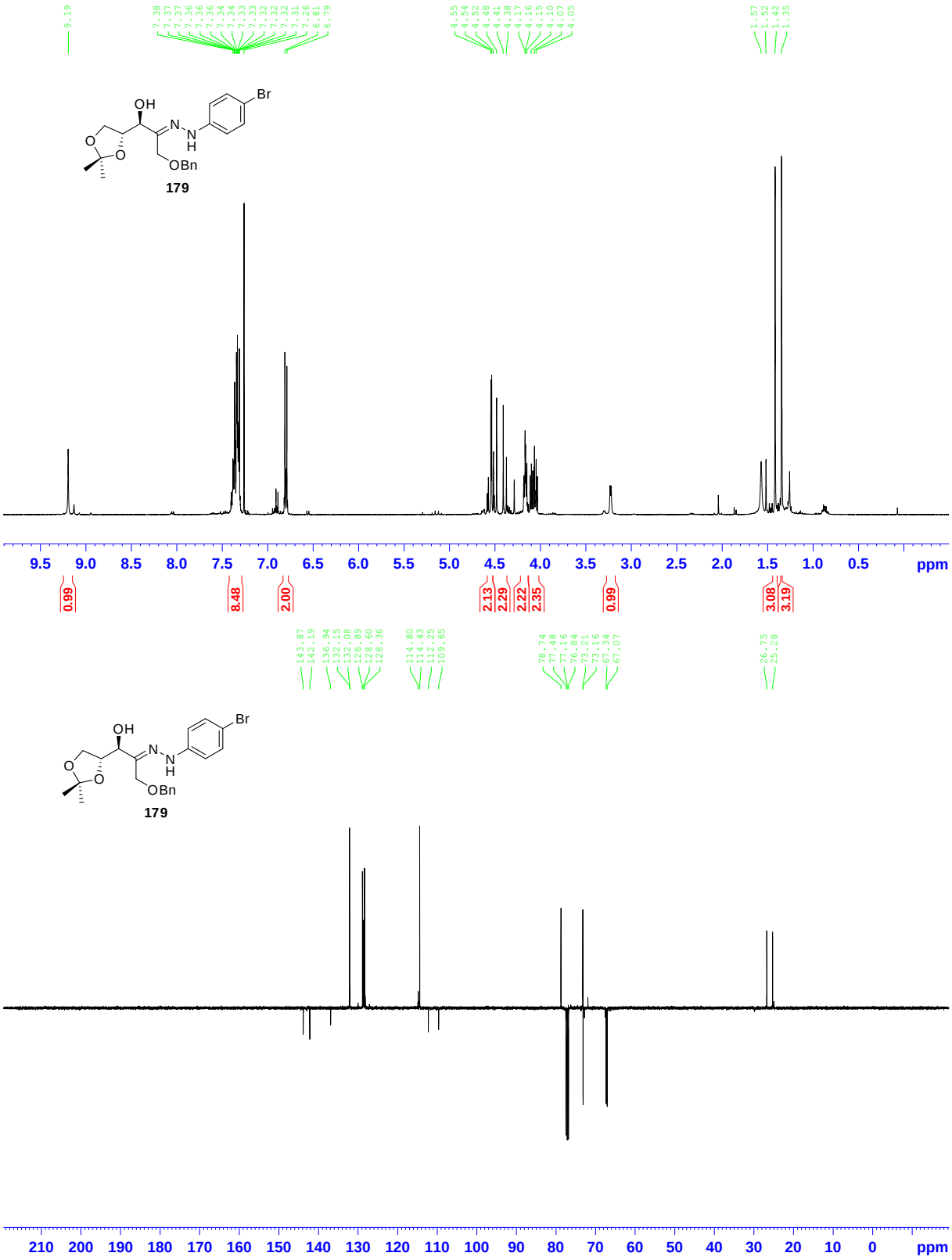
180b

To a solution of allene **194** (13 mg, 0.075 mmol) in dry pyridine (1 mL) and dry CH_2Cl_2 (0.9 mL), triethylamine (91 mg, 0.90 mmol) and *m*-chloro-*p*-nitrobenzoylchloride (98 mg, 0.448 mmol) were added at 0°C. The reaction was stirred for 18 hours at room temperature and

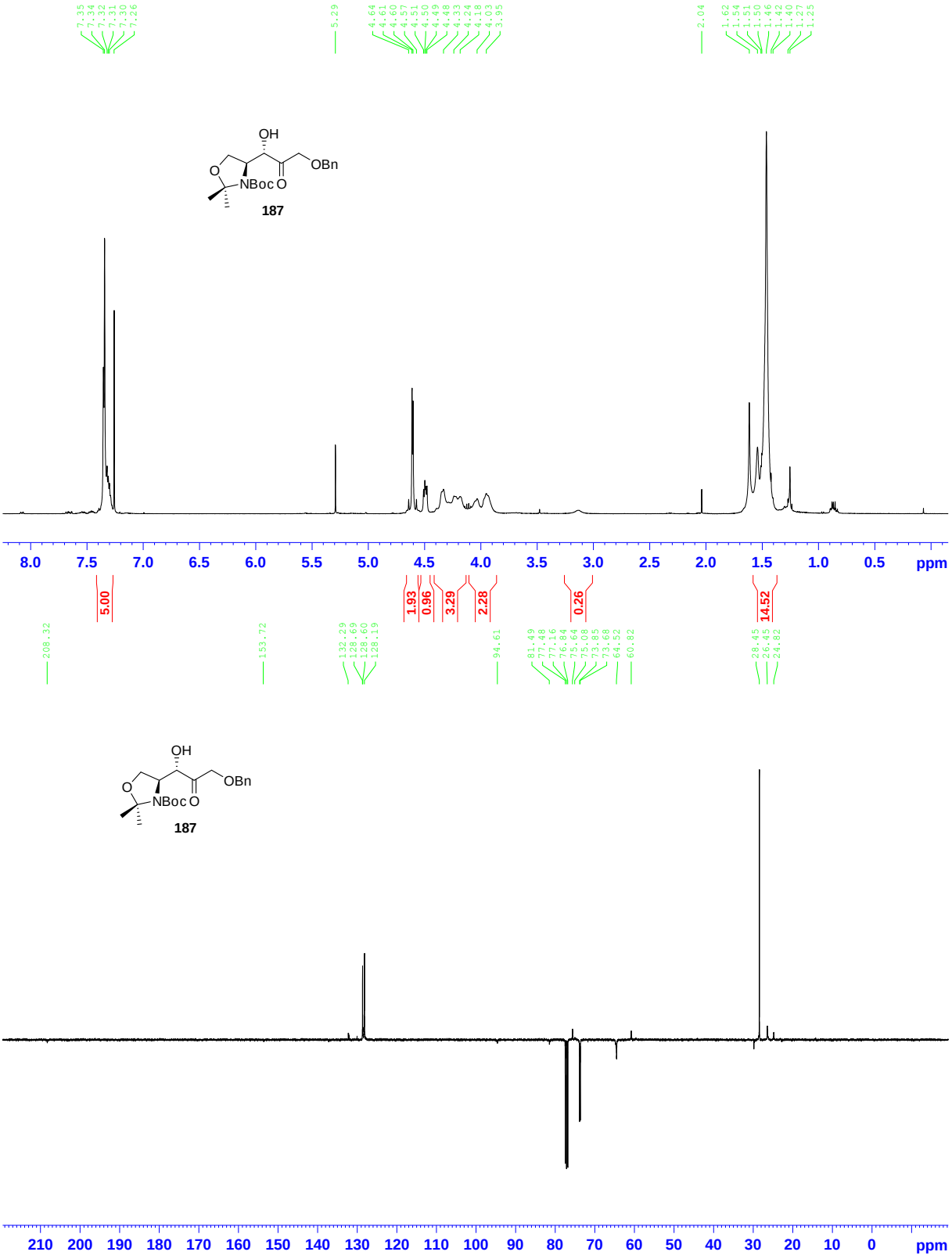
was quenched by addition of 1 M HCl solution (1 mL) and ethyl acetate (3 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 3 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated to give benzoyl protected allene **180b** (18 mg, 34%). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 8.41 (d, *J* = 2.0 Hz, 1H, CH), 8.40 (d, *J* = 2.0 Hz, 1H, CH), 8.09 (dd, *J* = 8.4 Hz. *J* = 2.0 Hz, 1H, CH), 8.07 (dd, *J* = 8.4 Hz. *J* = 2.0 Hz, 1H, CH), 8.02 (dd, *J* = 8.4 Hz. *J* = 2.0 Hz, 1H, CH), 7.61 (d, *J* = 6.1 Hz, 1H, CH), 7.59 (d, *J* = 6.2 Hz, 1H, CH), 7.56 (d, *J* = 8.4 Hz, 1H, CH), 5.97-5.91 (m, 1H, CH), 5.88-5.81 (m, 1H, CH) 5.07-4.94 (m, 2H, CH_2), 4.86 (dd, *J* = 12.4 Hz. *J* = 2.9 Hz, 1H, CH_2), 4.62 (dd, *J* = 12.3 Hz. *J* = 7.0 Hz, 1H, CH_2), 4.04 (dt, *J* = 11.3 Hz. *J* = 2.0 Hz, 1H, CH_2), 4.00 (dt, *J* = 11.4 Hz. *J* = 1.7 Hz, 1H, CH_2), 3.25 (s, 3H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$) δ = 208.0, 163.5, 163.0, 162.7, 148.1, 133.9, 133.8, 133.73, 133.70. 132.68, 132.56, 132.43, 129.34, 129.24, 127.0, 126.9, 126.8, 97.3, 79.6, 72.8, 71.8, 71.4, 63.4, 58.3.

Selected spectra:

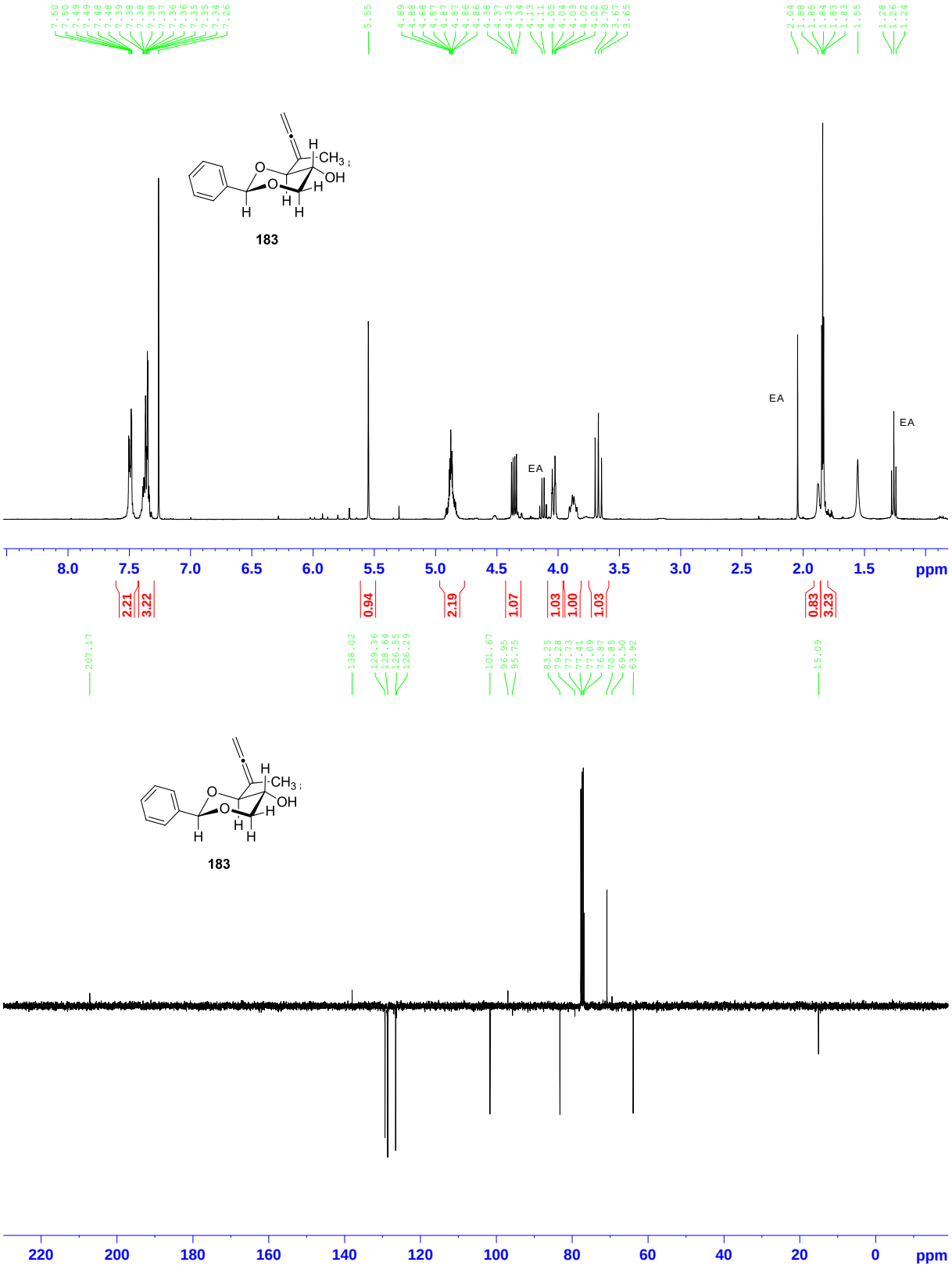
1-*O*-Benzyl-4,5-*O*-isoproylidene-D-erythro-pent-2-ulose *p*-bromophenylhydrazone (179):



1-*O*-Benzyl-4-*N*-*tert*-butyloxycarbonyl-4,5-*N*,*O*-isoproylidene-L-erythro-pent-2-ulose (187):



(*2R,4S*)-4-(buta-2,3-dien-2-yl)-2-phenyl-1,3-dioxane-5-ol (183):



PART D: Synthesis of 4-Fluoro-2-oxo-butanoic acid

D.1. Abstract

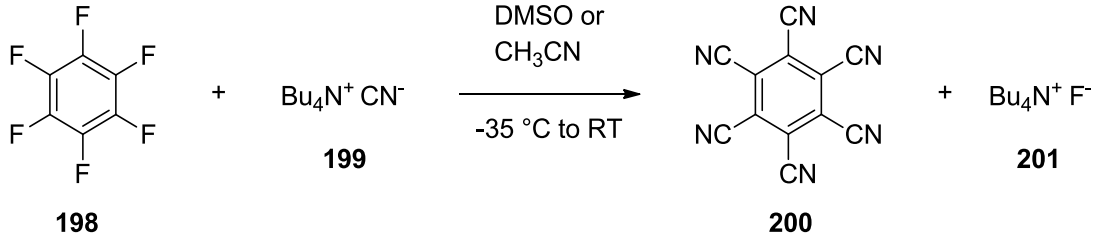
An efficient synthesis of 4-fluoro-2-oxo-butanoic acid has been developed. In the key step of this synthesis the fluorine atom was introduced by a homoallylic fluorination in moderate yield. These types of fluorinations are known to be extremely difficult and seldom used in organic synthesis. In an earlier report by O'Leary, 4-fluoro-2-oxo-butanoic acid was claimed to be unstable in D_2O solution with a half-life of decomposition to 2-oxo-3-butenoic acid of 162 s at pH = 7.0. These findings are in contrast of our results since 4-fluoro-2-oxo-butanoic acid was stable in D_2O solutions at pH = 7.0 for several hours. In our hands, 4-fluoro-2-oxo-butanoic acid decomposed to 3-fluoro-propanoic acid in ten days by loss of one carbon atom. Maybe the fast decomposition of 4-fluoro-2-oxo-butanoic acid O'Leary *et al*. reported originates from unrecognized impurities in their synthesis which catalyzed the fast elimination of hydrogen fluoride.

D.2. Introduction and Background

Nucleophilic fluorination

The most important route for the synthesis of fluorinated fine chemicals is by nucleophilic substitution of halogens or sulfonates by fluorine anions.[90] This reaction type is hampered by the fact that the fluorine anion acts more likely as a base instead of a nucleophile due to its high electronegativity, very small size (1.36 Å) and a low polarizability. The nucleophilic reactivity of alkali metal fluorides decreases in order CsF > RbF > KF > NaF > LiF, because of the increasing lattice energy in the reverse order and the formation of tight ion pairs in aprotic solvents.**Fehler! Textmarke nicht definiert.**,[91] Sterically demanding cations like CsF reduce ion paring by delocalizing the positive charge, making the fluoride anion more

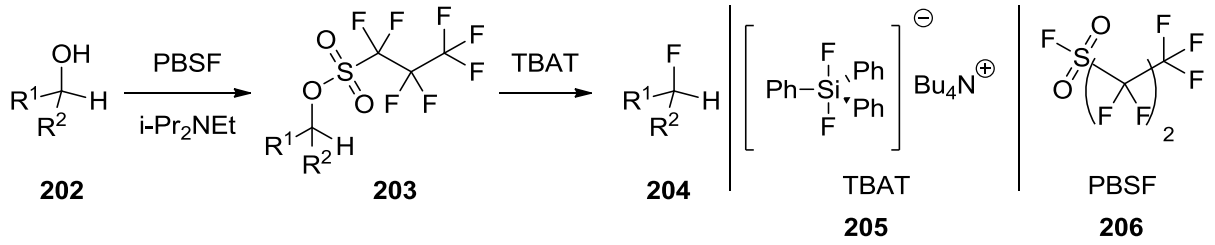
nucleophilic.[92] In addition, crown ethers (KF/18-crown-6)[93] and especially lipophilic cations like TBAF[94] are employed to enhance the nucleophilic character of fluoride anions ("naked fluorine"). Recently, DiMagno *et al*. reported a novel preparation of "truly" anhydrous TBAF (201) with a better reaction profile for nucleophilic substitution reactions than traditionally used TBAF (Scheme 69).[95]



198 199 200 201

Scheme 69. Preparation of "truly" anhydrous TBAF by DiMagno *et al*.Fehler! Textmarke nicht definiert.

Another highly selective protocol for the substitution of hydroxyl functionalities into fluorine atoms applied a combination of n-perfluorobutanesulfonyl fluoride (PBSF) (206) with tetrabutyl-ammonium triphenyldifluoro-silicate (TBAT) (205).[96] This strategy suppressed significantly elimination sidereactions in case of primary and secondary alcohols 202 (Scheme 70).



202 203 204 TBAT 205 PBSF 206

Scheme 70. Synthesis of primary and secondary alkyl fluorides by Zhao *et al*.Fehler! Textmarke nicht definiert.

Chi *et al*. published a remarkably enhanced reactivity for nucleophilic fluorinations with alkali fluorides using sterically demanding protic solvents like *tert*-butanol and amylalcohol.[97] Normally, protic solvents reduce the nucleophilicity of anions by hydrogen bonding and by interactions with their partial positive charges.[98] Chi *et al*. showed that the combination of CsF or TBAF in *tert*-butanol belong to the best nucleophilic fluorination methodologies to date.[99] According to Chi *et al*.,[99] *tert*-butanol enhances the reactivity for nucleophilic fluorination by four different effects (Figure 14): (i) The strength of the cesium fluoride ionic bond might be reduced by hydrogen bonding of the hydroxyl group of *tert*-butanol with the fluoride in the CsF lattice, resulting in selective solvation of the fluoride; (ii) The limited

coordination of bulky *t*BuOH molecules to fluoride anions might make the fluorine a good nucleophile; (iii) Hydrogen bonding of the *t*BuOH molecules of the sulfonate oxygen enhances their leaving group ability; (iv) The protic environment of the *tert*-butyl alcohol reduces the basicity of the fluoride anion avoiding side reaction as elimination and intramolecular alkylations.

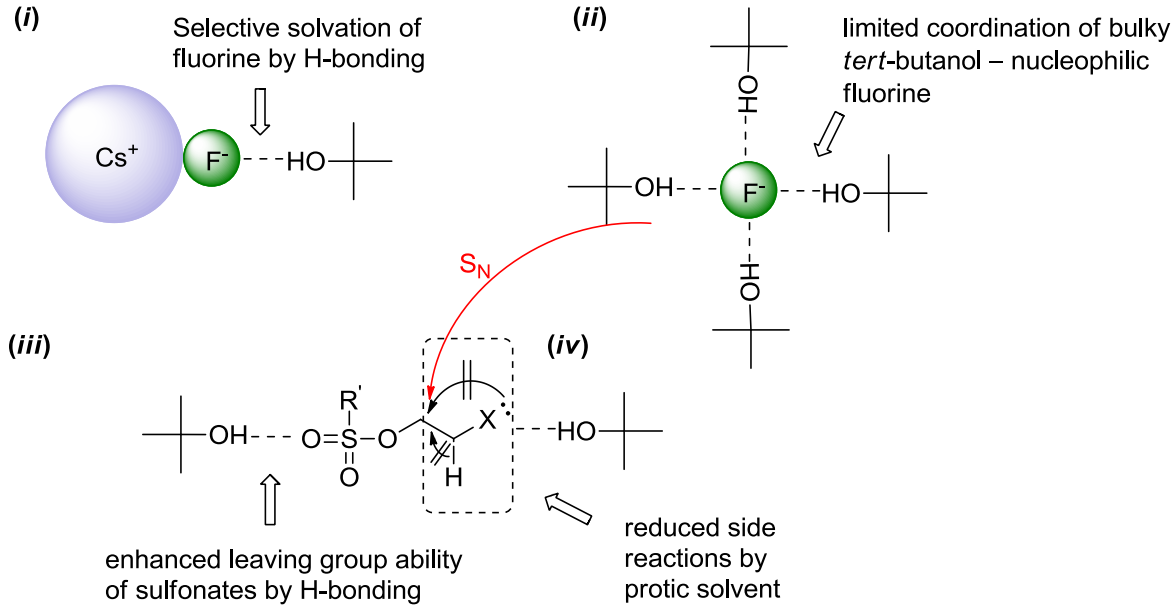


Figure 14. Chi's rationalizations for nucleophilic fluorination of CsF in *tert*-butanol[99]

Alternatively the nucleophilicity of fluorine can be enhanced by the combination of the fluoride anion (hard base) with a soft acid like sulfur as in the case of well known fluorodehydroxylation reagents DAST (**207**) and Deoxofluor (**208**) (Figure 16).[100] However, these two reagents are highly reactive (elimination as common side reaction), thermally unstable and highly explosive. Because of these hazardous properties, these reagents are considered to be unsafe for large scale application.

In the year 2010, a new class of deoxyfluorination reagents (aminodifluorosulfinium salts) have been reported by Couturier *et al.*[101] These salts (XtalFluor **209** and **210**), which can be synthesized directly from DAST are more selective, thermally stable and easier to handle that the former one's (Figure 15).

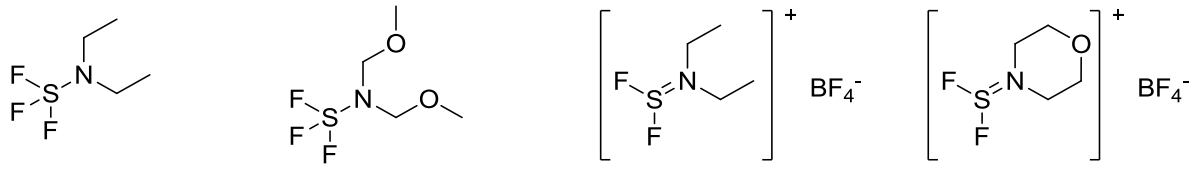


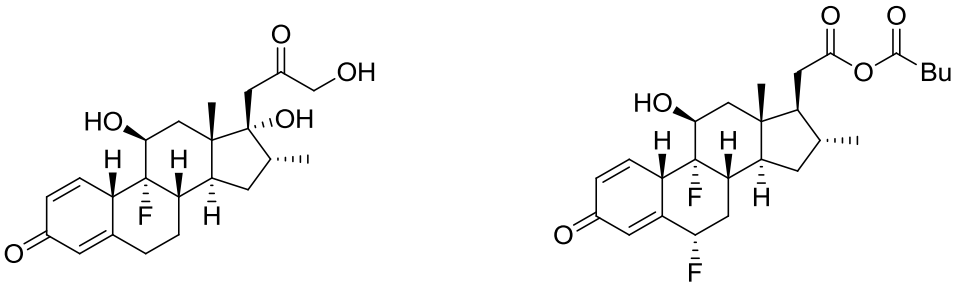
Figure 15. Deoxyfluorination reagents[100,101]

Homoallylic Fluorination

Volume 34 of Houben-Wiley's Science of Synthesis, published in 2005, covers the synthesis of compounds containing a single fluorine atom bonded to a sp^3 carbon.[102] One section of this reference book deals solely with homoallylic fluorides,[103] where the general introduction starts as following:

"There are very few examples of this class of compound in the literature, although the important corticosteroid analogues betamethasone [**1**]*and diflucortolone valerate* [**2**]*contain homoallylic fluoride functional groups.*

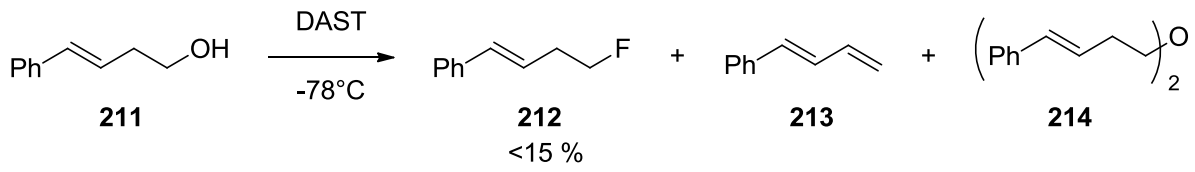
Scheme 1 Corticosteroids Containing the Homoallyl Fluoride Motif



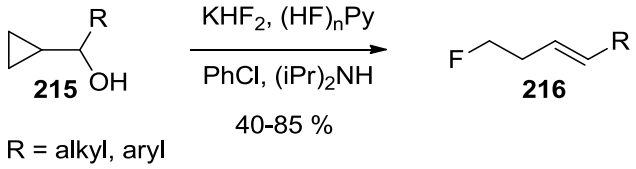
… This section will examine the extremely limited range of methods available for molecules which are strictly homoallylic fluorides …"[103]

This statement clearly points out that the synthesis of homoallylic fluorides belongs to one of the severe problems in fluorine chemistry. Normally hydrogen fluoride elimination and etherification are preferred to the desired fluorination. Unexpected rearrangements have been reported for this type of reaction as well.[104]

Two methods for the synthesis of strictly homoallylic fluorides were cited in the above mentioned Science of Synthesis Volume 34. A DAST mediated fluorodehydroxylation in low yield (Scheme 71) and ring opening reactions of cyclopropylmethanols **215** with Olah's reagent (70 % hydrogen fluoride/pyridine) were reported for the synthesis of homoallylic fluorides (Scheme 72).

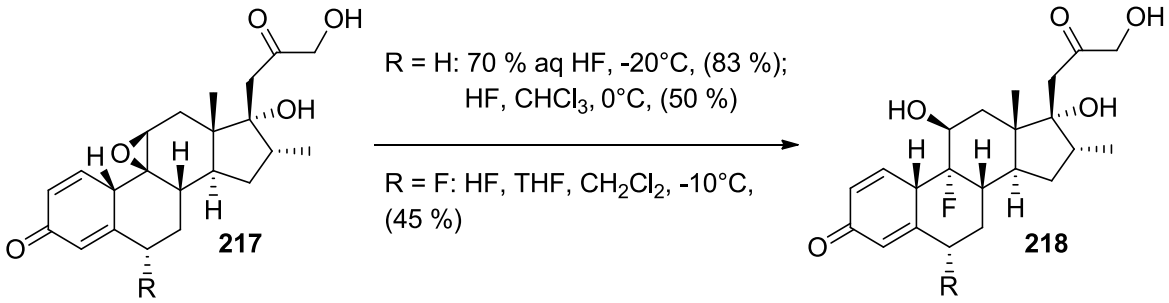


Scheme 71. DAST mediated fluorodehydroxylation for the synthesis of homoallylic fluorides (Ishikawa's reagent and TBAF-MsF did not yield any fluorinated product)[105]



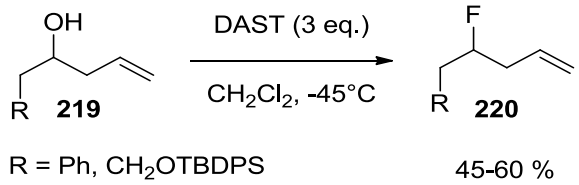
Scheme 72. Ring opening reactions of cyclopropylmethanols with Olah's reagent[105]

For the synthesis of secondary and tertiary homoallylic fluorides, ring-opening reactions of epoxides with hydrogen fluoride or with tetrabutylammonium dihydrogen trifluoride were used for the preparation of betasome and secondary fluoride **218** (Scheme 72).[106]



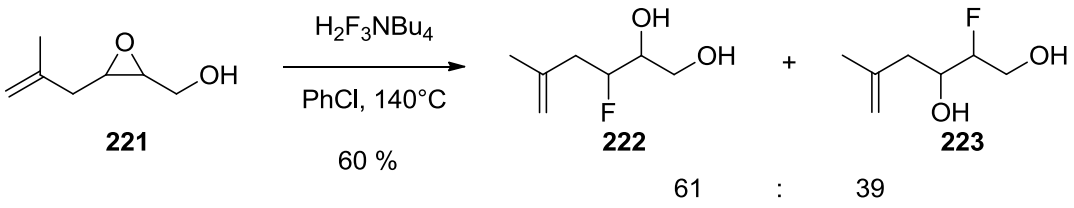
Scheme 73.[106]

Furthermore, homoallylic deoxofluorinations have been published for following other systems. The preparations of two secondary homoallylic fluorides **219** in moderate yields were reported by Cossy using three equivalents of DAST.[107] The bulky silyl protecting group TBDPS was tolerated under these reaction conditions (Scheme 74).



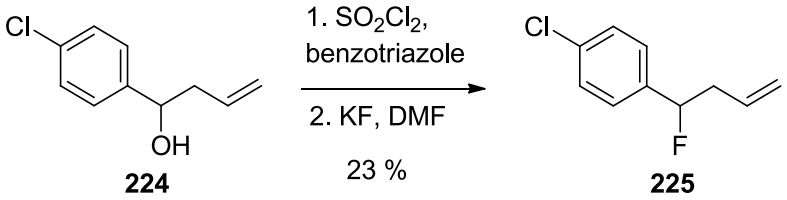
Scheme 74.[107]

Addition of various Lewis acids for the regioselectivity epoxide opening of **221** with ammonium hydrogen fluoride $H_2F_3NBu_4$ shifted the ratio of **222** to **223** towards allylic fluoride **222** (Scheme 74).[108]



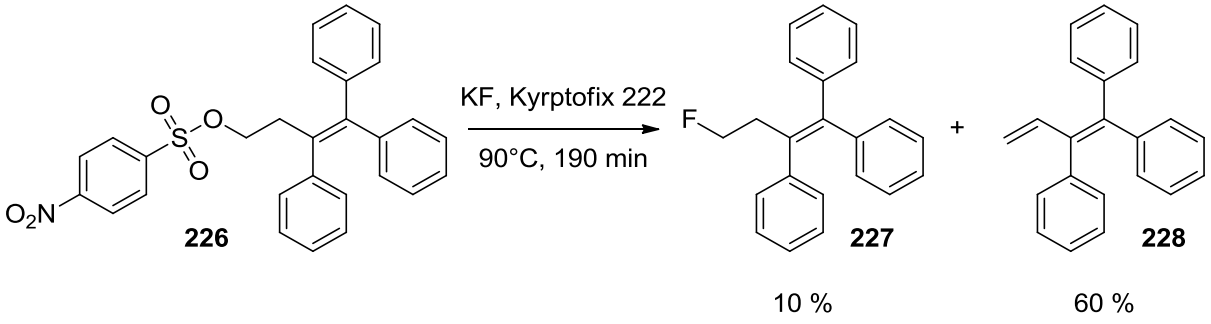
Scheme 75[108]

Alternatively a one-pot procedure using thionylchloride and potassium fluoride gave secondary homoallylic fluorine **225** in low yield (Scheme 76).[109]



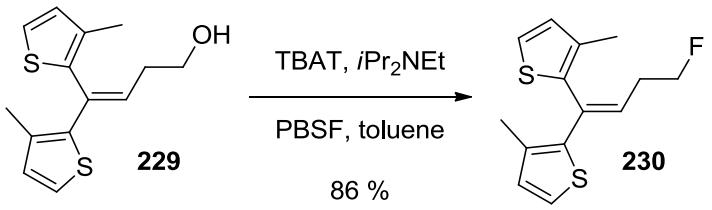
Scheme 76[109]

Primary homoallylic fluorides for positron emission tomography imaging of cyclooxygenase-2 expression in pathological disease were synthesized by nucleophilic displacement of *p*-nitrobenzenesulfonyl by potassium fluoride.[110] However, the major product **228** (60 %) formed was derived from elimination of hydrogen fluoride (Scheme 77).



Scheme 77.[110]

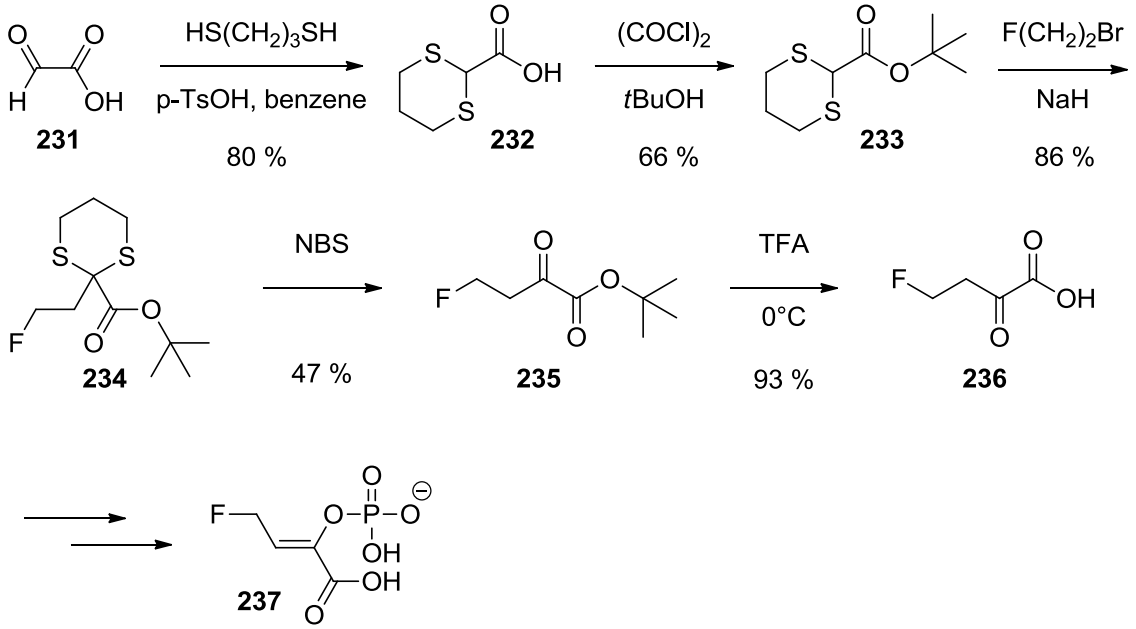
Recently Zhao *et al*. described an efficient method for the conversion of primary or secondary alcohols into fluorides with PBSF-TBAT.**Fehler! Textmarke nicht definiert.** This procedure was efficient for the synthesis of one homoallylic fluoride **230** as well (Scheme 78).



Scheme 78. Synthesis of homoallylic fluoride 230 by Zhao *et al*.Fehler! Textmarke nicht definiert.

Previous Synthesis

As depicted in scheme 79, 4-fluoro-2-oxo-butanoic acid (**236**) was previously prepared by O'Leary *et al*. as an intermediate in their synthesis of (*Z*)-3-(fluoromethyl)phosphenol-pyruvate (**237**).[111] Commercially available glycolic acid **231** was azeotropically thioacetalized with 1,3-propanthiol and the acid functionality was protected as *tert*-butyl ester. The sodium anion of this dithiane **233** was alkylated with 1-bromo-2-fluoroethane in high yield. Cleavage of the dithianyl protecting group by NBS and the *tert*-butyl ester afforded 4-fluoro-2-oxo-butanoic acid (**236**).

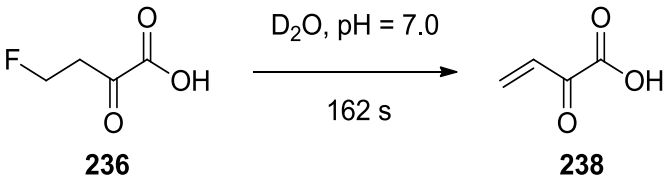


Scheme 79. Synthesis of4-fluoro-2-oxo-butanoic acid (236) by O'Leary *et al*. [111]

Stability of 4-fluoro-2-oxo-butanoic acid (236)

O'Leary *et al*.[111] observed a rapid decomposition of 4-fluoro-2-oxo-butanoic acid (**236**) to 2-oxo-3-butenoate (**238**) in D_2O with a half-life of decomposition of 162 s at pH = 7.0 (25°C)

and of 52 s at pH = 8.0 (Scheme 80). At pH = 2.1, decomposition occurred with a half-life of several hours.

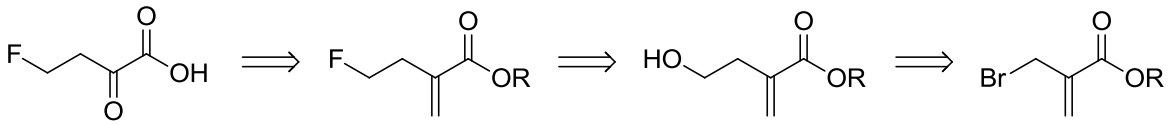


236 **238**

Scheme 80. Decomposition of 4-fluoro-2-oxo-butanoic acid reported by O'Leary.[111]

Concept and Strategy

From experiences of my diploma thesis,[112] we thought that the reported instability of 4-fluoro-2-oxobutanoate (**236**) might originate in mistakes in experimental setups. In this previous work[113] we synthesized *tert*-butyl 4-S-acetyl-2-oxobutanoate, a compound which is expected to eliminate more likely thioacetic acid than 4-fluoro-2-oxobutanoate (**236**) hydrogen fluoride. Therefore we believed 4-fluoro-2-oxobutanoate (**236**) to be more stable and potentially a useful compound for the incorporation of fluorine into proteins.



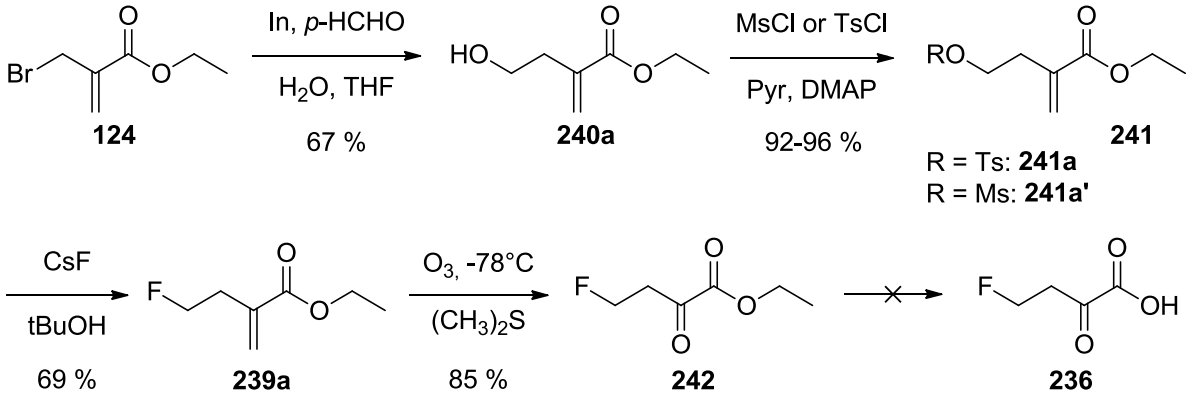
236 **239** **240** **124**

Scheme 81. Retrosynthetic analysis

In our retrosynthetic analysis we took the well documented general instability of free α-keto acids into account.[114] As a consequence, the acid and keto functionality has to be liberated as late as possible in the synthesis (Scheme 81). The fluorine atom was planned to be introduced by a homoallylic fluorination either via nucleophilic displacement of a sulfonate by a fluorine anion or by deoxofluorination of compound **240** with DAST. (The more selective XtalFluor reagents and the PBSF-TBAT system were not known to that time). Homoallylic alcohol **240** is accessible by our commonly used precursor **124** for the synthesis of isotope labeled α-keto acids.[113]

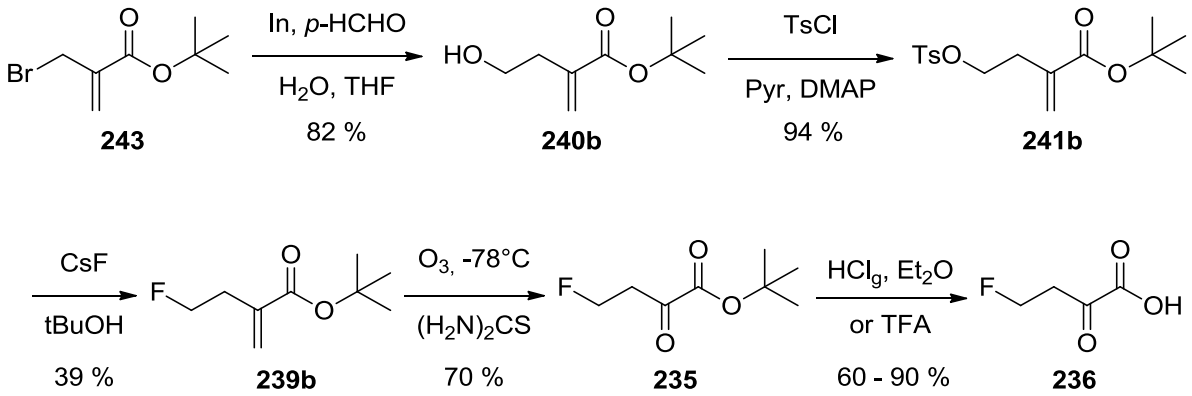
D.3. Results and Discussion

Starting form easily accessible ethyl α-bromomethylacrylate (**124**)[27], homoallylic alcohol **240a** was obtained by an indium mediated allylation between **124** and an aqueous solution of formaldehyde (Scheme 82).[113] Primary alcohol **240a** was transformed into the corresponding mesylate or tosylate under standard conditions.[113]



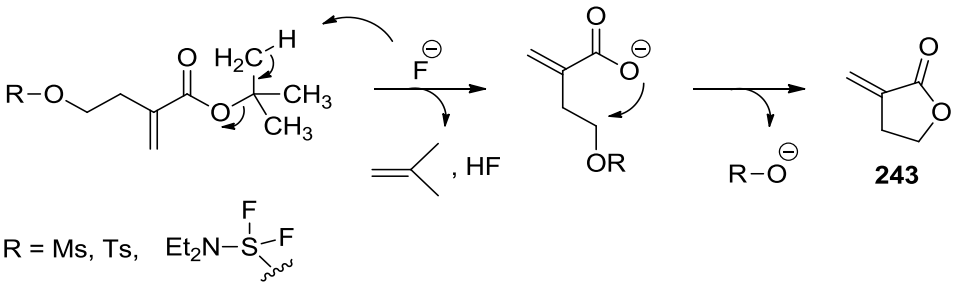
Scheme 82. Attempted synthesis of 4-fluoro-2-oxo-butanoic acid.

Nucleophilic displacement of the mesylate by KF with 18-crown-6 in acetonitrile did not yield any product. Interestingly this condition has been very efficient for synthesis of allylic fluoride **52**. Homoallylic fluoride **239a** was obtained by nucleophilic displacement of the mesylate or tosylate **241a** in good yield by using Chi's CsF/tBuOH system. This experiment was the first example that these conditions are efficient for the synthesis of homoallylic fluorides. Subsequently the double bond of compound **239a** was cleaved by ozonolysis to yield α-keto ester **242**. Unexpectedly the ozonide destroying reagent was crucial to isolate pure products or any product at all. Appling triphenylphosphine no products containing a fluorine atom could be purified. Dimethylsulfide gave the desired α-keto ester **242** along with seven percent 2-oxo-3-butenoate (**238**) and DMSO. A chromatographic purification was hampered by a concomitant elimination of hydrogen fluoride. Furthermore the boiling point of DMSO and the product was too similar to remove the solvent. Cleavage of the ethyl ester was unfortunately under all applied conditions unsuccessfully. No product could be isolated either by saponification under acid conditions (Dowex 50 H^+, 0.025 M HCl, CH_3CN) or by S_N2-type dealkylations (6.5 eq. LiI, $DMF_{abs.}$, reflux or 6.5 eq. LiI, dimethylpryidine, reflux). As a consequence, we protected the acid functionality by a *tert*-butyl ester which should be cleavable under slightly acidic conditions.



Scheme 83. Synthesis of 4-fluoro-2-oxo-butanoic acid.

Surprisingly the *tert*-butyl ester had a strong influence on the yield of the homoallylic fluorination (Scheme 83). The yield was diminished by two side reactions. A partial cleavage of the *tert*-butyl ester yielded γ-lactone **243** as major side product. A possible mechanism is depicted in scheme 84. The fluorine anion may act as a base, removing a proton of the *tert*-butyl ester and liberating isoprene. Subsequently the acid functionality attacks the leaving group and cyclize to the γ-lactone **243**. The other side product, the homoallylic alcohol **240b** originated from nucleophilic attack of hydroxide ions onto the tosylate **241b**.

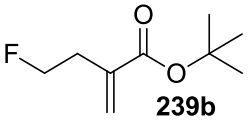
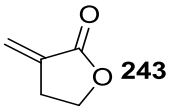
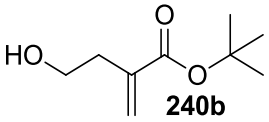


Scheme 84. Possible mechanism for γ-lactone formation.

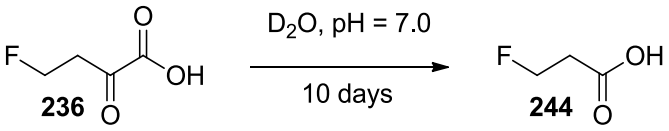
A screening of nucleophilic fluorination methods revealed that only the CsF/*t*BuOH system gave the desired homoallylic fluoride **239b** (Table 3). All other methodologies yielded quantitatively the γ-lactone **243**. These findings may be rationalized by the unique nucleophilic behavior of CsF in *t*BuOH as described above. Obviously under the other applied fluorination conditions the fluorine anion was less nucleophilic and acted as a strong base.

It became apparent that the work-up of the ozonolsyis is crucial for the synthesis of compound **235** as well. Triphenylphosphine gave no fluorinated product once again. In addition, using dimethylsulfide was hampered by removing the resulting DMSO. This severe

Table 3. Nucleophilic fluorination of compound 241b.

Conditions	 **239b**	 **243**	 **240b**
KF/18-crown-6		quant.	
KF/ionic liquid		quant.	
CsF/*t*BuOH	39 %	40 %	19 %
CsF/ionic liquid		quant.	
DAST		quant.	

problem could be solved by addition of thiourea to the intermediate formed ozonide. The resulting precipitate which is insoluble in methanol can be filtered off via normal filter paper. Using celite substantial amounts of elimination products were formed. The *tert*-butyl ester was cleaved off by two different methods. Primarily, the ester moiety was removed by gaseous HCl in an ether/dichloromethane solution. Interestingly, beside the desired α-keto acid **236**, the major product was 3-fluoropropionic acid (**244**) as shown by ^{1}H-NMR in D_2O. Only minor amounts (< 5%) of the elimination product 2-oxobut-3-enoic acid (**238**) were formed. Prolonged standing of the reaction solution simplified the spectra and after ten days 4-fluoro-2-oxo-butanoic (**236**) acid was fully converted into 3-fluoropropionic acid (**244**) (Scheme 85). In contrast to O'Leary *et al*., we observed a slow decarbonylation instead of HF-elimination of 4-fluoro-2-oxo-butanoic acid (**236**). Furthermore this loss of carbon prolonged over ten days compared to extremely fast elimination reaction (162 s at pH = 7) O'Leary has published.



Scheme 85. Decomposition of 4-fluoro-2-oxobutanoate 236.

Using dry TFA at 0°C, the *tert*-butyl ester was cleanly removed to yield 4-fluoro-2-oxo-butanoic acid (**236**).[111] The corresponding ^{1}H-NMR spectrum in D_2O is depicted in figure 16, demonstrating that the keto functionality is hydrated to a large excess (keto/*gem*-diol = 1/2.4).

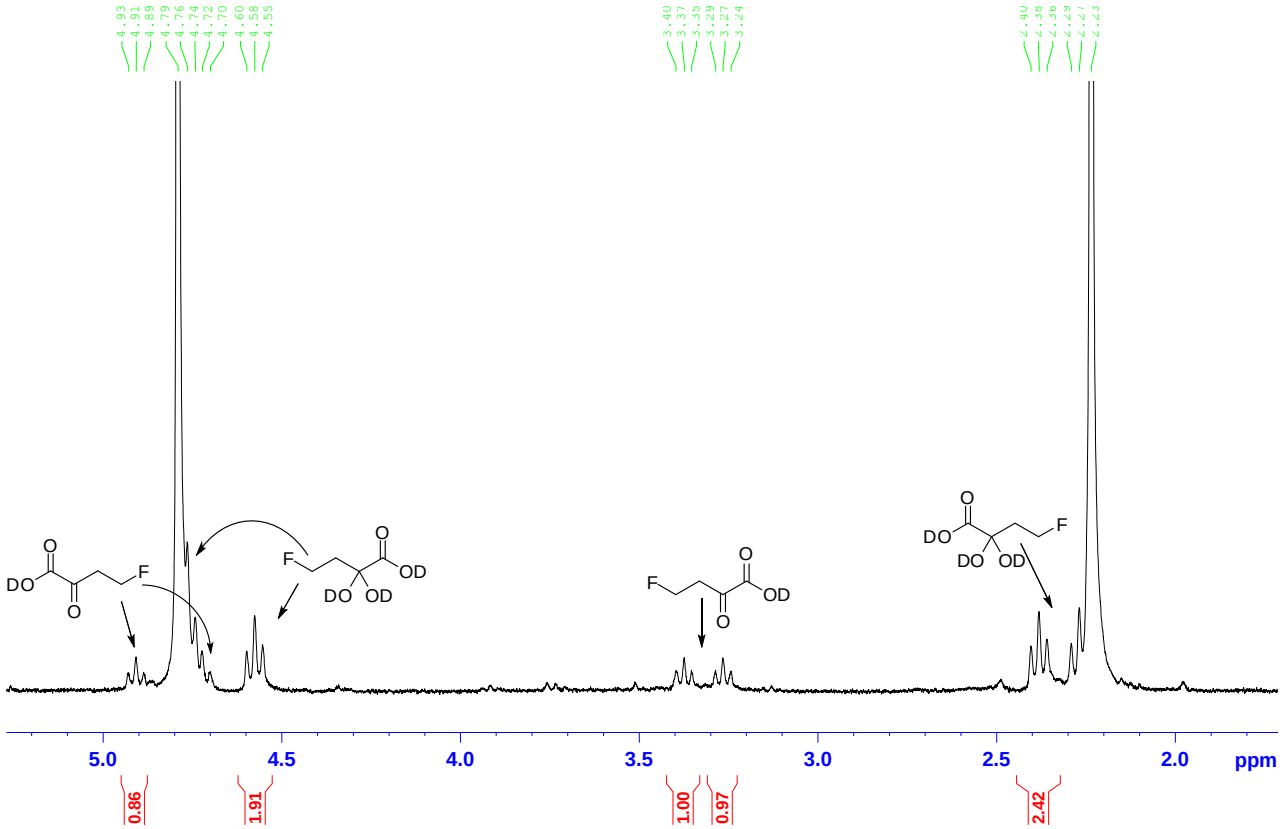


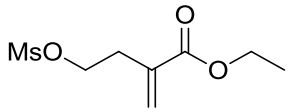
Figure 16. ^{1}H-NMR spectrum of 4-fluoro-2-oxo-butanoic acid in D_2O.

D.4. Experimental Procedures

Following compounds were synthesized according to literature procedures:

- Ethyl 4-hydroxy-2-methylenebutanoate (**240a**)[112]
- Ethyl 2-methylene-4-(tosyloxy)butanoate (**241a**)[112]
- *tert*-Butyl 4-hydroxy-2-methylenebutanoate (**240b**)[112]
- *tert*-Butyl 2-methylene-4-(tosyloxy)butanoate (**241b**)[112]
- 4-Fluoro-2-oxobutanoic acid (**236**)[111]

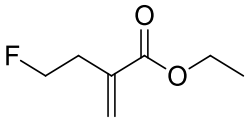
Ethyl 2-methylene-4-(mesyloxy)butanoate (**241a'**):



241a'

To a solution of ethyl 4-hydroxy-2-methylenebutanoate (**240a**) (446 mg, 3.09 mmol) in dry CH_2Cl_2 (10 mL), triethylamine (1.03 mL, 7.4 mmol) and *p*-meyslchloride (425 mg, 3.7 mmol) were added under an argon-atmosphere at 0 °C. After 1 hour of stirring at room temperature (TLC: toluene/ethyl acetate 2:1; stain solution: aqueous KMnO4 -solution) CH_2Cl_2 (15 mL) and 2M NH_4Cl-solution (5 mL) were added. The aqueous phase was separated and extracted with CH_2Cl_2 (3 x 20mL) and the combined organic layers were washed with water, dried over $MgSO_4$, filtered and the solvent was evaporated. Subsequent column chromatography over silica gel (toluene/ethyl acetate 2:1) provided ethyl 2-methylene-4-O-mesyl-butanoate (**241a'**) (658 mg, 96 %). ^{1}H NMR (250 MHz; $CDCl_3$) δ = 6.31 (s, 1H, CH) 5.74-5.70 (m, 1H, CH), 4.37 (t, *J* = 6.7 Hz, 2H, CH_2), 4.22 (q, *J* = 7.0 Hz, 2H, CH_2) 2.98 (s, 3H, CH_3) 2.76 (dt, *J* = 6.6 Hz, *J* = 0.8 Hz, 2H, CH_2), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3).

Typical procedure for homoallylic fluorination: Synthesis of ethyl 4-fluoro-2-methylene-butanoate (239a):

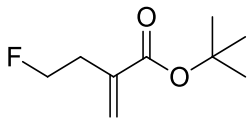


239a

To a solution of ethyl 2-methylene-4-(tosyloxy)butanoate (**241a**) (1.69 g, 5.66 mmol) in dry *t*BuOH (40 mL), cesium fluoride (2.58 g, 17 mmol) was added at room temperature. The reaction mixture turned into a white gel and was stirred for two days at 80°C. The reaction was quenched by addition of a half saturated chloride solution and the aqueous phase was extracted with diethyl ether. The product was carefully concentrated under reduced pressure (the product co-evaporated with *t*BuOH) and was purified by distillation over a Vigreux column (75°C, 750 mbar) to give ethyl 4-fluoro-2-methylenebutanoate (**239a**) (605 mg, 69%). Using ethyl 2-methylene-4-(mesyloxy)butanoate (**241a'**) as an educt gave identical yields. ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 6.29 (s, 1H, CH), 5.71-5.69 (m, 1H, CH), 4.56 (dt, *J* = 47.2

Hz, *J* = 6.1 Hz, 2H, CH_2), 4.22 (q, *J* = 7.1 Hz, 2H, CH_2) 2.71 (dtd, *J* = 21.4 Hz, *J* = 6.1 Hz, *J* = 1.1 Hz, 2H, CH_2), 1.30 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$): δ = 166.7, 136.0 (*J* = 5.0 Hz), 127.7, 82.1 (*J* = 167.4 Hz), 61.0, 33.3 (*J* = 21.0 Hz), 14.3.

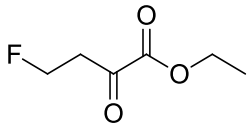
***tert*-Butyl 4-fluoro-2-methylenebutanoate** (**239b**):



239b

Starting from *tert*-butyl 2-methylene-4-(tosyloxy)butanoate (**241b**) (2.13 g, 6.53 mmol), *tert*-butyl 4-fluoro-2-methylenebutanoate (**239b**) (455 mg, 40 %;) was synthesized like described for **239a**. ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 6.19 (s, 1H, CH), 5.64-5.60 (m, 1H, CH), 4.55 (dt, *J* = 47.2 Hz, *J* = 6.2 Hz, 2H, CH_2), 2.71 (dtd, *J* = 21.2 Hz, *J* = 5.9 Hz, *J* = 0.7 Hz, 2H, CH_2), 1.50 (s, 9H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$): δ = 166.6, 137.4 (*J* = 5.1 Hz), 82.3 (*J* = 167.2 Hz), 81.1, 33.4 (*J* = 21.0 Hz), 28.2; ^{19}F-NMR (400 MHz; $CDCl_3$): δ = -218.4 (tt, J = 47.1 Hz, *J*= 24.3 Hz).
Sideproducts: *tert*-butyl 4-hydroxy-2-methylenebutanoate (**240b**) (238 mg); 3-methylenedihydrofuran-2(3H)-one (**243**) (552 mg) (Spectroscopical data were identical with Nakatsu *et al*.[115]).

Ethyl 4-fluoro-2-oxobutanoate (242):

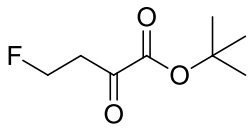


242

Ozone was bubbled through a solution of ethyl 4-fluoro-2-methylenebutanoate (**239a**) (20 mg, 0.137 mmol) in CH_2Cl_2 (2 mL) at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of dimethylsulfide (32 mg, 0.55 mmol), the mixture was allowed to reach room temperature over night. The solvent was carefully removed under reduced pressure (300 mbar at 30°C) to yield ethyl 4-fluoro-2-oxobutanoate (**242**) (approximately: 16 mg, 78 % after subtraction of DMSO and 2-oxo-3-butenoate (**238**) by integration of ^{1}H-NMR resonances). A complete removed of DMSO was not possible due to co-evaporation of the product and DMSO. A

chromatographic purification over silica gave more elimination product. ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 4.75 (dt, *J* = 46.4 Hz, *J* = 5.7 Hz, 2H, CH_2), 4.32 (q, *J* = 7.1 Hz, 2H, CH_2) 3.23 (dt, *J* = 24.0 Hz, *J* = 5.7 Hz, 2H, CH_2), 1.35 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$): δ = 191.2 (*J* = 4.3 Hz), 160.7, 136.0 (*J* = 5.0 Hz), 78.2 (*J* = 167.0 Hz), 63.2, 40.2 (*J* = 22.1 Hz), 14.3.

tert-Butyl 4-fluoro-2-oxobutanoate (235)

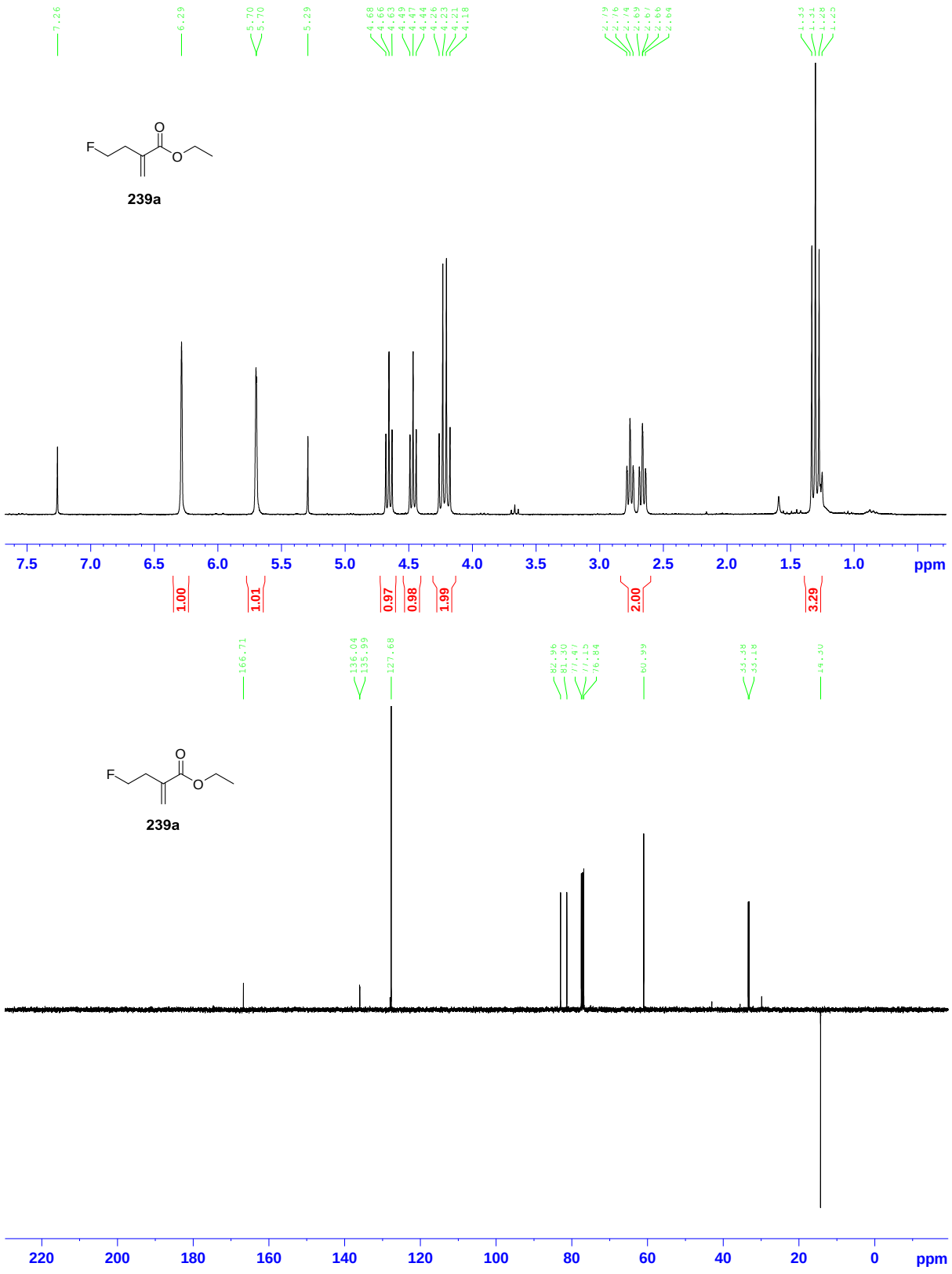


235

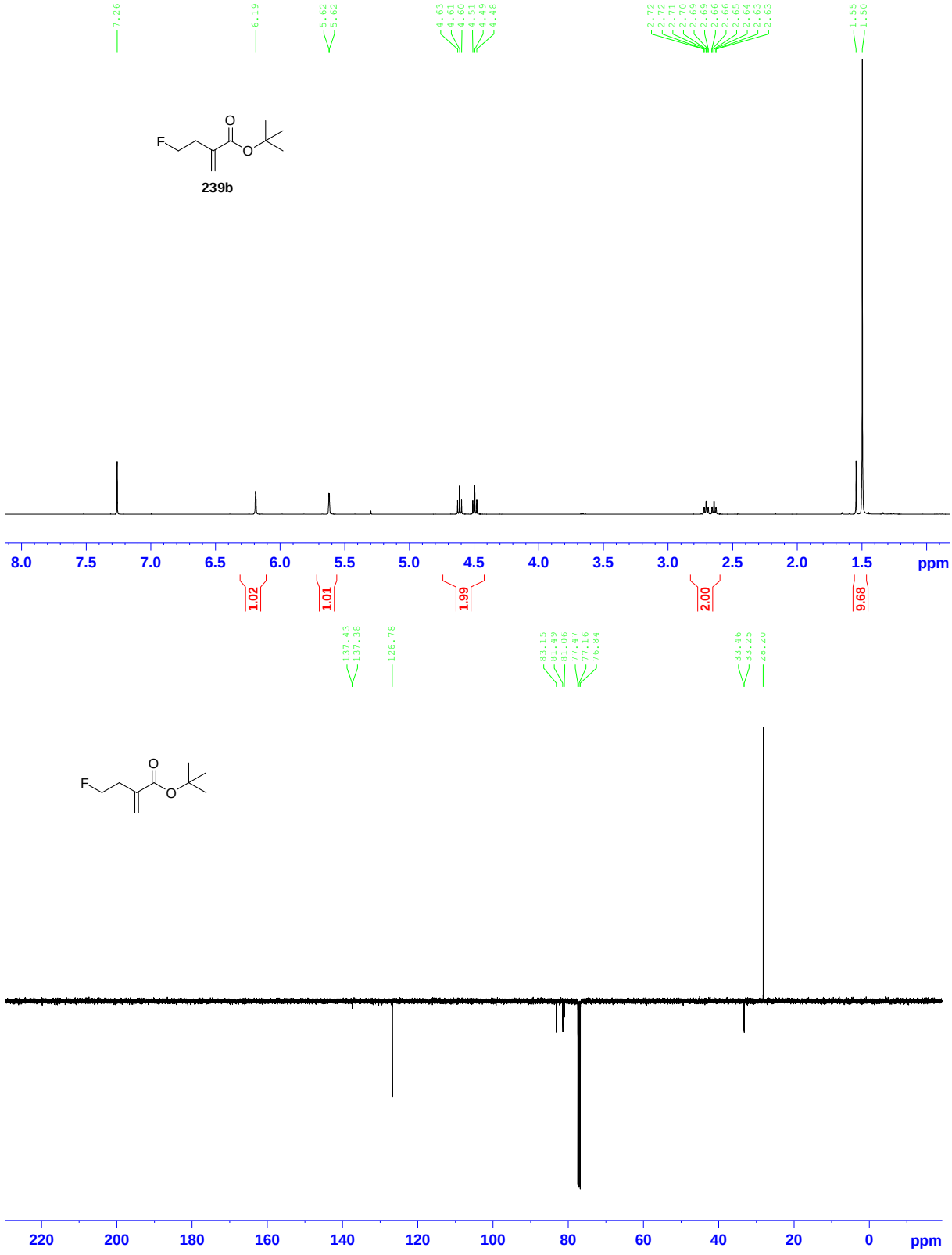
Ozone was bubbled through a solution of *tert*-Butyl 4-fluoro-2-methylenebutanoate (**239b**) (10 mg, 0.057 mmol) in methanol (0.5 mL) at -30°C for two minutes. Dry air was bubbled through the solution for three minutes. After addition of thiourea (2.5 mg, 0.032 mmol) the mixture was allowed to reach room temperature and a white solid precipitated. After filtration over paper, the solvent was removed under reduced pressure to yield *tert*-butyl 4-fluoro-2-oxobutanoate (**242**) (7 mg, 70 %). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 4.76 (dt, *J* = 46.4 Hz, *J* = 5.8 Hz, 2H, CH_2), 3.20 (dt, *J* = 23.7 Hz, *J* = 5.8 Hz, 2H, CH_2), 1.55 (s, 9H, CH_3); ^{13}C-NMR (400 MHz; $CDCl_3$): δ = 192.0 (*J* = 4.4 Hz), 159.9, 84.6, 78.1 (*J* = 166.8 Hz), 39.8 (*J* = 22.1 Hz), 27.9. ^{19}F-NMR (600 MHz; $CDCl_3$): δ = -221.6 (tt, *J* = 46.4 Hz, *J* = 23.5 Hz).

Selected spectra:

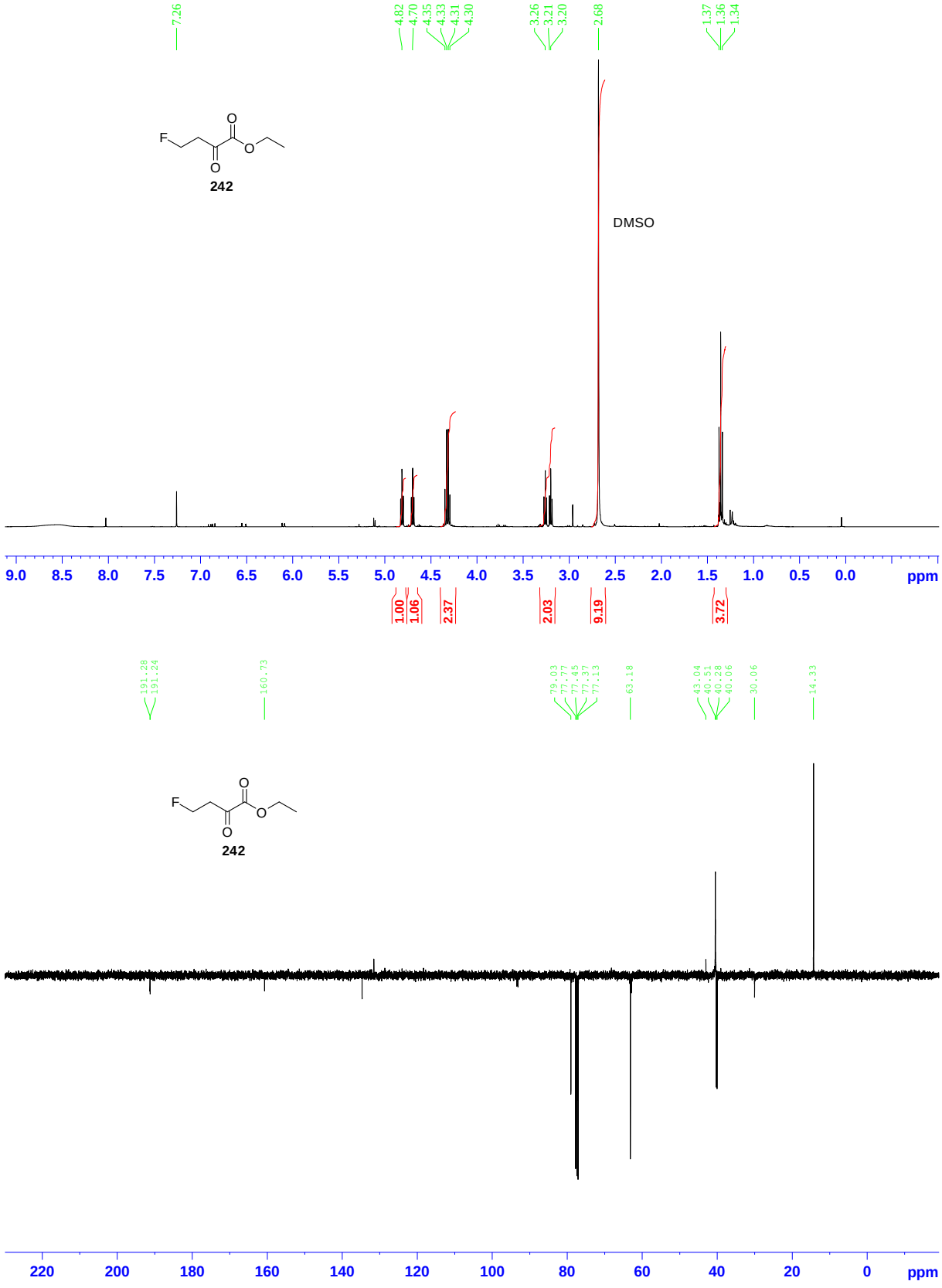
Ethyl 4-fluoro-2-methylenebutanoate (**239a**):



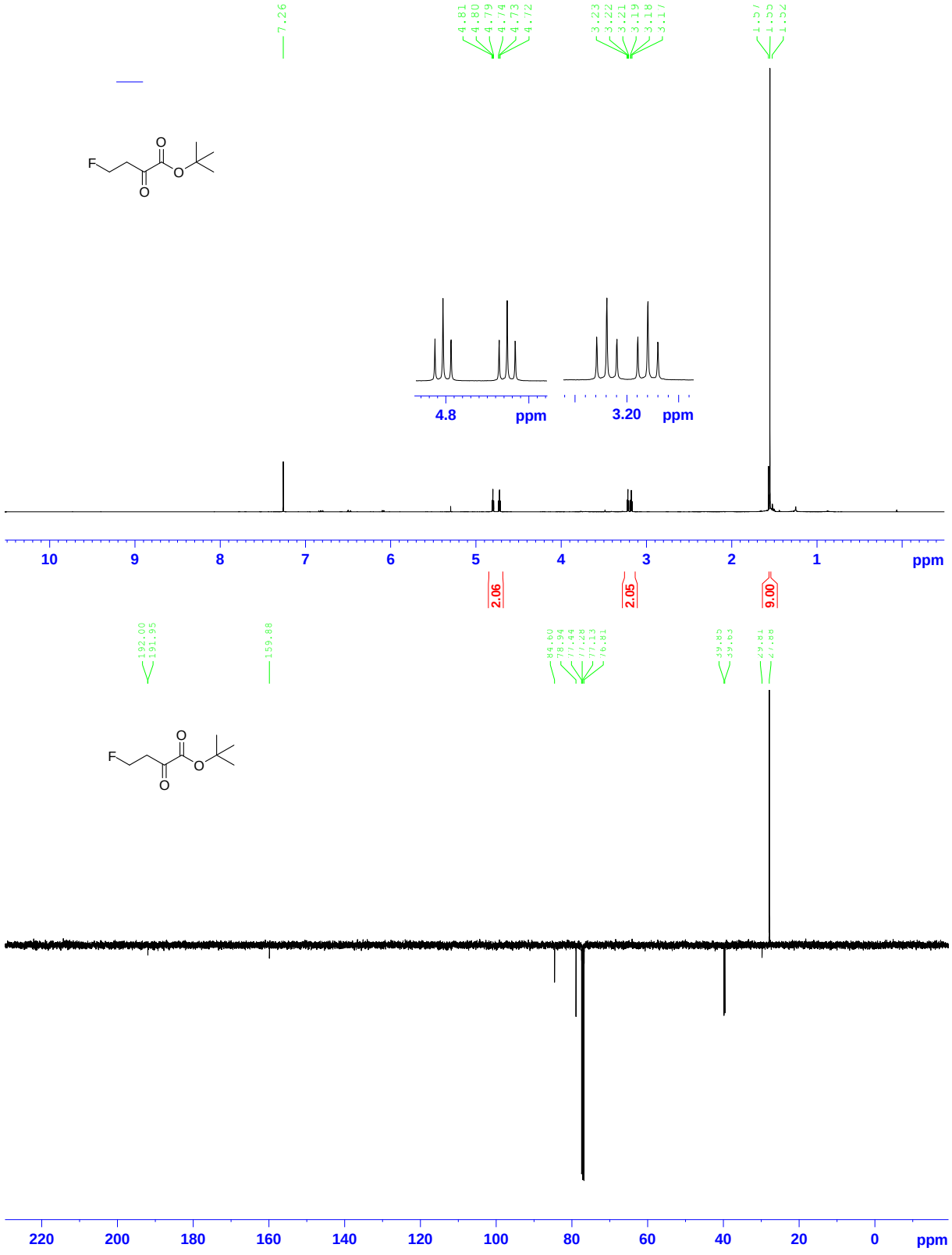
tert-Butyl 4-fluoro-2-methylenebutanoate (**239b**):



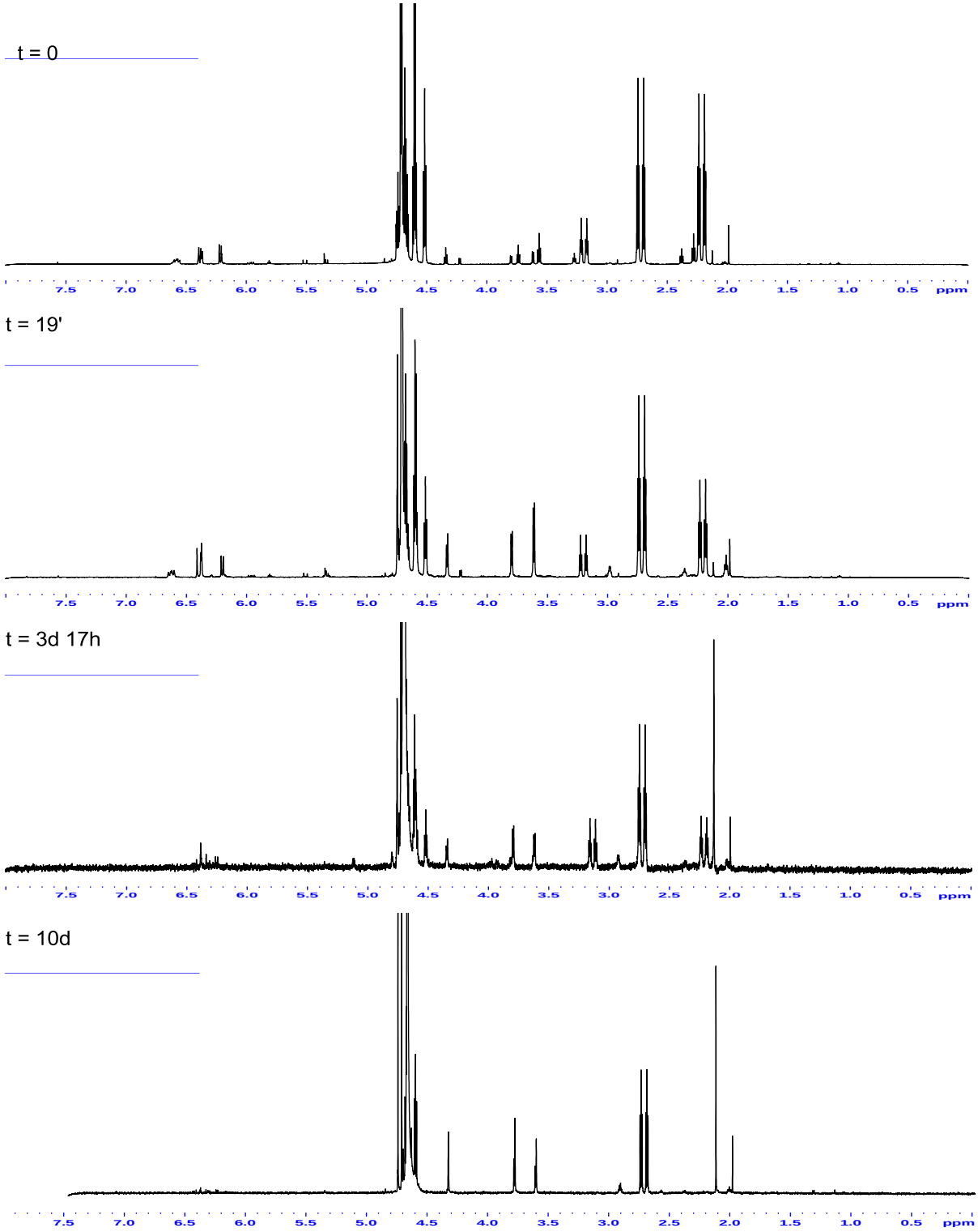
Ethyl 4-fluoro-2-methylenebutanoate (**242**):



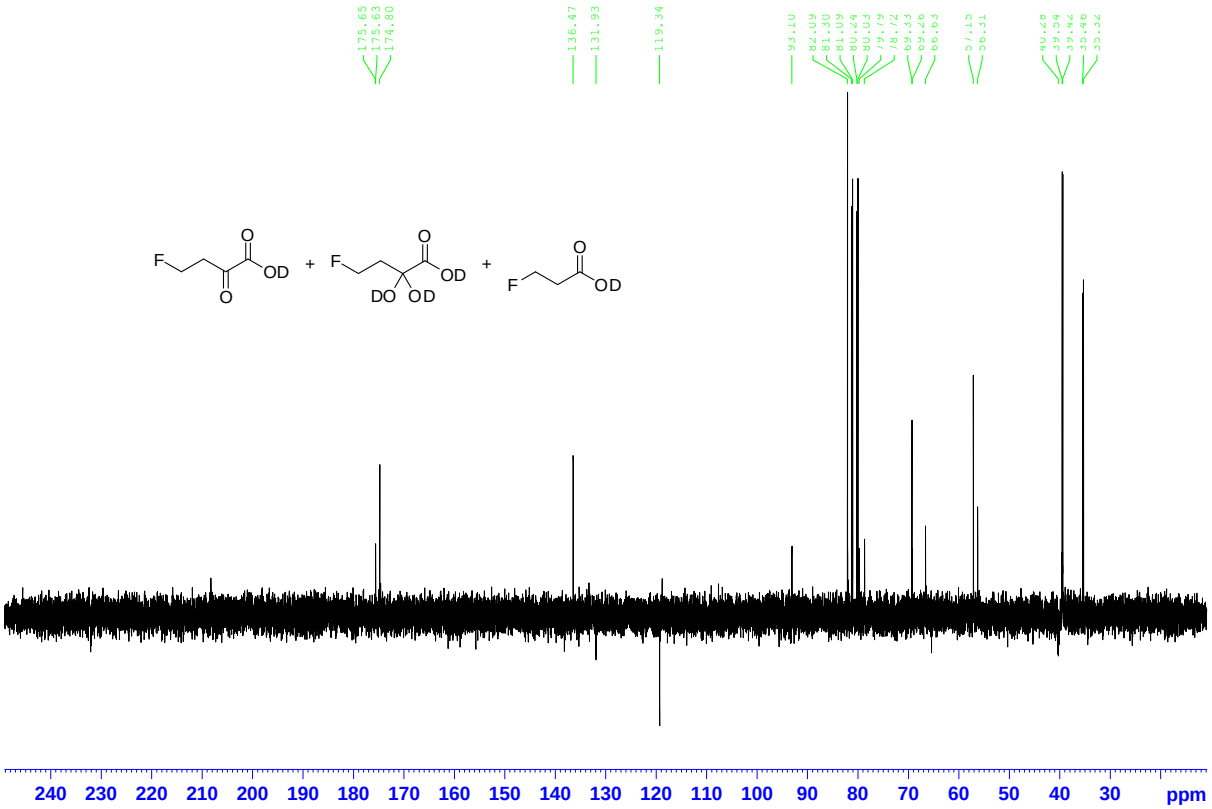
tert-Butyl 4-fluoro-2-oxobutanoate (**235**):



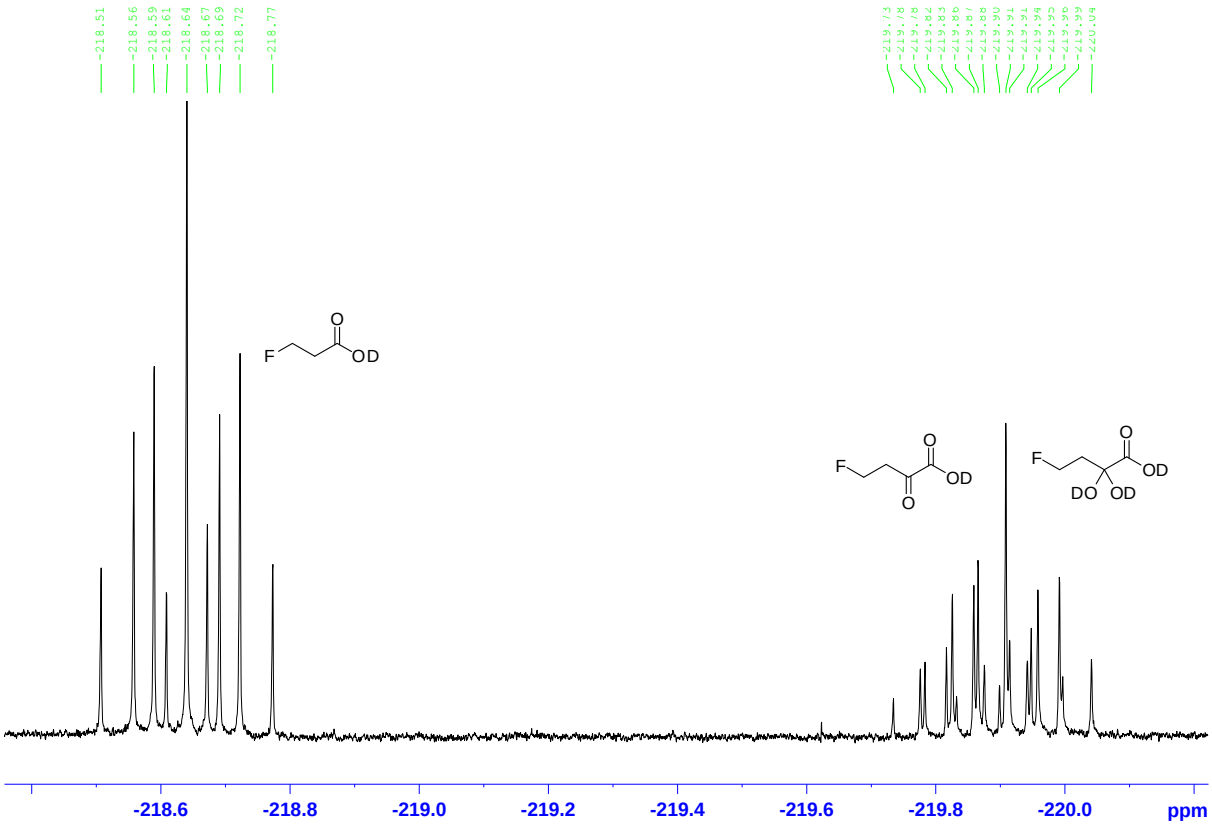
4-Fluoro-2-oxobutanoic acid (**236**) (^{1}H-NMR): ^{1}H-NMR spectra after deprotection of *tert*-butyl 4-fluoro-2-oxobutanoate (**235**) with gaseous HCl in CH_2Cl_2/diethyl ether (1/1) – prolonged standing of the reaction mixture in the NMR tube at room temperature



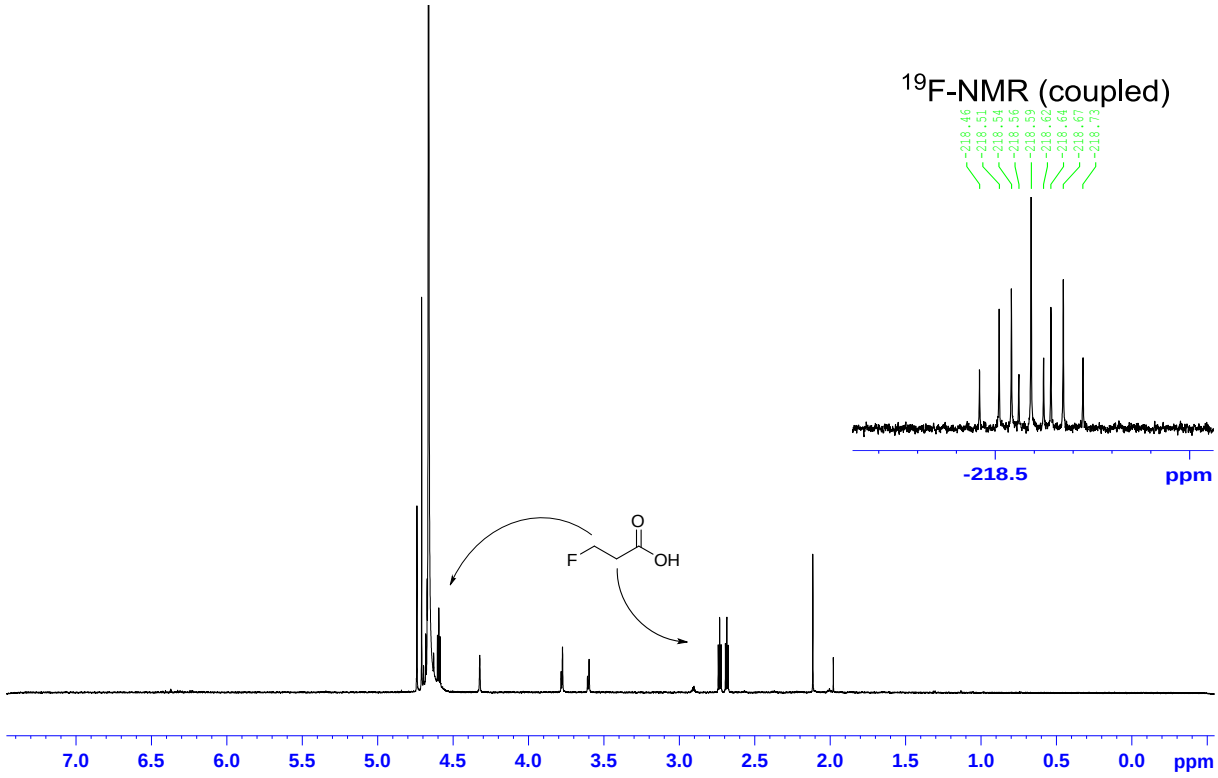
4-Fluoro-2-oxobutanoic acid (235) (^{13}C-NMR) after three hours:



4-Fluoro-2-oxobutanoic acid (**235**) (^{19}F-NMR; coupled) after three hours:



3-Fluoropropanoic acid (**244**) (^{1}H-NMR and ^{19}F-NMR; coupled) after ten days:



PART E: Preliminary Experiments Regarding the Synthesis of Selectively Labeled Imidazol-5-yl Pyruvic Acid

E.1. Abstract

The aim of this project was the development of a selectively ^{13}C and deuterium labeled precursor compound for the synthesis of histidine labeled proteins. The α-keto acid imidazol-5-yl pyruvic acid, usually involved in the degradation of histidine, was chosen as a possible precursor for the *in vivo E. coli* mediated synthesis of histidine. A convergent synthesis of imidazol-5-yl pyruvic acid was attempted to label the imidazole ring and the resulting peptide backbone from cheap carbon-13 enriched precursors.

E.2. Introduction and Background

Histidine adopts a unique position among the 20 proteinogenic amino acids because of the chemical properties of the imidazole side chain. The possibility of the imidazole moiety to switch between the protonation states is essential for the catalytic function of many enzymes which rely on a general acid/base mechanism. As a consequence, histidine residues are often found in the active site of enzymes such as proteases and ribonuclease A. Another general function of the nitrogen atoms of the imidazolium ring is the chelation of metal ions like zinc, copper, iron and cobalt in metalloenzymes.[116] In addition, in past decade an increasing number of publications described the importance of phosphorylation and dephosphorylation of the imidazole nitrogen moiety for signal transduction.[117] To study these manifold properties of histidine it is essential to provide special isotope labeled histidine for protein expression by bacteria or by cell-free methods.[118] Besides isotope labeled amino acids, suitable isotope labeled biosynthetic precursors were used for the overexpression of selectively labeled proteins and enzymes by *E. coli*. Among these groups of compounds the most versatile precursors have been the α-keto acids.[119] The in situ

transformation of α-keto acids into the corresponding α-amino acid by *E. Coli* circumvents the necessary multistep asymmetric synthesis of the L-amino acid.

Protein Labeling with α-Keto Acids[120]

The first α-keto acid applied for protein labeling was pyruvate in D_2O.[121] Protein expression resulted in complete deuteration at C^{α} and 80% deuteration at C^{β} position of nearly all amino acids. The methyl groups of Ala, Val, Leu and Ile (γ2 only) remained highly protonated. Unfortunately the protons of the methyl group of pyruvate exchanged with the solvent D_2O leading to isotopomers (CH_2D, CHD_2 and CD_3) which were transferred to the methyl groups of the biosynthesized protein. Wang *et al*. used [3-^{13}C]-pyruvate[122] for the labeling (>90 %) of Leu^{δ}, Val^{γ}, Ile^{γ} and Ala^{β} methyl groups. In a more precise study Tugarinov *at al.* applied [1,2-$^{13}C_2$]-pyruvate[123] for selective backbone labeling. Since pyruvate is metabolized in the citric acid cycle twelve of the 20 amino acids were labeled. High labeling efficiency was obtained for Ala, Val, Lys, Tyr, Phe, Trp, Ser, Gly (50-100%) and low efficiency for Thr, Met, Asn and Asp (20-40 %).

A more effective strategy for the labeling of Val, Leu and Ile applied the direct biosynthetic precursors for these amino acids. Isotope labeled α-ketoisovalerate was used for the labeling of Leu and Val[124] and α-ketobutyrate for Ile.[125] Recently, an efficient and versatile synthetic concept for these compounds was published by Lichtenecker *et al*.[126] Additionally the methyl group of methionine was labeled via its corresponding isotope labeled α-keto acid.[113]

Isotope Labeling of Histidine Residues of Proteins

[*ring*-2-^{13}C]-Histidine was incorporated in vivo into the galactose-H^+ symport protein by overexpression in histidine auxotrophic strain of *E. Coli*. A detailed MS analysis showed that the ^{13}C-label was selectively incorporated into the membrane transport protein to an extent of 80 %.[127] Uniformly labeled $^{13}C_6$-histidine was used to monitor side-chain dynamics and protonation of histidine in *A. variabilis* plastocyanin by ^{13}C NMR relaxation dispersion measurement.[128]

Alternatively the aromatic amino acids Phe, Tyr, His and Trp can be simultaneously labeled with [1-^{13}C]-glucose to a theoretical extent of 50% at specific positions.[129]

The Biosynthesis of Histidine

The amino acid histidine is synthesized by an extremely complex sequence of nine enzyme-catalyzed transformations starting from adenosine triphosphate (ATP) (**246**) and phosphor-ribosylpyrophosphate (PRPP) (**247**) (Scheme 86). For the de novo synthesis of histidine (**245**) approximately 41 molecules of ATP (**246**) are necessary,[130] emphasizing its extraordinary importance among the 20 proteinogenic amino acids. In the first step, a transferase catalyzes the displacement on C1 of PRPP (**247**) by N1 of the purine ring of ATP (**246**) under liberation of pyrophosphate. The ribose moiety derived from PRPP (**247**) is inverted from the α to the β configuration. Subsequently the triphosphate **250** is irreversible hydrolyzed to the N'-5'-phosphoribosyl-adenosin monophosphate (PR-AMP) (**251**) which is ring-opened by a PR-AMP cyclohydrolase. In the next step, an isomerase catalyzes the interconversion of the phosphoribosyl moiety into a phosphoribulosyl unit by an Amadori rearrangement. The imidazol ring of imidazoleglycerol phosphate (IGP) (**254**) and 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) are generated from phosphoriboslyl formino-5-aminoimidazole-4-carboxamide ribonucleotide (PRFAR) (**253**) by a synthetase. A Mn^{2+} ion-dependent dehydratation of IGP (**254**) combined with a keto-enol tautomerisation produces imidazolacetol phosphate (IAP) (**255**). The final precursor histidinol (**257**) is synthesized by a reversible transamination of IAP (**255**) with glutamate, followed by a dephosphorylation of histidinol phosphate (**256**).

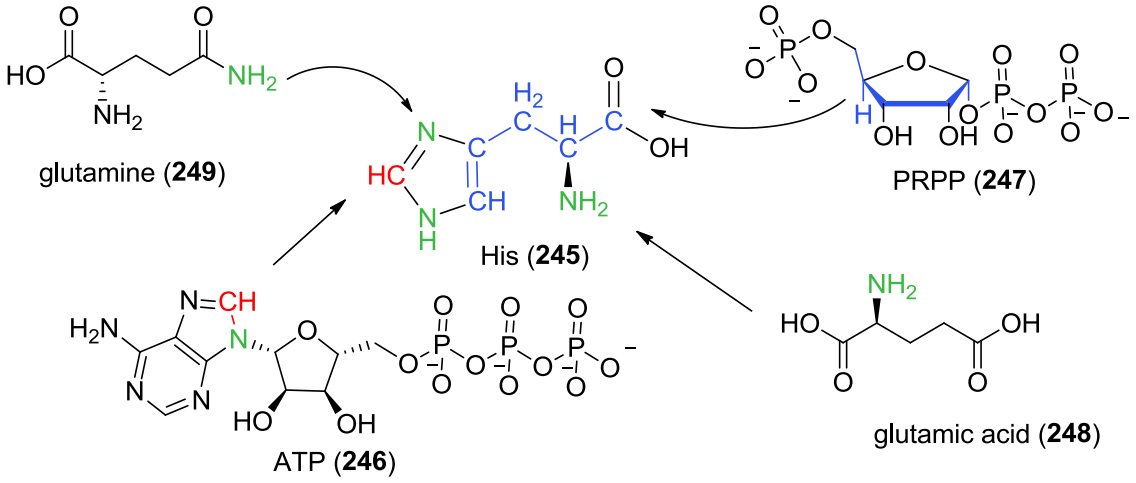
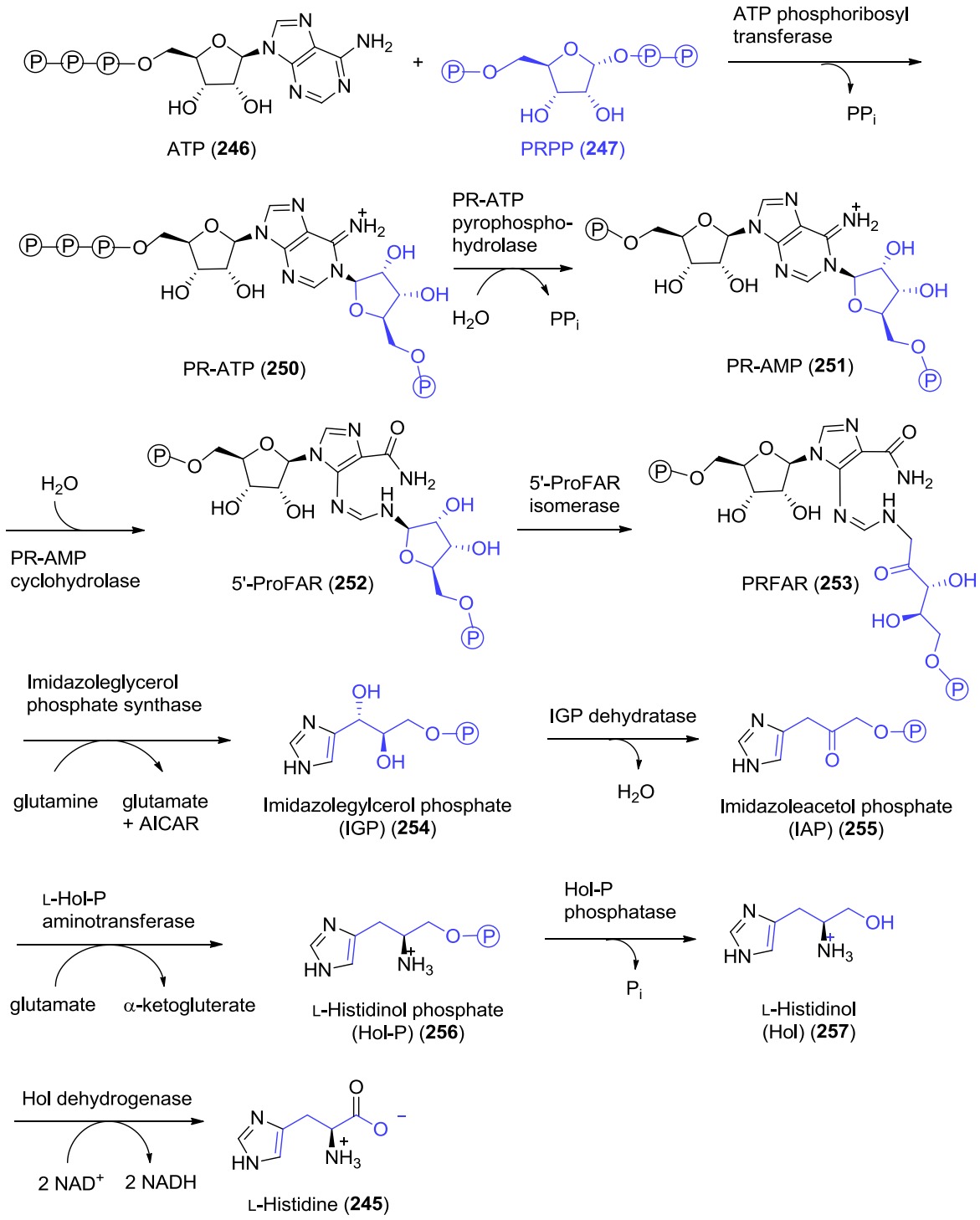


Figure 17. Biosynthetic precursors of histidine[130]

In the last step histidine (**245**) is generated by a NAD^+- and Zn^{2+}-dependent oxidation of the primary alcohol functionality to the carboxylic acid. For the sake of clarity the origin of the nitrogen and carbon atoms of histidine are summarized in figure 17.



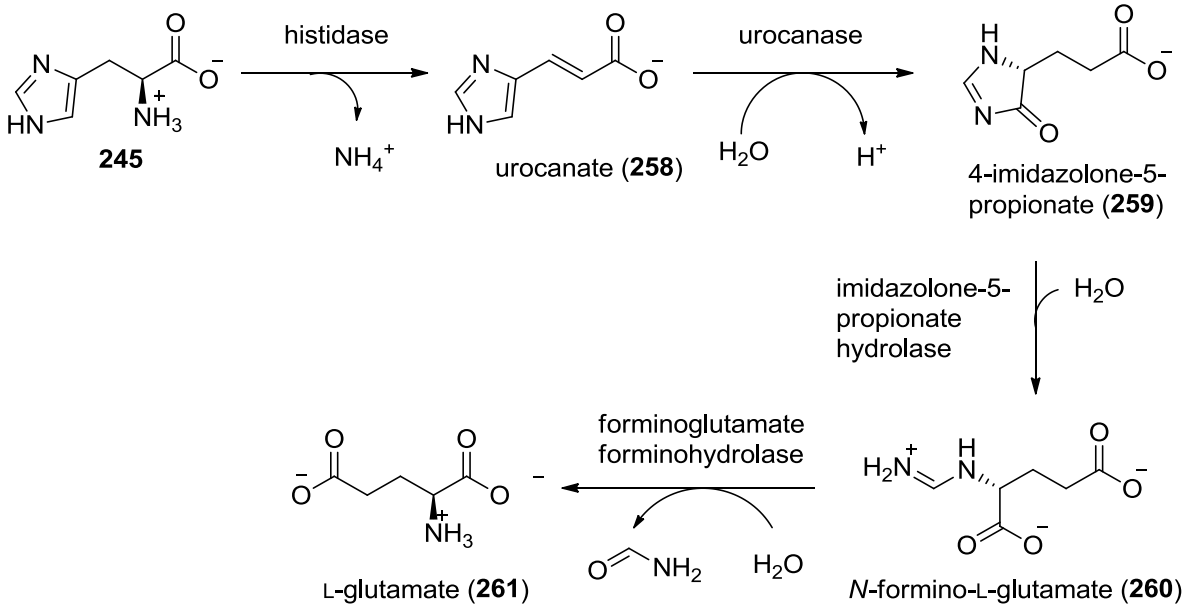
Scheme 86. Biosynthesis of L-histidine (245)[130]

As depicted in Scheme 85, a suitable precursor for protein labeling might be the last intermediate L-histidinol (**257**). All the other biosynthetic intermediates are either more

complicated to synthesize than histidine (**245**) or are probably too polar for cell-wall permeation. Compared to histidine (**245**), the synthesis of histidinol (**257**) might be as complicated since both compounds constitute out of one chiral center. Alternatively it might be possible to use an intermediate of the degradation pathways of histidine for protein labeling.

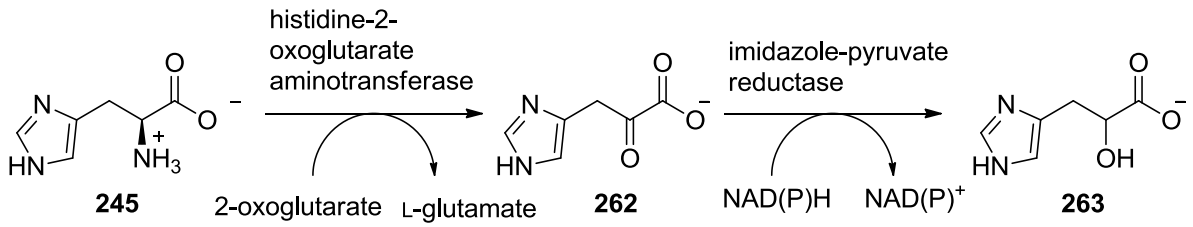
Histidine Degradation

The main degradation pathway of histidine (**245**) leads to urocanate (**258**) by elimination of ammonium (Scheme 87).[131] In two further enzyme catalyzed steps urocanic acid (**258**) is converted into L-glutamate (**261**). From a synthetic point of view only urocanate (**258**) is a valuable precursor for the synthesis of isotope labeled proteins. Unfortunately the elimination of ammonium from histidine (**245**) is practically irreversible in the bacterium and therefore urocanate (**258**) is not suitable for protein labeling.



Scheme 87. Histidine degradation (Path B)[132]

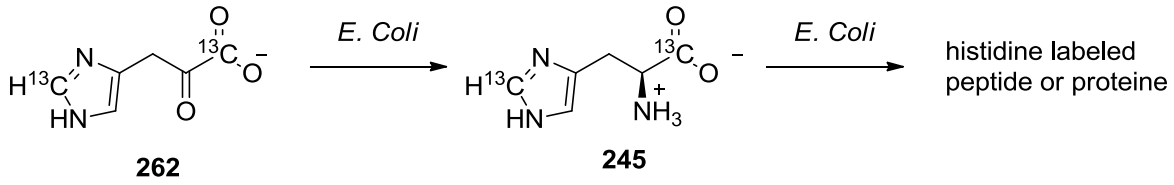
Alternatively, the minor degradation pathway of histidine (**245**) starts with the transamination of histidine (**245**) to imidazol-5-yl pyruvic acid (**262**) which is further reduced to imidazol-5-yl lactate (**263**). These two reactions are the only ones that have been elucidated of this degradation pathway (Scheme 88).[132]



Scheme 88. Histidine degradation (Path B)[132]

Concept and Strategy

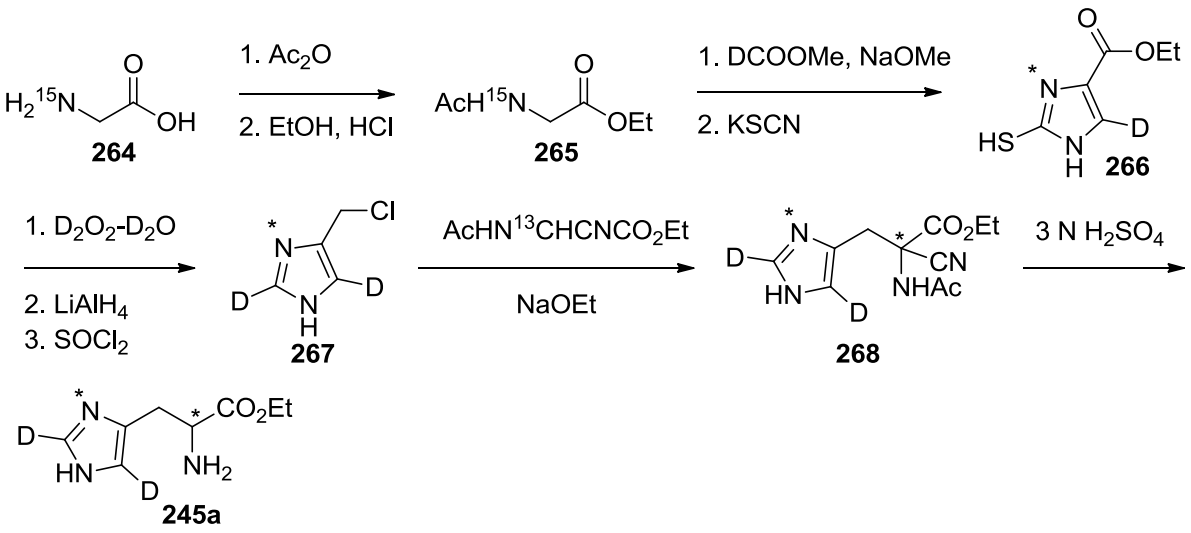
In our opinion, considering the synthetic accessibility and biological production of histidine (**245**), the most promising precursor for protein labeling might be imidazol-5-yl pyruvic acid (**262**) (Scheme 89). As described above, α-keto acids have been used successfully as precursors for several other amino acids for protein labeling and showed similar or higher incorporation values than the corresponding labeled amino acids. Subsequently, the previous reported strategies for the synthesis of multi-labeled histidine[133] (**245a-245g**) are discussed which were the basis for our retrosynthetic considerations towards imidazol-5-yl pyruvic acid (**262**).



Scheme 89. Strategy for the synthesis of selectively isotope labeled histidines in proteins (one possible isotope pattern is indicated)

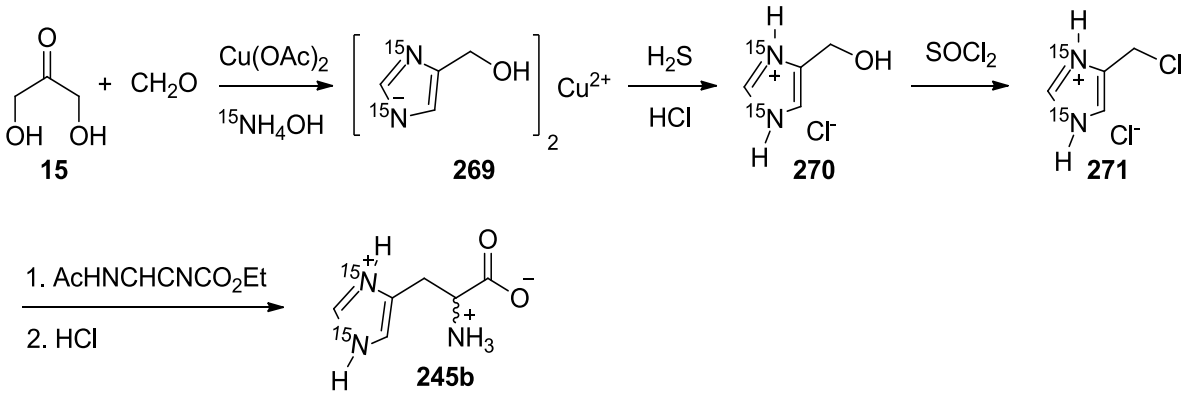
Previous Syntheses of Multi-Labeled Histidine

The first isotope labeled synthesis of histidine (**245a**) was reported by SooHoo *et al*. in 1976 (Scheme 90).[134] Histidine (**245a**) was synthesized carbon-13 labeled at the α-carbon, N-15 labeled at the N1 and deuterium labeled at C2 and C4 of the imidazole ring. This sequence of seven steps afforded racemic multi-labeled histidine (**245a**) in 3% overall yield.



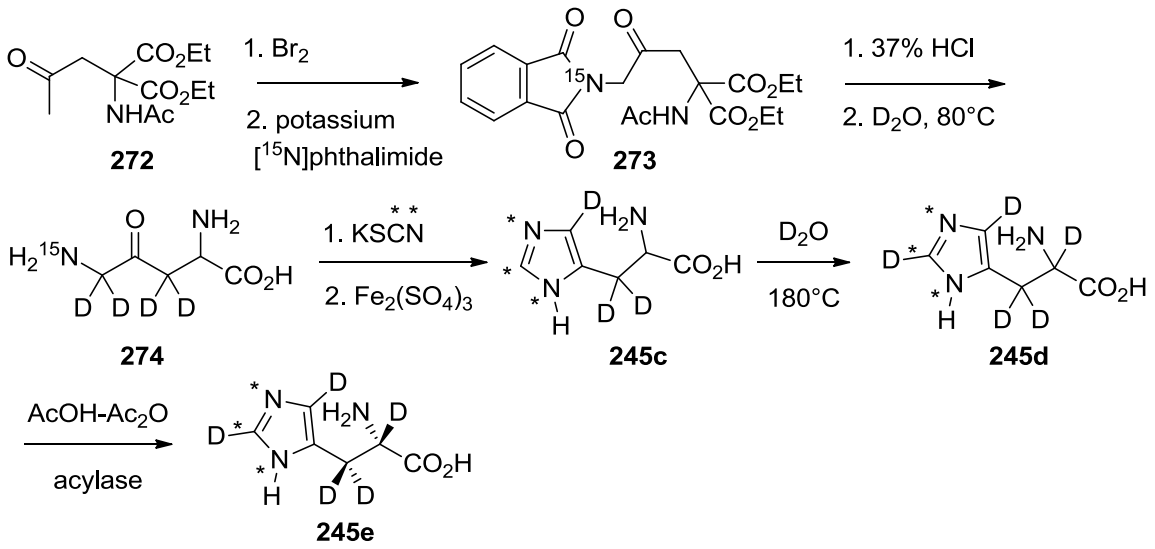
Scheme 90. Synthesis of multi-labeled histidine 245a by SooHoo *et al.*[134]

One year later Silks *et al*. published a short and efficient synthesis of racemic multi-labeled histidine (**245b**).[135] Imidazol-4-ylmethanol (**270**) was isolated as a water insoluble copper salt after condensation of dihydroxyacetone with ammonia (Scheme 91). Copper sulfide precipitation with hydrogensulfide afforded primary alcohol (**270**) in 59% yield based on dihydroxyacetone (**15**) or 37% on $^{15}NH_4OH$. Conversion of imidazole (**270**) was performed identical to SooHoo *et al*. and gave racemic multi-labeled histidine (**245b**) in 47 % overall yield based on dihydroxyacetone or 30% on $^{15}NH_4OH$.



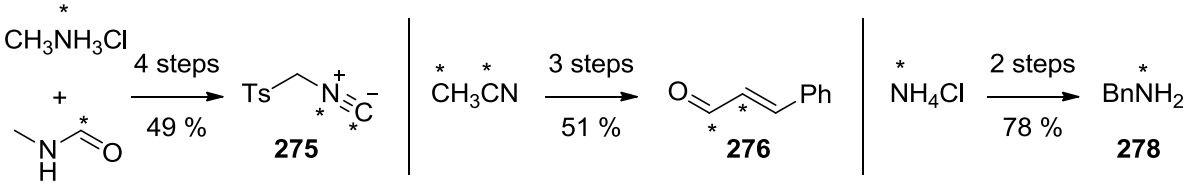
Scheme 91. Synthesis of multi-labeled histidine 245b by Silks III *et al.*[135]

Furuta *et al*. synthesized multi-labeled L-histidine (**245e**) after enzymatic resolution of (**245d**) in 4% overall yield (Scheme 92).[136] The N1 of the imidazolium ring was nitrogen-15 labeled via a Gabriel synthesis, C2 and N3 of the imidazolium ring were labeled via $KS^{13}C^{15}N$ and deuteration was performed in D_2O at elevated temperatures.



Scheme 92. Synthesis of multi-labeled histidine 245c-e by Furuta *et. al.*[136]

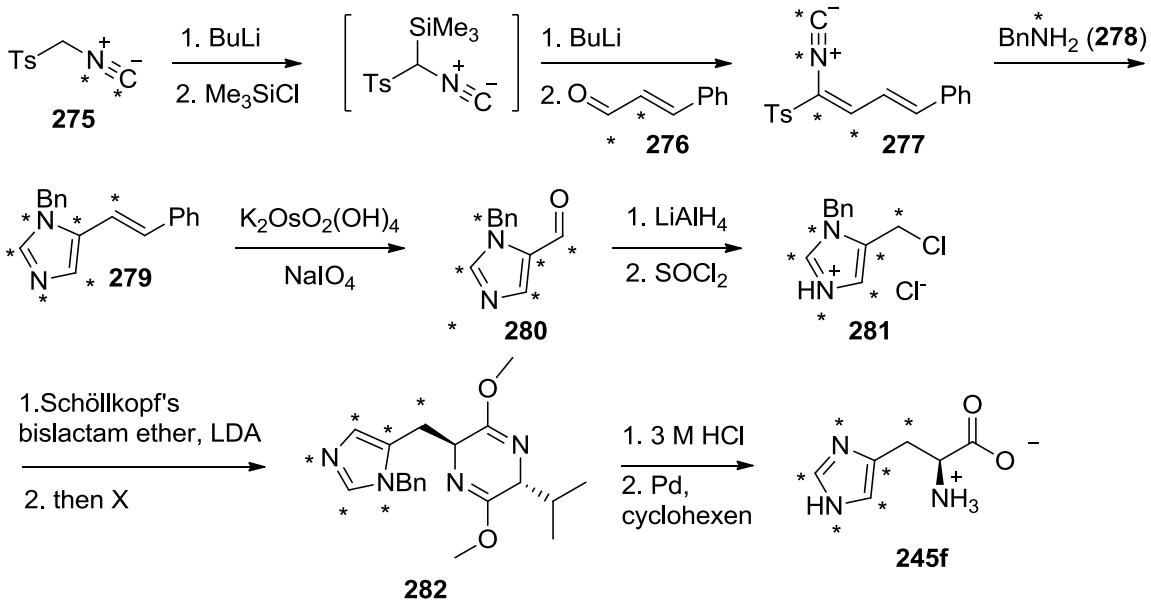
The first enantioselective synthesis of multi-labeled histidine was published by Cappon *et al*. in 1994 (Scheme 94).[137] The imidazole ring was constructed by the Van-Leusen Three Component Reaction.[138] The reactants for this imidazole synthesis were synthesized from isotope labeled methylammonium chloride, N-methylformamide, acetonitrile and ammonium chloride (Scheme 93). After transformation of **279** in three steps into benzyl protected chloromethylimidazole (**281**), histidine (**245f**) was enantioselective synthesized by the Schöllkopf bislactim ether method.[139] This strategy virtually allows a selective incorporation of several carbon-13 and nitrogen-15 atoms at each desired position and afforded multi-labeled histidine (**245f**) in 35% overall yield based on tosylmethyl isocyanide (**275**).



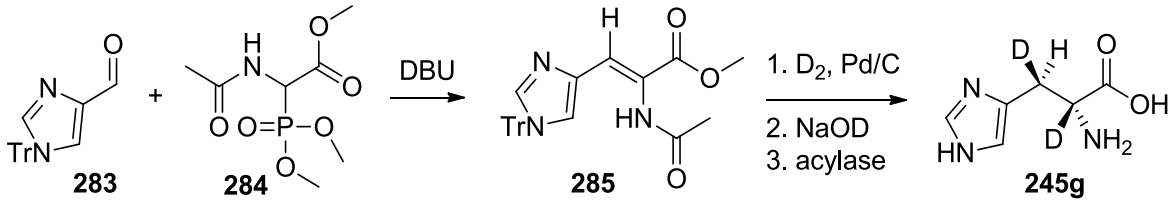
Scheme 93. Reactants for the Van Leusen Three-Component Reaction.[137]

Nishiyama *et al*. reported a general methodology for the synthesis of L-*threo* and L-*erythro*-[1-^{13}C, 2,3-$^{2}H_2$]amino acids (Scheme 95).[140] Methyl *N*-acetyl-2-(dimethoxy-phosphoryl)glycinate[141] (**284**) was condensed with 1-tritylimidazole-4-carbaldehyde (**283**) to afford compound (**285**) in 49% yield. Stereoselective incorporation of deuterium was

accomplished by catalytic deuteration. Enzymatic resolution after cleavage of the methoxy ester gave multi-labeled histidine (**285g**) in 24% overall yield.



Scheme 94. Synthesis of multi-labeled histidine by Cappon *et al*.[137]

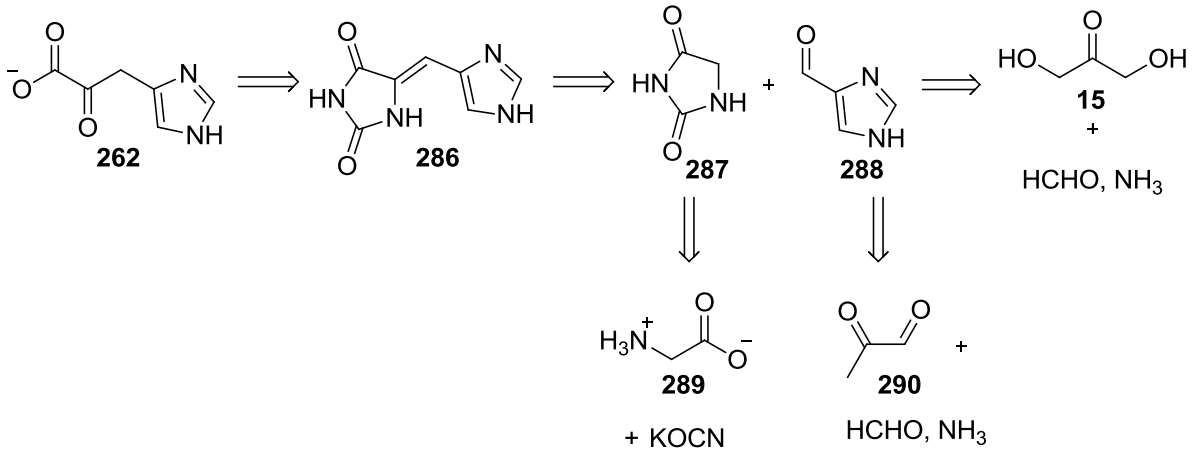


Scheme 95. Synthesis of multi-labeled histidine by Nishiyama *et al*.[140]

Retrosynthetic Analysis

The same retrosynthetic considerations which had been regarded in the synthesis of 4-fluoro-2-oxobutanoate (**236**) have been taken as a basis for the synthesis of imidazol-5-yl pyruvic acid (**262**). The α-keto functionality was planned to be liberated at the same time as the carboxylic acid from hydantoin derivative **286** (Scheme 96). Compound **286** is accessible via the condensation of hydantoin (**287**) with imidazole-4-carbaldehyde (**288**).[142,143] Hydantoin (**287**) can be prepared from glycine (**289**) and potassium cyanate by a slight modification of the Bucherer-Bergs reaction.[144] Imidazole-4-carbaldehyde (**288**) is accessible by an oxidation of the cyclization products from 2-oxopropanal[145] (**290**) or dihydroxyacetone

(**15**) with formaldehyde and ammonia.[146,147] These approaches would allow the labeling of histidine (**245**) from cheap carbon-13 and nitrogen-15 sources (glycine, formaldehyde and NH_4Cl).



Scheme 96. Retrosynthetic analysis

Deuterium labels can be introduced by D_2O at elevated temperatures at C2 and C4 of the imidazolium ring[136] and under slightly basic conditions at the β carbon of imidazol-5-yl pyruvic acid (**262**) (Figure 18). A very similar strategy was used for the synthesis of 4-hydroxyphenyl- and phenylpyruvic acid by R. Lichtenecker.[148]

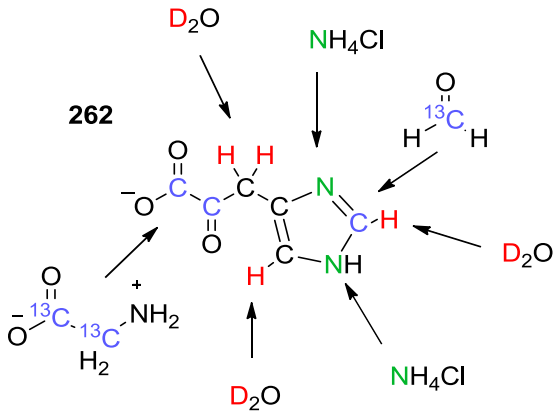
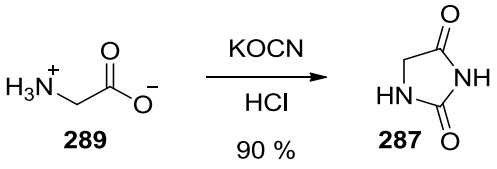


Figure 18. Possible isotope label patterns of imidazol-5-yl pyruvic acid (262)

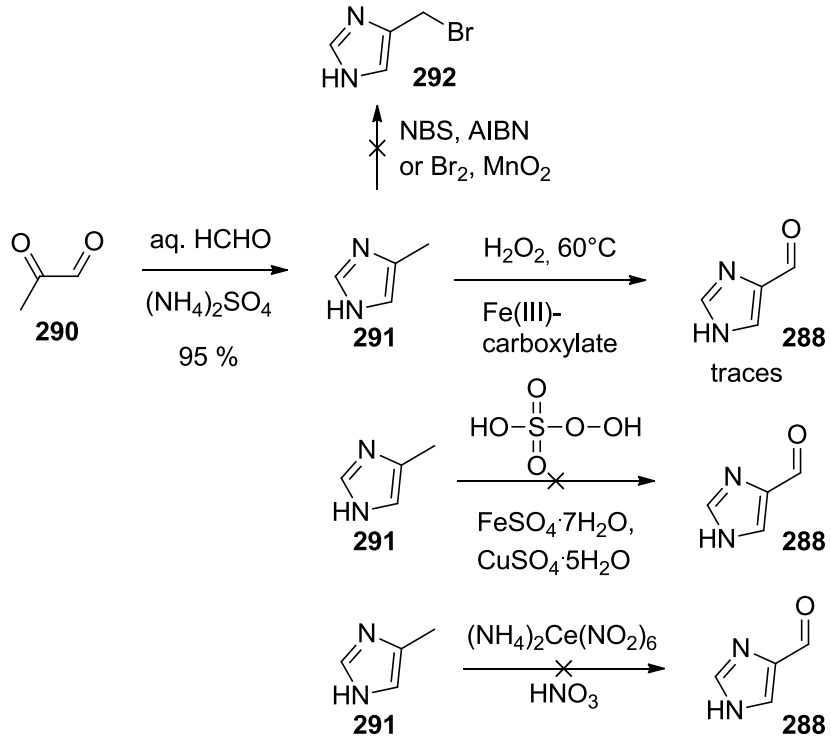
E.3. Results and Discussion

According to literature procedures, the hydantoin (**287**) was synthesized form glycine and potassium cyanate in high yield.[144] The optimum pH-value for the cyclization is below pH 1 (Scheme 97).



Scheme 97. Hydantoin synthesis[144]

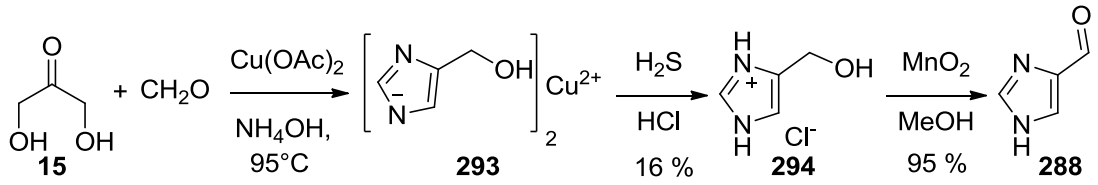
For the synthesis of imidazole-4-carbaldehyde (**288**) two different approaches were tested. A cyclization of 2-oxopropanal (**290**) with ammonium sulfate or ammonium chloride and freshly prepared formaldehyde under acidic conditions (pH = 0-1) afforded 4-methyl-imidazol (**291**) in excellent yield (Scheme 98).[145] The literature known $FeSO_4$ and $CuSO_4$ catalyzed oxidation of a methyl group with permonosulfonic acid did not yield any product. According to Hamazaki *et al.*,[149] this oxidation gives imidazole-4-carbaldehyde (**288**) in 95 % yield.



Scheme 98. First approach towards imidazole-4-carbaldehyde (288) or 4-(bromomethyl)imidazole (292).

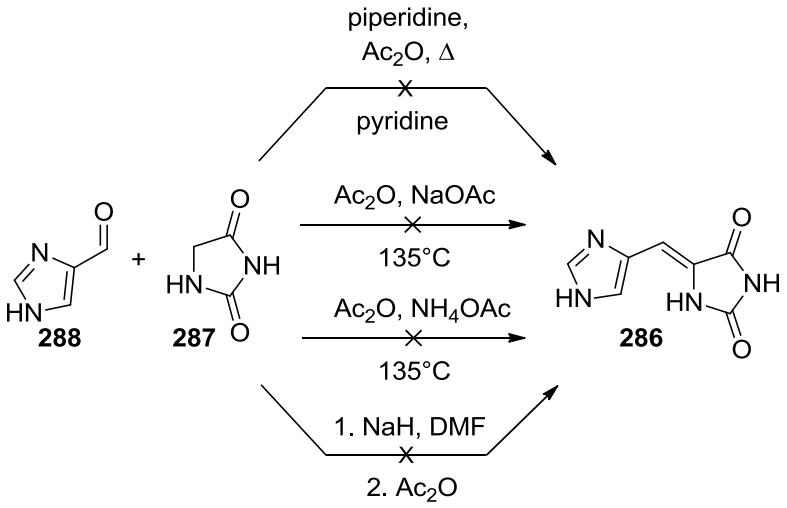
A iron(III)-carboxylate catalyzed oxidation with hydrogen peroxide gave the desired product (**288**) only in trace amounts. The original procedure described by Schmid *et al*.,[150] which was developed for the oxidation of unactivated cycloalkanes, was used. For further studies lower reaction temperatures may be beneficial. Cer(IV)ammonium nitrate in nitric acid did not yield the desired product.[151] A radical bromination with NBS or bromine with MnO_2[152] of the methyl group only led to a decomposition of the imidazole ring system.

In the second approach, imidazole-4-carbaldehyde (**288**) was synthesized by oxidation of imidazol-4-ylmethanol (**294**) with manganese dioxide in excellent yield (Scheme 99). The alcohol **294** was prepared in low yield by cyclization of dihydroxyacetone **15** with formaldehyde and copper(II)acetate in ammoniacalic solution.[135] The resulting imidazolium copper complex **293** was filtered and freed from copper by bubbling hydrogen sulfide through the reaction mixture. The low yield may be enhanced by further experimentation and by heating the reaction mixture under microwave conditions.



Scheme 99. Second approach towards imidazole-4-carbaldehyde (288)

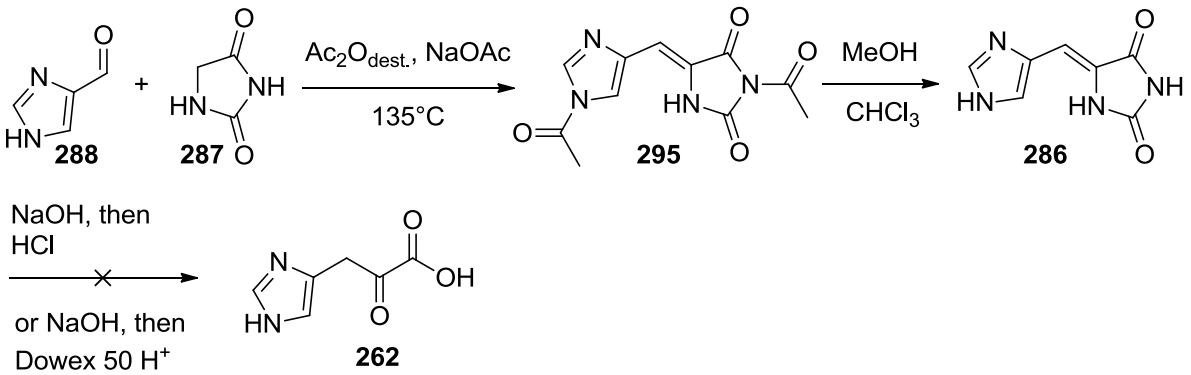
After obtaining hydantoin (**287**) and imidazole-4-carbaldehyde (**288**), various literature known coupling procedures which were successful for aldehyde **288** or benzaldehyde derivatives were performed. All initial attempts, even the conditions from Deulofeu *et al*. (Ac_2O, NaOAc, 135°C) for exactly the same coupling were unsuccessful (Scheme 100).[142]



Scheme 100. Attempted condensation of hydantoin (287) with aldehyde 288

Applying ammonium acetate instead of sodium acetate, which was beneficial for the coupling of hydantoin with *p*-methoxybenzaldehyde did not give any product (Scheme 100).[148]

For a successful coupling, the quality of the acetic anhydride proved to be essential. The desired coupling product was exclusively obtained by using freshly distilled acetic anhydride in combination with sodium acetate at elevated temperatures. The acetyl protecting groups were easily cleaved during work-up with methanol (Scheme 101).



Scheme 101. Primarily studies towards the synthesis of imidazol-5-yl pyruvic acid (262)

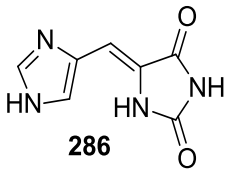
The final opening of the hydantoin ring system could not be solved. Reaction conditions which have been very efficient for the opening 5-(*p*-hydroxybenzal)hydantoin were uneffective.[148,153] In the original procedure, the α-keto acid was continuously extracted under acidic conditions in an atmosphere of argon for several hours. In our case no product isolation was possible, since under acidic conditions the imidazole ring is protonated and the free α-keto acid displays extraordinarily good water solubility. Changing the work-up conditions from hydrochloric acid to an ion exchange chromatography did not enhance the procedure. Imidazol-5-yl-pyruvate (**262**) is presumably too unstable for these reaction conditions and has to be synthesized by a different procedure. Furthermore, the generation of the imidazole ring has to be improved for an isotope label synthesis.

E.4. Experimental Procedures

Following compounds were synthesized according to literature procedures:

- Hydantoin (**287**)[144]
- 4-Methylimidazole (**291**)[145]
- Imidazol-4-ylmethanol (**294**)[134]
- Imidazole-4-carbaldehyde (**288**)[135b] (Solvent: MeOH)

5-(Imidazol-5-yl)methylenehydantoin (286):

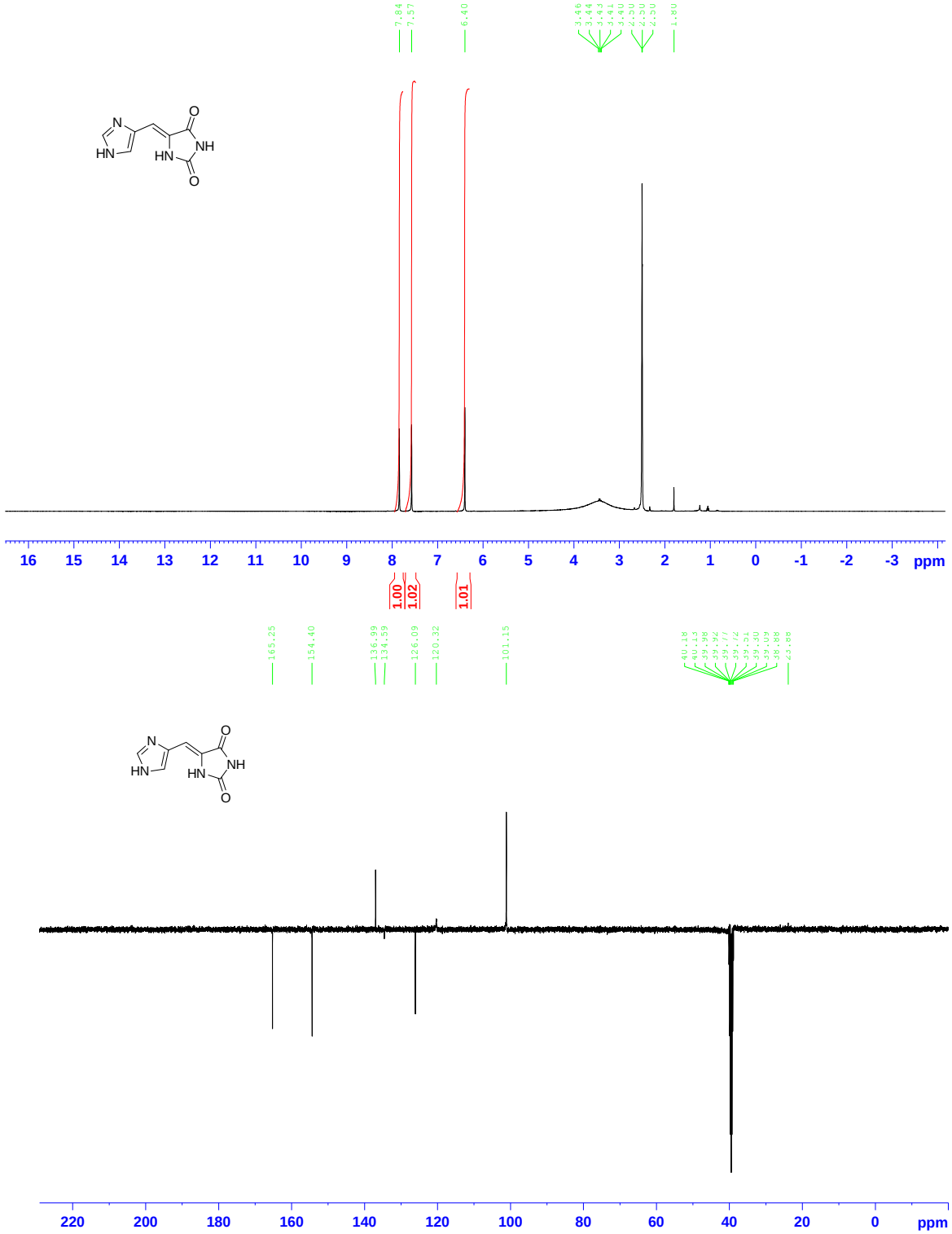


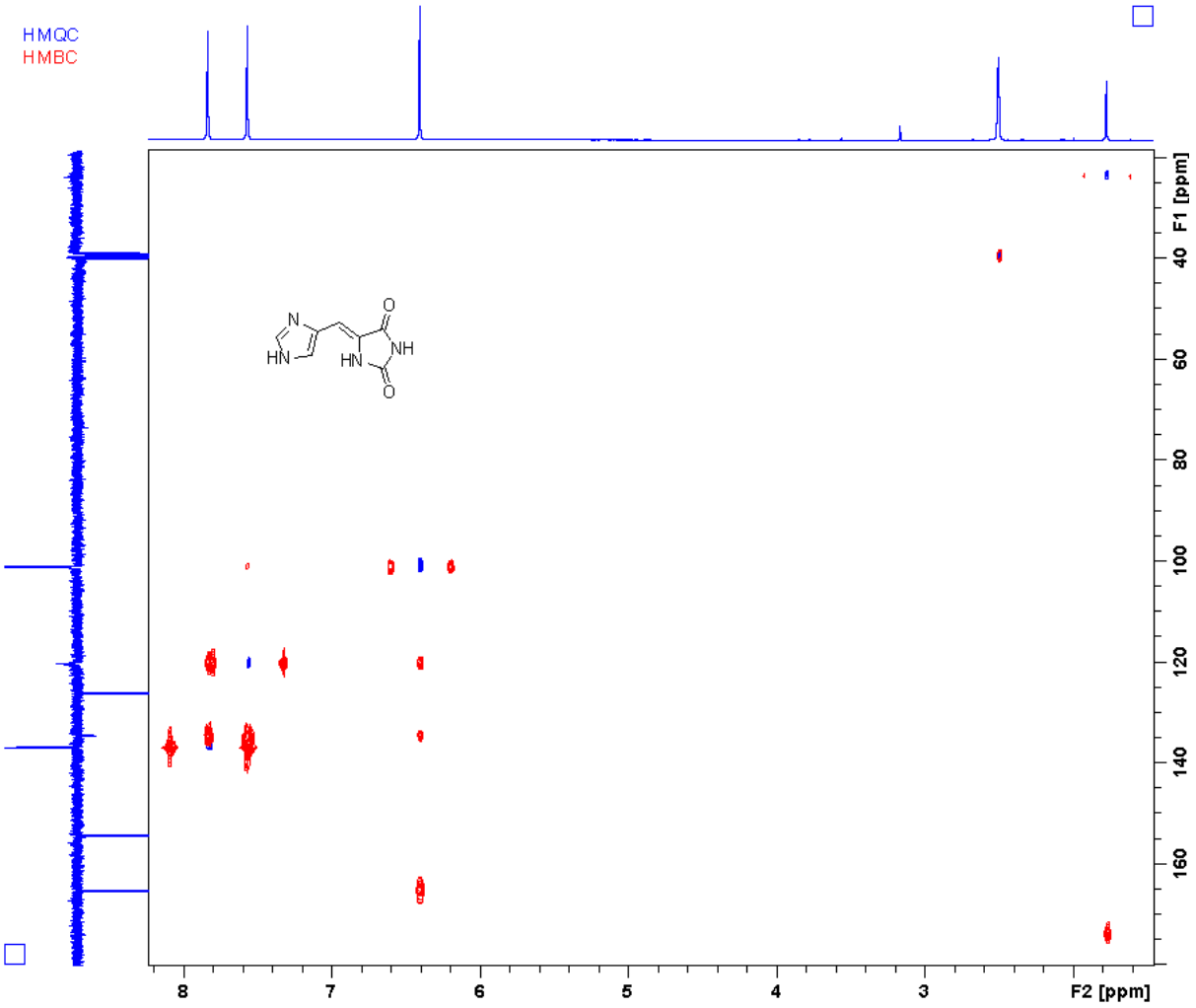
286

5-(Imidazol-5-yl)methylenehydantoin was prepared very similar to Doulofeu *et al.*[142] Imidazoyl-4-carbaldehyde (50 mg, 0.52 mmol) and dry sodium acetate (53 mg, 0.65 mmol) were suspended in freshly distilled acetanhydride (206 µL) and hydantoin (52 mg, 0.52 mmol) was added. The reaction mixture was heated to 135°C, until a yellow solid precipitated. The mixture was cooled to 0°C and after 12 hours the precipitate was filtered. The solid was dissolved in a mixture of $CH_3OH/CHCl_3$ (1/1) and heated to 50°C. After 1 minute a yellow solid precipitated, the mixture was cooled to 0°C and after 12 hours the precipitate was filtered to yield 5-(Imidazol-5-yl)methylenehydantoin (**286**) (37 mg, 40%).^{1}H-NMR (400 MHz, DMSO): δ = 7.84 (s, 1H, CH) 7.57 (s, 1H, CH) 6.40 (s, 1H, CH); ^{13}C-NMR (400 MHz, DMSO): δ =165.2, 154.4, 137.0, 134.6, 126.1, 120.3, 101.1.

Selected spectra:

5-(Imidazol-5-yl)methylenehydantoin: (**286**):




HMQC
HMBC
F1 [ppm]
40
60
80
100
120
140
160
8
7
6
5
4
3
F2 [ppm]
N
HN
HN
NH
O
O

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Education

Jun. 2006 –	**PhD Thesis** *"*Organic chemistry as a useful tool in structural biology*"* Supervisor: Prof. Walther Schmid Institute of Organic Chemistry, University of Vienna
Oct. 1999 – Jan. 2006	**Master of Science** Studies of Chemistry at the University of Vienna Focus: organic chemistry, analytical chemistry Passed with distinction
	Diploma Thesis (Jan 13th, 2006) *"Synthesis of [4-^{13}C]-methylthio-2-oxobutanoate – Protein expression of the selective isotope labeled SH2 domain of PLC-γ1"* Supervisor: Prof. Walther Schmid Institute of Organic Chemistry, University of Vienna

Employment history

2008 – to date	**Teaching assistant** Department of Organic Chemistry, University of Vienna
2006 – 2008	**Project employee** Department of Organic Chemistry, University of Vienna
2005	Participant in the 2005 Minidistillation Round Robin ASTM D02.08
2004/2005	Tutor in the "Chemical laboratory course for biologists"
2004	Summer scholarship at the *Novartis Institutes for BioMedicinal Research* under the guidance of Dr. Karl Baumann (2 months); compartment: Global Discovery Chemistry (GDC)

2000/2001 Summer employment at the *Environment Institute of Vorarlberg* under the guidance of Dr. Christoph Scheffknecht (2 ½ months); Compartment: environmental and analytical chemistry

List of publications

M. Fischer, H. Kählig, W. Schmid, in preparation. "*Homoallylic fluorination - Synthesis of 4-fluoro-2-oxobutanoic acid*"

M. Fischer, H. Kählig, W. Schmid, *Chem. Commun.* **2011,** *47*, 6647-6649; ''*Gram Scale Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate: A Novel Donor Substrate in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions*".

Cover story: M. Fischer, C. Schmölzer, C. Nowikow, W. Schmid, *Eur. J. Org. Chem.* **2011**, 1645-1651; "*Indium-Mediated Allenylation of Aldehydes and its Application in Carbohydrate Chemistry: Efficient Synthesis of D-Ribulose and 1-Deoxy-D-ribulose".*

C. Schmölzer, M. Fischer, W. Schmid, *Eur. J. Org. Chem.* **2010**, 4886-4892; "*Permanganate Oxidation Revisited: Synthesis of 3-Deoxy-2-uloses via Indium-Mediated Chain Elongation of Carbohydrates".*

K. Kloiber, M. Fischer, K. Ledolter; M. Nagl, W. Schmid, R. Konrat; *Journal of Biomolecular* NMR **2007**, *38*, 125-131; *"Generation and Relaxation of High Rank Coherences in AX_3 Systems in a Selectively Methionine Labeled SH2 Domain"*

Cover story: M. Fischer, K. Kloiber, J. Häusler, K. Ledolter, R. Konrat, W. Schmid; *ChemBioChem* **2007**, *8*, 610-612; *"Synthesis of a ^{13}C-Methyl Group Labeled Methionine Precursor as a Useful Tool for Simplifying Protein Structural Analysis by NMR Spectroscopy"*

Oral and poster presentations

2011 Oral presentation, 15th Austrian Carbohydrate Workshop, **2011**, Feb 15th, Graz, Austria*, "A Novel Donor Substrate in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions – Gram Scale Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate".*

2010 Oral presentation, 3rd EuCheMS Chemistry Congress 2010, Aug 29th-Sept 2nd, Nürnberg, Germany, "*Indium-Mediated Allenylation of Aldehydes: 4-Bromo-2-butyn-1-ols as Versatile α-Hydroxyacetyl Anion Equivalents".*

2010 Oral presentation, 14th Austrian Carbohydrate Workshop, **2010**, Feb 11th, Vienna, Austria, "*Indium-Mediated Allenylation: A Novel C2-Synthon for the Preparation of Ketoses".*

2009 Poster presentation at the 15th European Carbohydrate Symposium, July 19th-24th, Vienna, Austria.

2007 Oral presentation, 12th Austrian Chemical Days 2007, Sept 10th–13th Klagenfurt, Austria, *"Isotope Labeled Methionine as a Useful Tool for Quantifying Protein Dynamics by NMR Spectroscopy".*

2007	Poster presentation at the "8th Tetrahedron Symposium 2007", June 26th–29th, 2007, Berlin, Germany.
2006	Poster presentation at the "3rd Central European Conference Chemistry Towards Biology", Sep 8th–12th 2007, Kraków, Poland.

Achievements

2008	Research Scholarship 2008: University of Vienna
2007	Sponsorship- and Research Scholarship 2007: University of Vienna
2004	Performance Scholarship: University o Vienna

Special Skills

Language	German: native speaker English: excellent French: basics
Computing	Proficient computer skills (MS Office, Topspin, Chem Office, Beilstein, SciFinder, Isis Draw and other chemical data bases)
Interests	Music, various sports (table tennis, soccer, basketball), cooking outdoor activities: hiking, skiing

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