Mixing Chemo- and Biocatalysis for Rare Monosaccharide Production by Combining Aldolase and N-Heterocyclic Carbene Gold Catalysts

Cédric Gastaldi[a], Ghozlene Mekhloufi[b], Claude Forano[a], Arnaud Gautier[a]* and Christine Guérard-Hélaine[a]*

[a] *Université Clermont Auvergne, CNRS, INP Clermont Auvergne, Institut de Chimie de Clermont-Ferrand (ICCF), F-63000 Clermont-Ferrand, France.*

[b] *Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 92296 Châtenay-Malabry, France*

E-mail : christine.helaine@uca.fr

Supplementary Information

Table of contents

Material

Formaldehyde, glycolaldehyde, propanal, butanal, propargyl alcohol, 3-hydroxypropanal, D-fructose-6-phosphate dipotassium salt, β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH) and sodium phosphate monobasic dihydrate were purchased from Sigma-Aldrich whereas hydroxyacetone was purchased from Fluka (purity 90%). FSA was produced as previously reported[1] giving an enzymatic powder composed of FSA and glycylglycine buffer. Glycerol dehydrogenase was obtained as described by A.K. Samland.[2]

Nuclear magnetic resonance (NMR) spectra were recorded using D_2O as solvent on a Bruker AC-400 spectrometer, operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm relative to TMS signal. Coupling constant values (*J*) are given in Hertz. The purities of the ligand and gold catalyst were determined by ^1H qNMR as described by Pauli[3] using trimethylsilyl propanesulfonic acid sodium salt (Aldrich).

Circular dichroism measurements were performed using a Jasco model J810 spectropolarimeter at 20 ± 1 °C, the temperature being controlled with a thermal bath (Lauda, Germany). Quartz cuvettes (Hellma GmbH & Co., Germany) with path lengths of 0.1 cm and 1 cm were used for measurements in the far-UV region (250 – 190 nm) and in the near-UV region (320 – 250 nm), respectively.

Methods

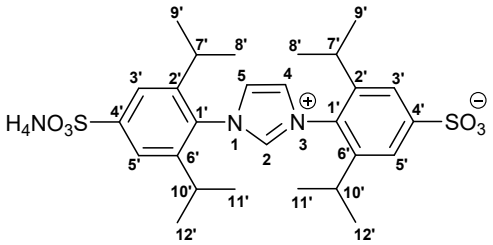
1. Synthesis and purification of the gold catalyst

1.1. Preparation of ligand **4'**

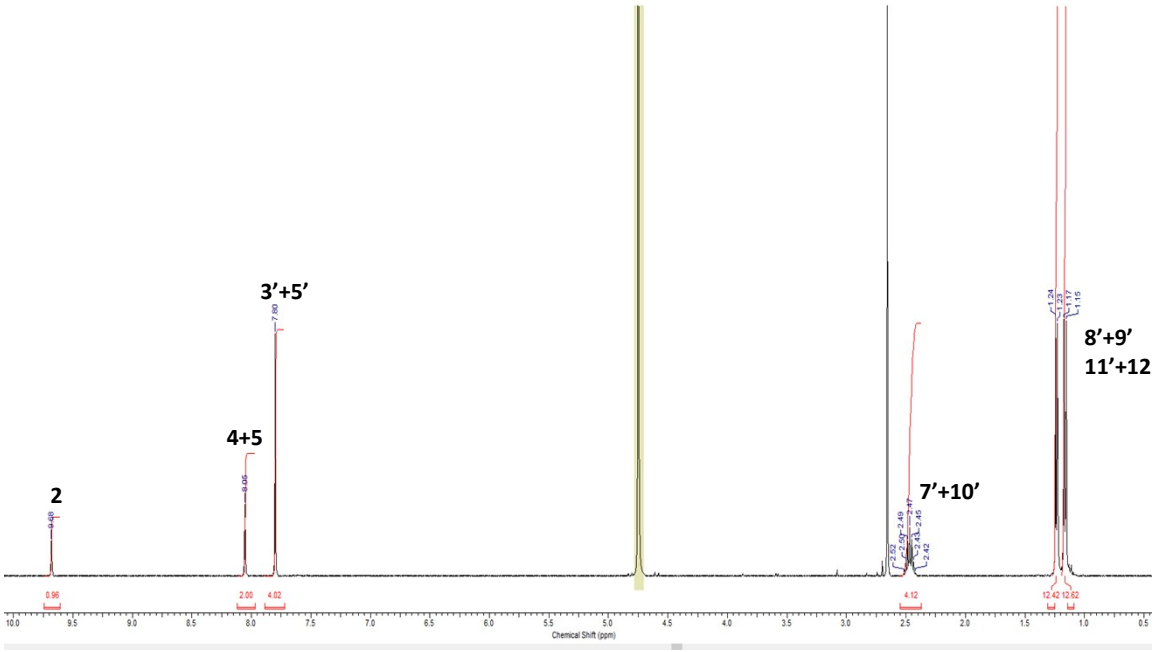
The synthesis of the imidazolium was achieved using the Plenio's protocole[4]. For the purification, 1.5 g of ligand was loaded on a C18 silica column (30 cm × 4 cm, 10 cm of silicagel). The column was washed with distilled water until the fractions became coloured. The ligand was eluted with 600 mL of water followed by 200 mL of methanol 20%. The column was then washed with 50, 80 and 100% methanol mixtures. The positive fractions, ie containing the ligand, revealing on TLC by UV light (254 nm), were collected, evaporated and dried under vacuum. 980 mg of an orange powder with a purity of 70% were obtained.

22 ml of QAE Sephadex^{TM} A-25 resin were swollen in water for 1 hour. This resin was poured in a column (30 cm × 4 cm), washed with 10 volumes of water, 10 volumes of 0.5 M ammonium bicarbonate and 10 volumes of water. Then, 737 mg of ligand dissolved in water were loaded. The column was washed with 5 volumes of water and then the product **4'** was eluted with 0.5 M ammonium bicarbonate. The fractions were tested by TLC and then collected and evaporated under vacuum. The powder obtained was then freeze-dried. Then, 403 mg of a white powder with a purity of 94% were obtained.

NMR ^{1}H (400 MHz, D_2O): δ 9.68 (s, 1H, 2), 8.05 (s, 2H, 4+5), 7.80 (s, 4H, 3'+5'), 2.47 (h, $^{3}J_{H-H}$ = 7.0 Hz, 4H, 7'+10'), 1.23 (d, $^{3}J_{H-H}$ = 7.0 Hz, 12H, 8'+9'+11'+12'), 1.15 (d, $^{3}J_{H-H}$ = 7.0



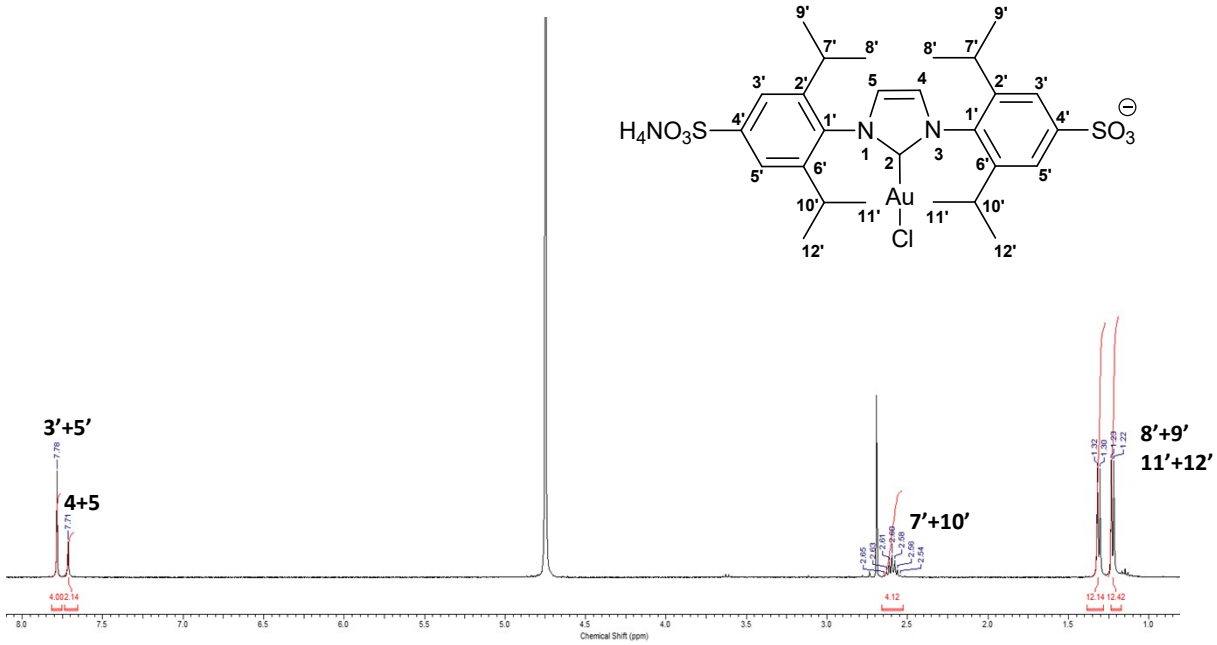
Hz, 12H, 8'+9'+11'+12').



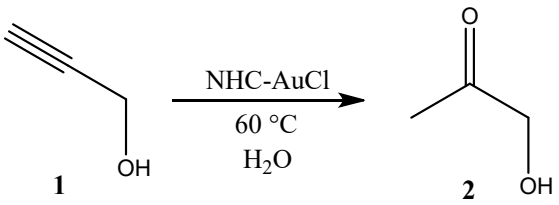
1.2. Preparation of [1,3-Bis(2,6-diisopropyl-4-ammoniumsulfonatophenyl)imidazol-2-ylidene]gold(I) chloride **5** :

In a 25 mL flask, a solution of imidazolium salt **4'** (0.708 mmol, 403 mg) and chloro(tetrahydrothiophene)gold (0.708 mmol, 228 mg)[5] in 12 mL ethanol was stirred for 20 minutes. Then, 0.918 mL (13.77 mmol) of aqueous ammonia (15 mol/L) were added dropwise and the mixture was stirred for 3 hours. The final solution was filtered on celite and the filtrate was evaporated under vacuum. The solid was then taken up again in water, filtered on a 0.2 µm-nylon filter and then evaporated again and dried under vacuum. Product **5** was obtained as a white solid (yield 77%, 0.442 g, 0.54 mmol, purity 94%).

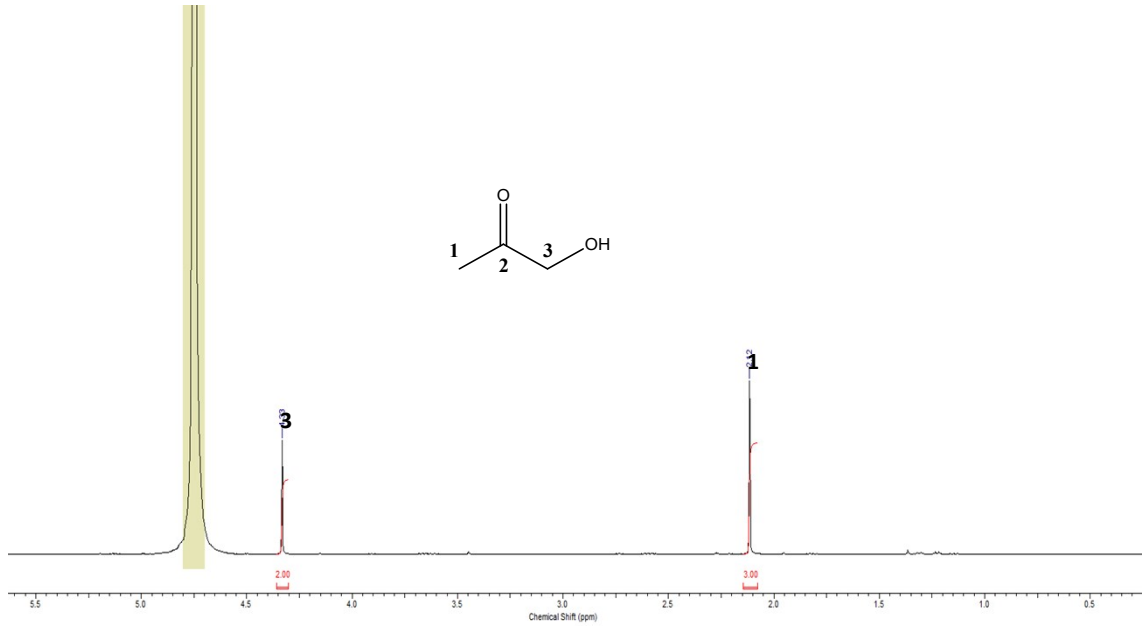
NMR ^{1}H (400 MHz, D_2O): δ 7.78 (s, 4H, 3'+5'), 7.71 (s, 2H, 4+5), 2.60 (h, $^{3}J_{H-H}$ = 7.0 Hz, 4H, 7'+10'), 1.30 (d, $^{3}J_{H-H}$ = 7.0 Hz, 12H, 8'+9'+11'+12'), 1.22 (d, $^{3}J_{H-H}$ = 7.0 Hz, 12H, 8'+9'+11'+12').



1.3. Activity assay of the NHC-AuCl catalyst



1.5 mg of catalyst (0.75 mol%) was added to a 2 mL flask containing 500 µL of 0.5 M propargyl alcohol solution (in water). The solution was stirred at 60 °C in a sand bath. The reaction was monitored by ^{1}H NMR, 100% conversion was obtained after 1h30.



^{1}H NMR (400 MHz, D_2O): δ 4.27 (s, 2H, 3), 2.05 (s, 3H, 1).

2. FSA structuring study

The secondary and the tertiary structures of FSA suspension at a concentration of 0.1 mg/mL (in water or in 0.5 M propargyl alcohol solution) were assessed in, respectively, far-UV and near-UV regions. The scanning conditions were 0.5 nm of data interval, 1 nm of bandwidth, and 50 nm min^{-1} of scan speed, corrected by subtracting the appropriate blank baseline spectrum.

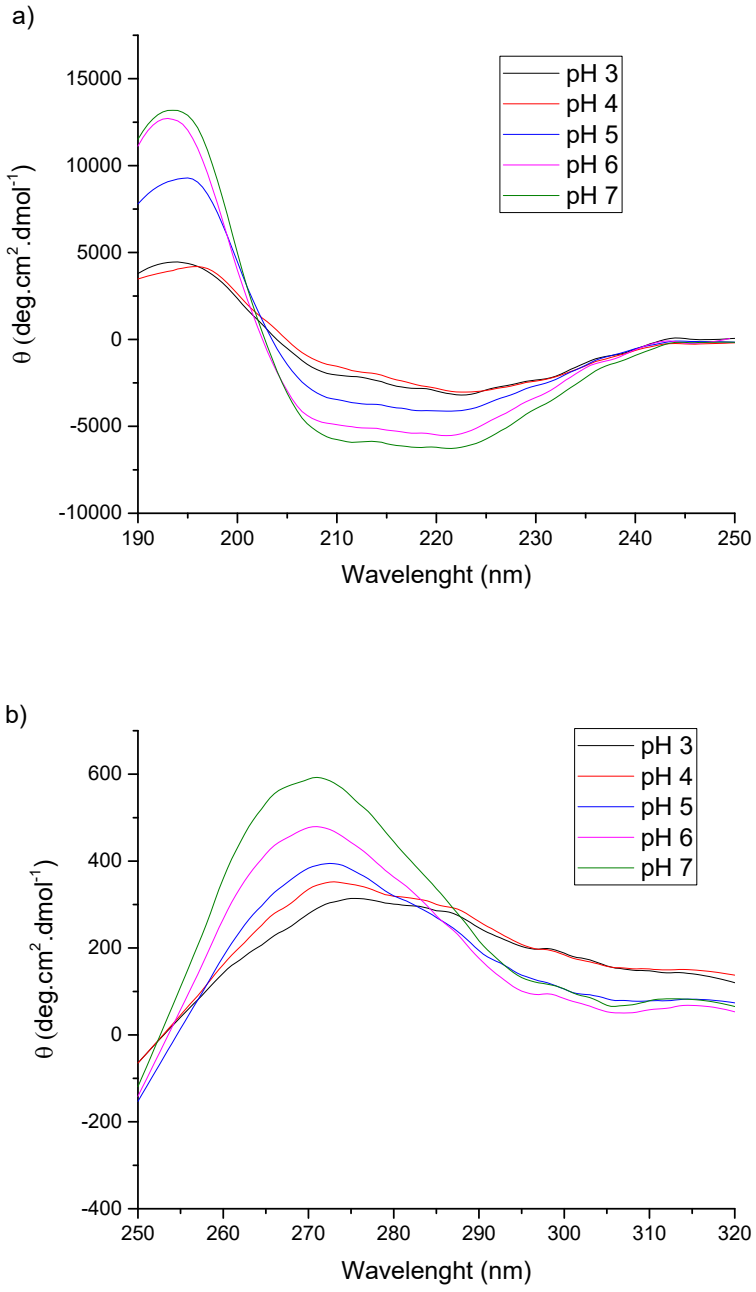
The results were expressed in mean residue ellipticity [θ] (deg cm^2 $dmol^{-1}$)[6]. [θ] has been calculated using the formulae:

$$[\theta] = \frac{MRW \times \theta}{C.l.10}$$

where MRW is the mean residue weight (g mol^{-1}), θ is the ellipticity (mdeg), C the concentration (g ml^{-1}) and l the path length (cm).

2.1. Effect of the pH

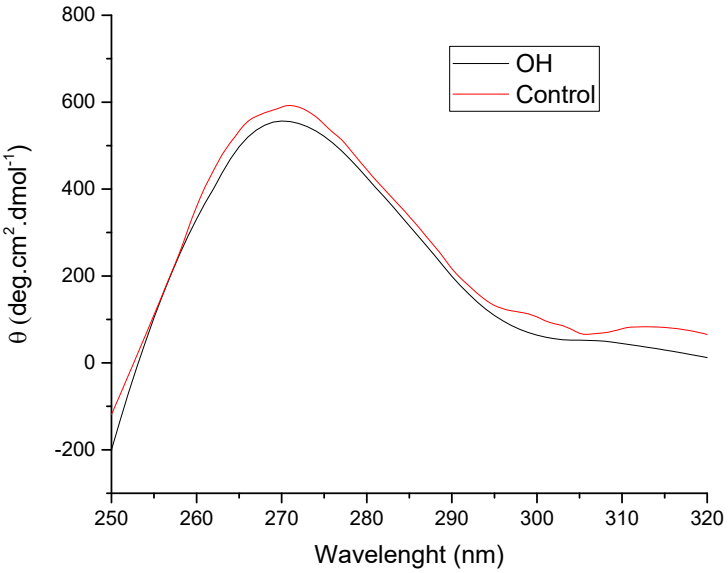
A suspension of 0.1 mg/mL of FSA was prepared in water and, the pH of the solution was adjusted in the range of 3 to 7. Then, these solutions were used for the measurements. Time had no effect on results. Indeed, the same spectra were obtained at least 1 h after the preparation of the solution, whatever its pH. Below are presented the evolution of spectra as a function of pH for the far-UV (a) and the near-UV (b) regions.



2.2. Effect of the presence of propargyl alcohol

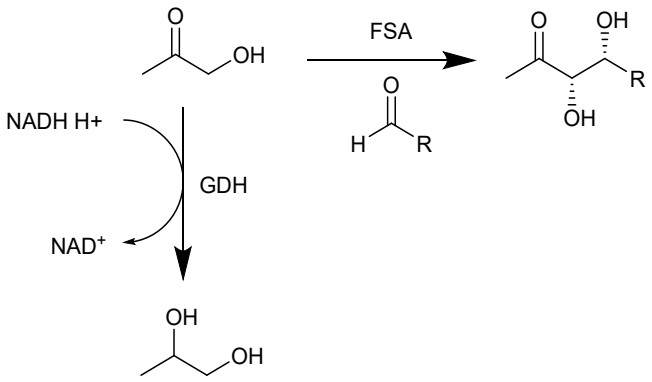
A suspension of 0.1 mg/mL of FSA in 0.5 M of propargyl alcohol was prepared in water and the pH of the solution was adjusted to 7. Then, this solution was used for the measurement in near-UV region. Time had no effect on the results, as the same spectra were obtained even 1 h after the preparation. For measurements in the far-UV region, the absorbance was too high and results were not usable.

Below are presented the spectra for the near-UV regions in the presence of alcohol (OH) and without any alcohol (control).



3. FSA activity study

3.1. FSA assay



General procedure: to a solution of hydroxyacetone (500 mM) were added the aldehyde (500 mM) and 2 mg of FSA powder (50% glycylglycine) in 1 mL of water. The pH was adjusted to 7 and the solution was stirred. The reaction was monitored by spectrophotometry at 340 nm following the disappearance of HA *via* NADH consumption: to a solution of glycerol dehydrogenase GDH (20 µL) in 50 mM glycyl-glycine buffer pH 7.5 at 25°C, were added NADH (0.5 mM) and 8 µL of the reaction medium. The final volume was 1 mL. One mmol of NADH oxidized was equivalent to 1 mmol of hydroxyacetone used in the aldolisation reaction.

3.2. Effect of both substrates' concentrations

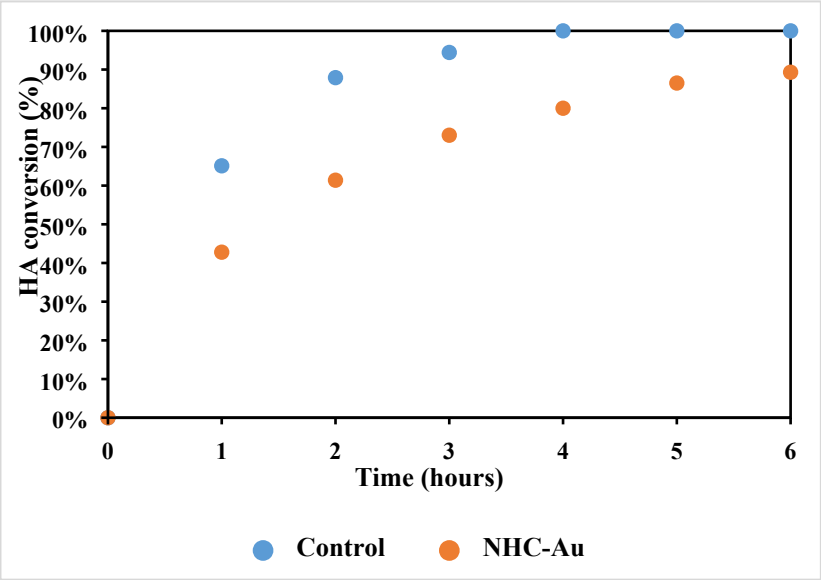
A stock solution was prepared containing 1 M of both substrates (hydroxyacetone and formaldehyde) with a fixed pH of 7. This solution was used to prepared 5 solutions of 500 µL with final concentrations of 1 M, 0.8 M, 0.5 M, 0.3 M and 0.1 M. In each vial was added 15 µL of a solution of 60 mg/mL of FSA. The pH was adjusted to 7. The progress of the reaction was then monitored by following the disappearance of the hydroxyacetone using UV-visible spectrophotometry and auxiliary enzyme (see 3.1). Samples for measurements were taken at: 0.5, 1, 2, 4, 6 and 24 h of reaction time. The results are shown in figure 1.

3.3. Effect of the pH

In a vial, 0.5 M of both substrates (hydroxyacetone and formaldehyde) were added to water for a final volume of 500 µl. The pH of the solution was adjusted with HCl or NaOH. Then 2 mg/mL of FSA was added. The pH was adjusted again and the vial was shaken at room temperature. The progress of the reaction was monitored by following the disappearance of the hydroxyacetone using UV-visible spectrophotometry and auxiliary enzyme (see 3.1). Samples for measurements were taken at: 2, 4, 6 and 20 h of reaction time. The results are shown in figure 2a.

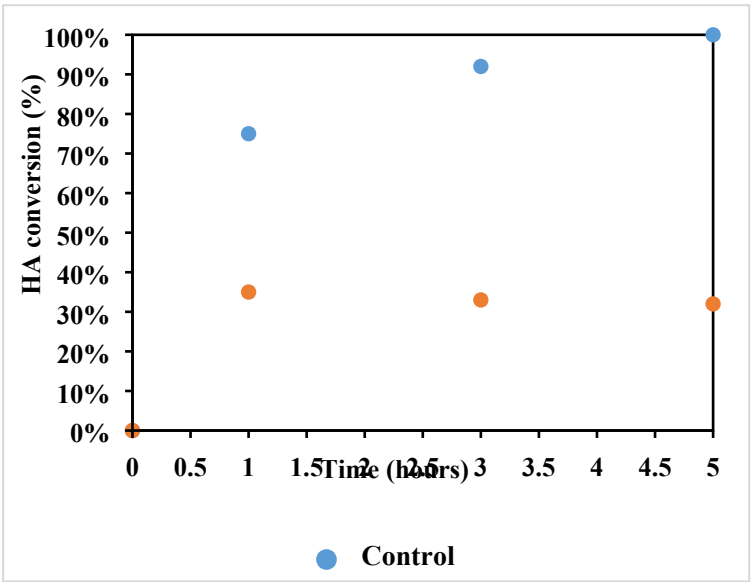
3.4. Effect of the presence of NHC-AuCl **5**

A 1 mL stock solution containing 0.5 M of both hydroxyacetone and formaldehyde was prepared and the pH of the solution was adjusted to 7. Two vials were then prepared containing either 1 mg of FSA (control) or 1 mg of FSA plus 1.5 mg of **5**. 500 µL of stock solution were added to each of the vials and the pH was readjusted to 7. The vials were shaken at room temperature. The pH was checked regularly and the progress of the reaction was monitored by following the disappearance of the hydroxyacetone using UV-visible spectrophotometry and auxiliary enzyme (see 3.1). Samples for measurements were taken at: 1, 2, 3, 4, 5 and 6 h of reaction time.



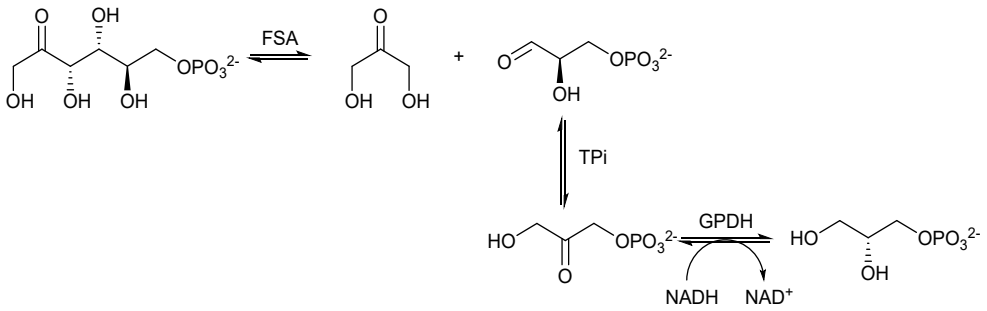
3.5. Effect of propargyl alcohol **1**

Two vials were prepared containing 0.5 M of both hydroxyacetone and formaldehyde (control) for the first one, or 0.5 M of hydroxyacetone, formaldehyde and propargyl alcohol for the second one. The pH was adjusted to 7 and 2 mg/mL of FSA was added. The solutions were stirred at room temperature and the progress of the reaction was monitored by following the disappearance of the hydroxyacetone using UV-visible spectrophotometry and auxiliary enzyme (see 3.1).

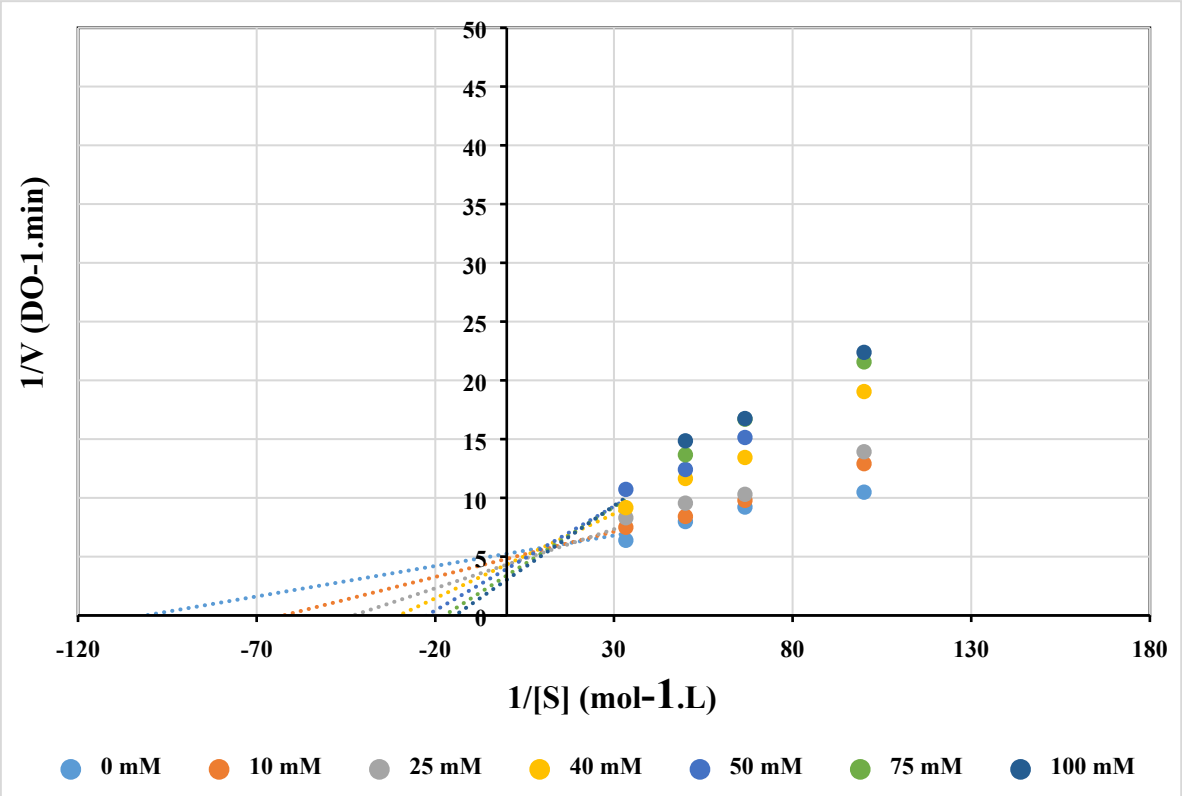


3.6. FSA inhibition by propargyl alcohol **1** study

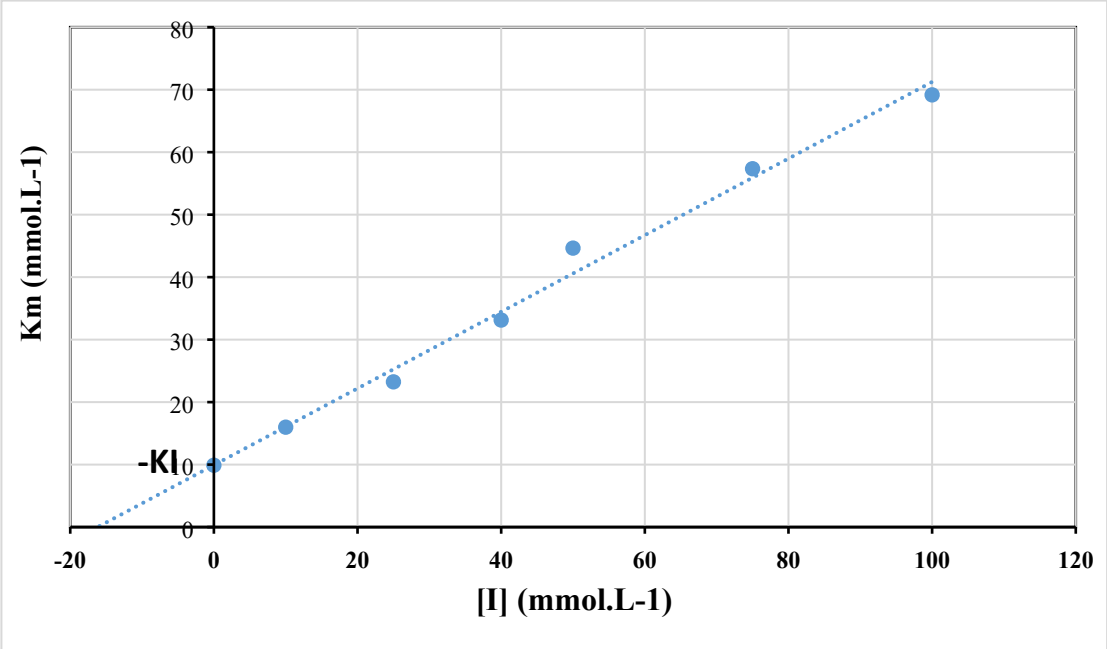
FSA Steady-state kinetic measurements were determined as previously described.[7]



To a solution of D-fructose-6-phosphate ([S]=5-30 mM) was added FSA (5 µL, 4 mg/mL) in 50 mM glycyl-glycine buffer pH 7.5 at 25°C. NADH (0.5 mM), auxiliary enzymes triose-phosphate isomerase and glycerol-3-phosphate dehydrogenase were then added to first isomerise D-glyceraldehyde-3-phosphate into dihydroxyacetone phosphate (DHAP) and then to reduce this latter into glycerolphosphate. Then, propargyl alcohol was added ([I]=0-100 mM) for a final volume of 1 mL. The reaction was monitored by spectrophotometry at 340 nm following the consumption of NADH. One mmol of NADH oxidized was equivalent to 1 mmol of D-fructose-6-phosphate cleaved.



[I] (mM)	KmApp(mM)
0	10
10	16
25	23
40	33
50	45
75	57
100	69



4. Catalyst 5 activity study

4.1. Effect of the pH

A range of 0.1 M citric acid/sodium citrate buffers (50 mL) were prepared according to the following table:

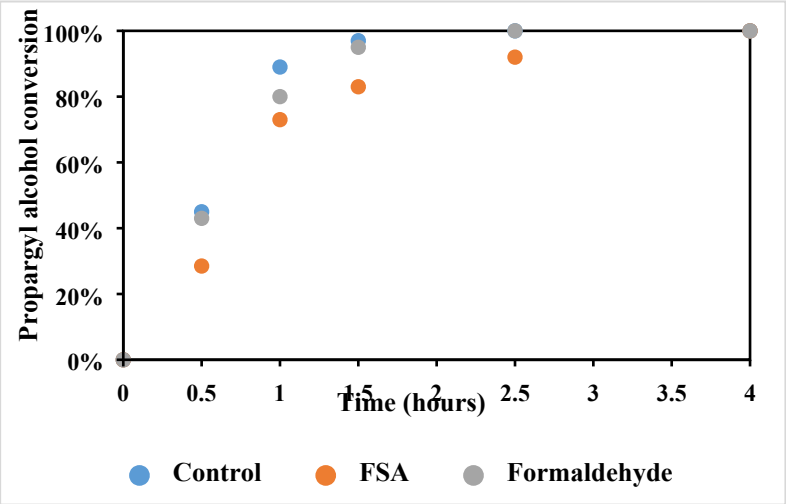
pH	Sodium citrate (mg)	Citric acid (mg)
3.5	317.0	753.5
4	496.5	636.0
4.5	675.5	519.0
5	855.0	402.0

For each pH, 1.5 mg of gold catalyst and 15 µL of propargyl alcohol were added to the buffer to give a 500 µL final solution. The resulting solutions were then stirred at 60°C. The sample pH value of 3 corresponds to the normal pH of the reaction without adding any acid or base. In this case, the pH started around 3, decreased at 2.7 during the reaction and then increased to 3.2 at the end of the reaction. The pH values were regularly monitored and the progress of the

reaction was followed by ^{1}H NMR. Samples for measurements were taken at: 1, 2, 4, 6 and 22 h of reaction time. The results are shown in figure 2b.

4.2. Effect of the presence of FSA or substrate **6**

A solution of 500 µL containing 1.5 mg of gold catalyst and 0.5 M of propargylic alcohol was prepared (control). To a similar solution, 1 mg of FSA or 0.5 M of formaldehyde **6** was added. The pH was set to 3. Then the solution was stirred at 60°C. The conversion rate of the reaction



was followed by ^{1}H NMR.

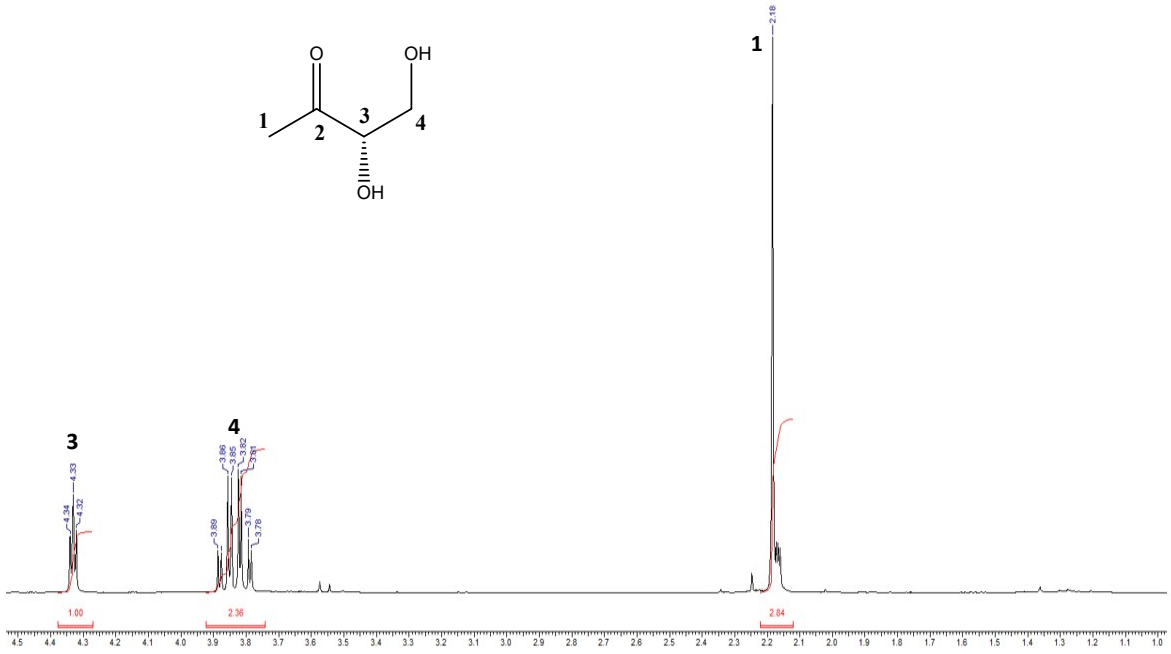
5. One-pot two-step hybrid reaction

General protocol: 3 mg of catalyst (0.75 mol%) was added to a 2 mL vial containing 1 mL of 0.5 M propargyl alcohol solution. The solution was stirred at 60°C in a sand bath. The reaction was followed by ^{1}H NMR. After hydration (2 h), the pH of the solution was raised to 7. 4 mg/mL of FSA and the amount of aldehyde for a final concentration of 0.5 M were added to the vial. The pH was controlled and the solution was stirred at room temperature. The progress of the reaction was monitored by following the disappearance of the hydroxyacetone using UV-visible spectrophotometry and auxiliary enzyme (see 3.1). At the end of the reaction, the solution was filtered through a Sephadex-type anion exchange resin (bicarbonate form) to remove the catalysts. The remaining solution was then evaporated under vacuum and the product was recovered pure as an oil.

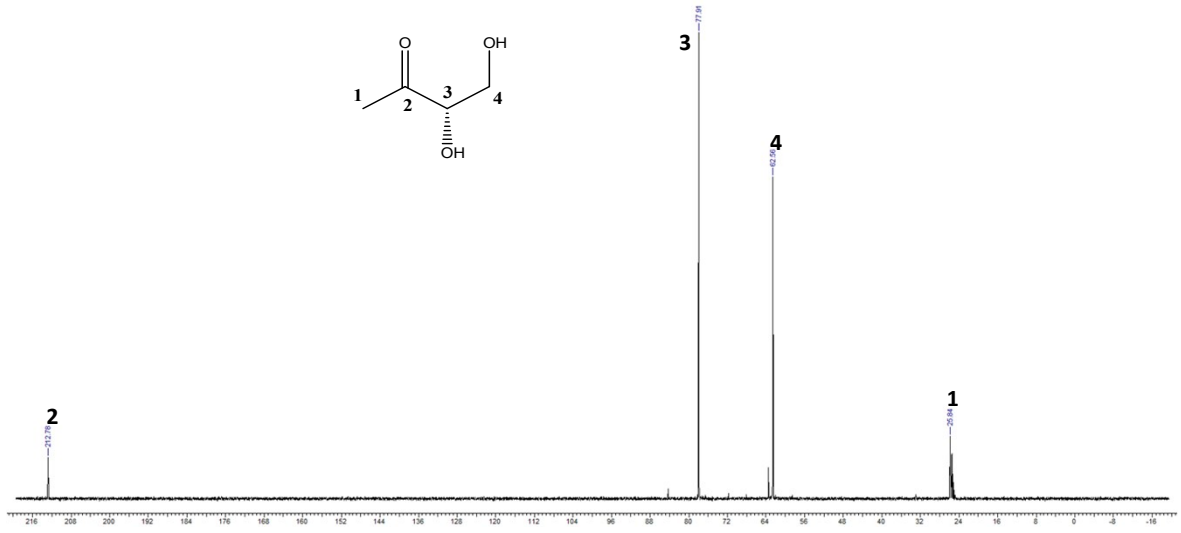
5.1. (3*S*)-3,4-dihydroxy-2-butanone **11**

^{1}H and ^{13}C NMR spectra (identical to those obtained by Schürmann et al.)[8]

^{1}H NMR (400 MHz, D_2O): δ 4.33 (t, 1H, *J*=3.8 Hz, 3), 3.83 (2dd, 2H, *J*=3.8 Hz and *J*=12.4 Hz, 4_A, 4_B), 2.18 (s, 3H, 1).



^{13}C NMR (100 MHz, D_2O): δ 212.78 (2), 77.91 (3), 62.56 (4), 25.84 (1).

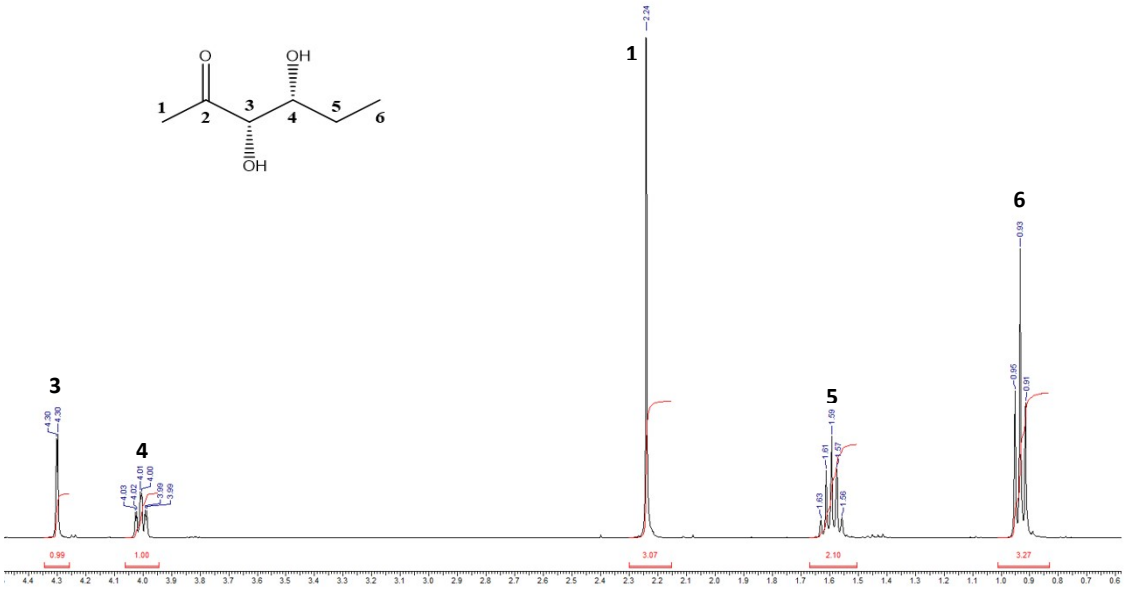


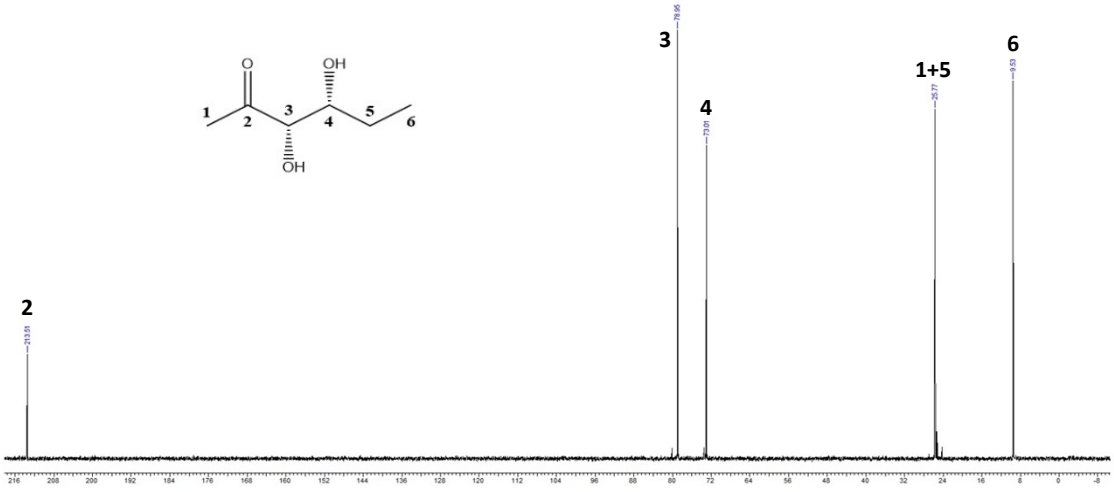
5.2. (3*S*,4*R*)-3,4-dihydroxy-2-hexanone **12**

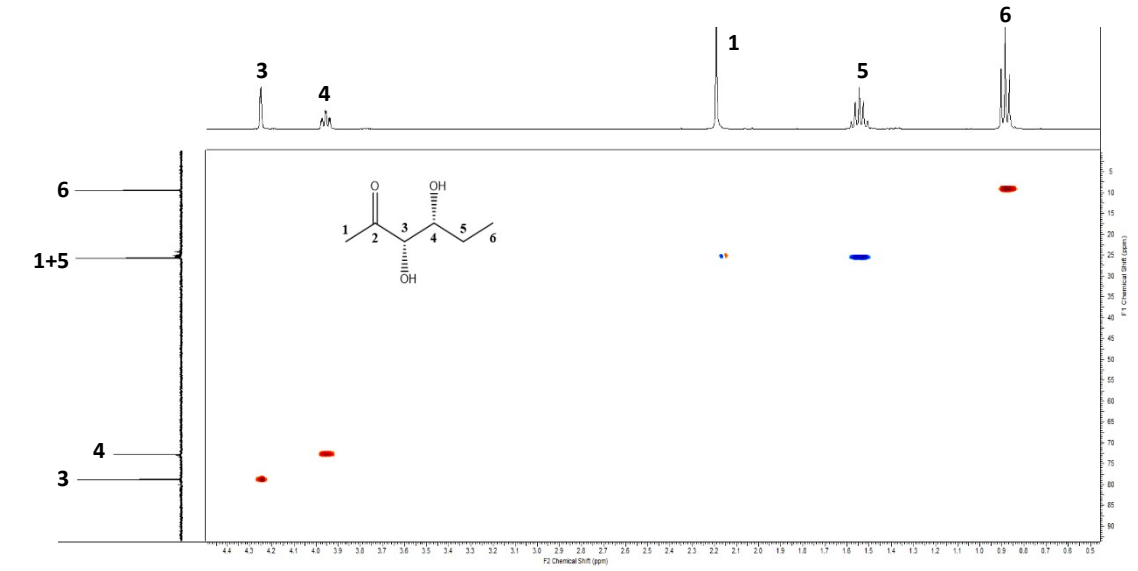
^{1}H, ^{13}C, (identical spectra to those obtained by Rale et al.)[9], HSQC and HMBC NMR spectra

^{1}H NMR (400 MHz, D_2O): δ 4.30 (d, 1H, *J*=2.1 Hz, 3), 4.00 (dt, 1H, *J*=2.1 Hz and *J*=7.2 Hz, 4), 2.24 (s, 3H, 1), 1.59 (m, 2H, 5), 0.93 (t, 3H, *J*=7.2 Hz, 6).

^{13}C NMR (100 MHz, D_2O): δ 213.51 (2), 78.95 (3), 73.01 (4), 25.77 (1 and 5), 9.53 (6).


O
OH
1
2
3
4
5
6
OH
1
3
4
5
6

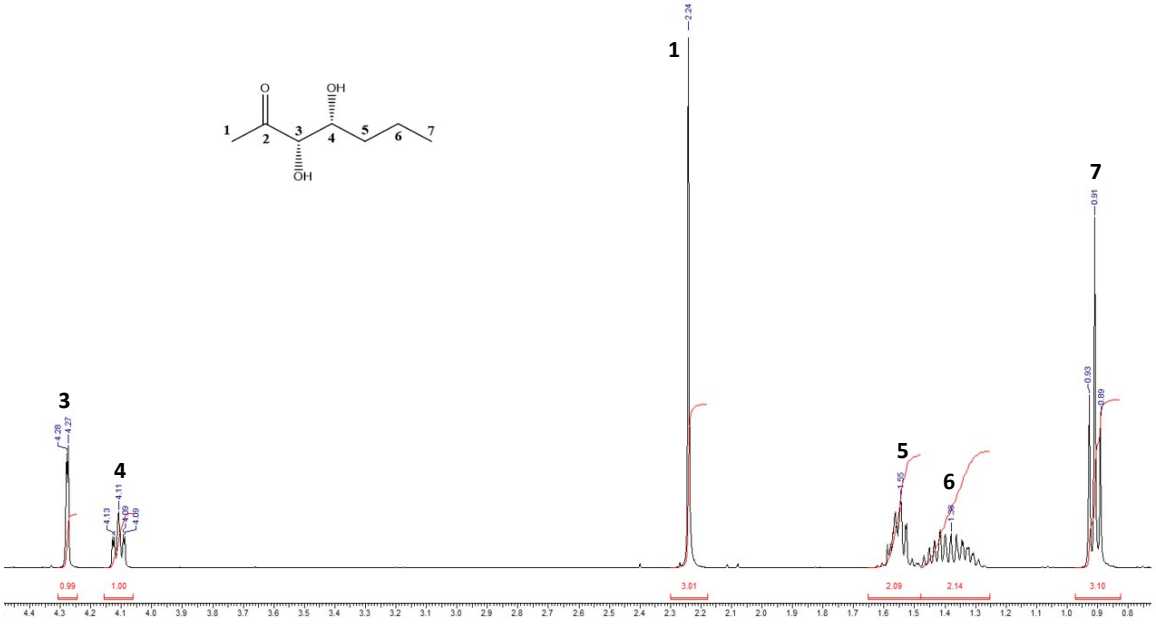

O
OH
1
2
3
4
5
6
OH
2
3
4
1+5
6



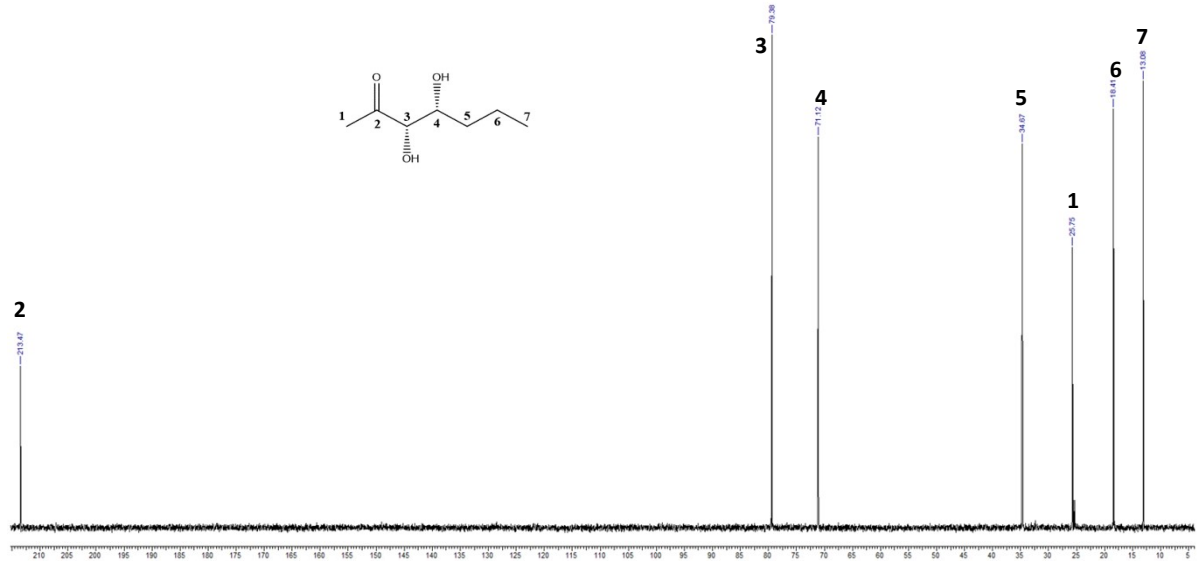
5.3. (3*S*,4*R*)-3,4-dihydroxy-2-heptanone **13**

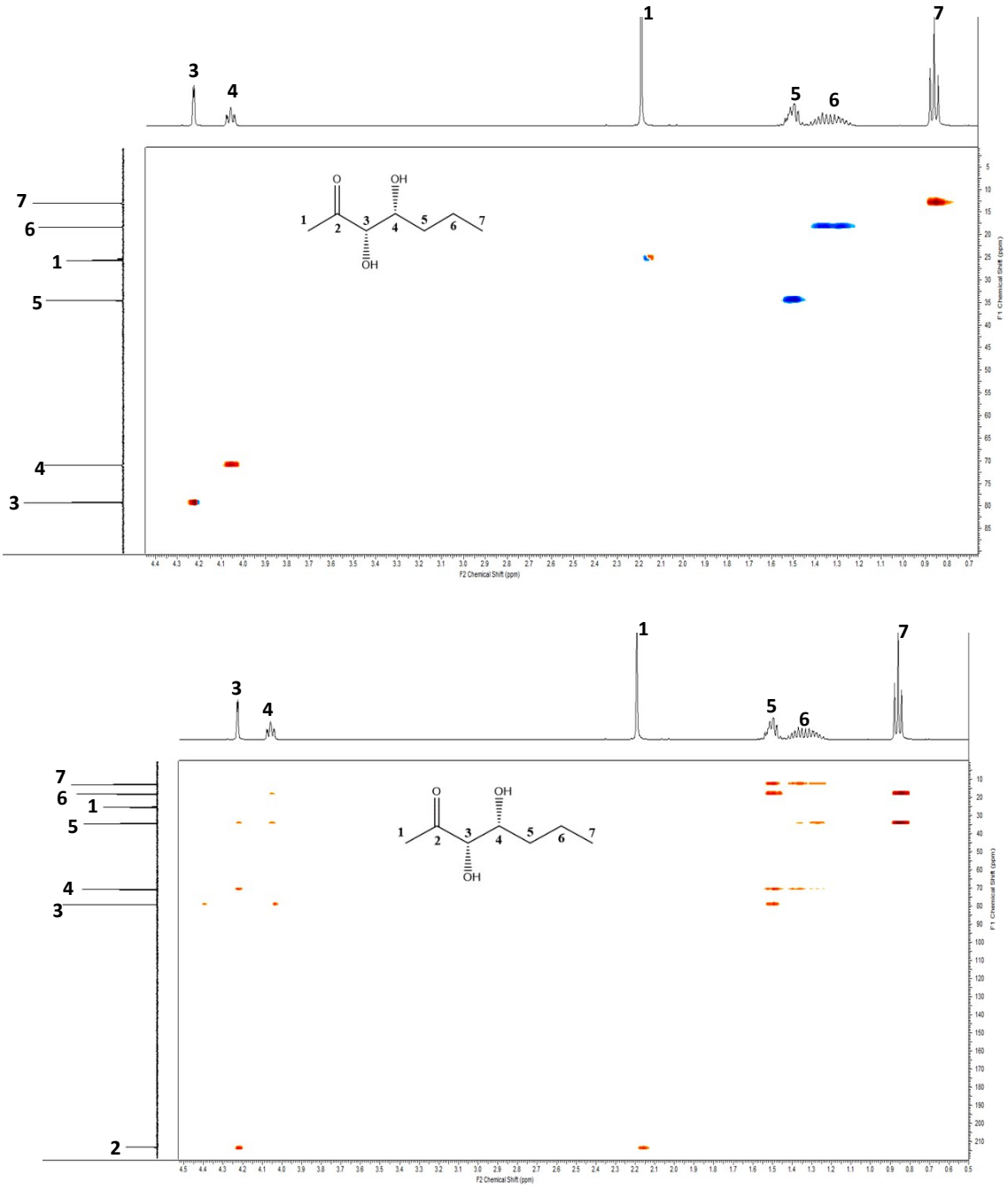
^{1}H, ^{13}C, (identical to those obtained by Zang et al.),[10] HSQC and HMBC NMR spectra.

^{1}H NMR (400 MHz, D_2O): δ 4.28 (d, 1H, *J*=2.0 Hz, 3), 4.11 (td, 1H, *J*=7.0 Hz and *J*=2.0 Hz, 4), 2.24 (s, 3H, 1), 1.55 (m, 2H, 5), 1.38 (m, 2H, 6), 0.91 (t, 3H, *J*=7.0 Hz, 7).



^{13}C NMR (100 MHz, D_2O): δ 213.47 (2), 79.38 (3), 71.12 (4), 34.67 (5), 25.75 (1), 18.41 (6), 13.08 (7)

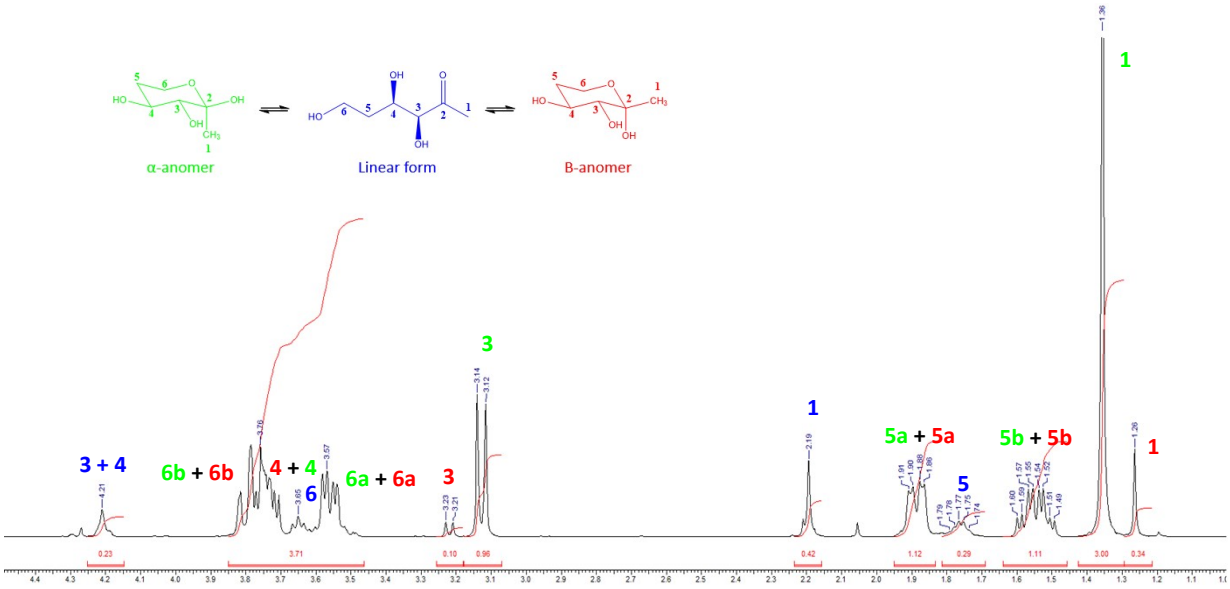




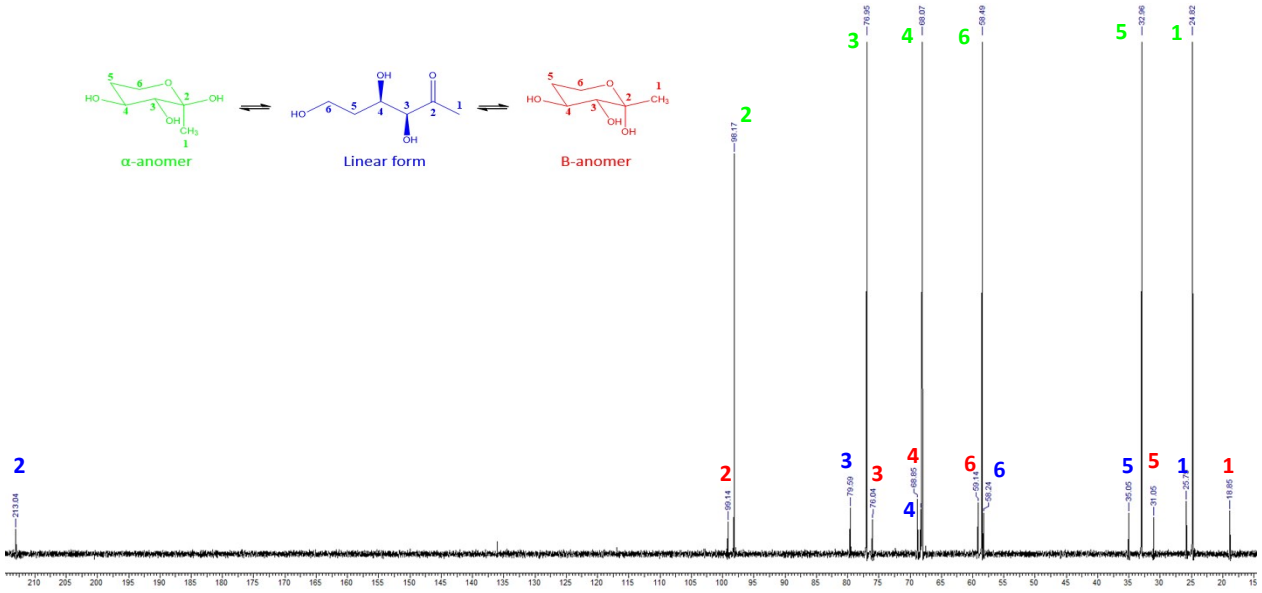
5.4. 1,5-dideoxy-D-*threo*-2-hexulose **14**

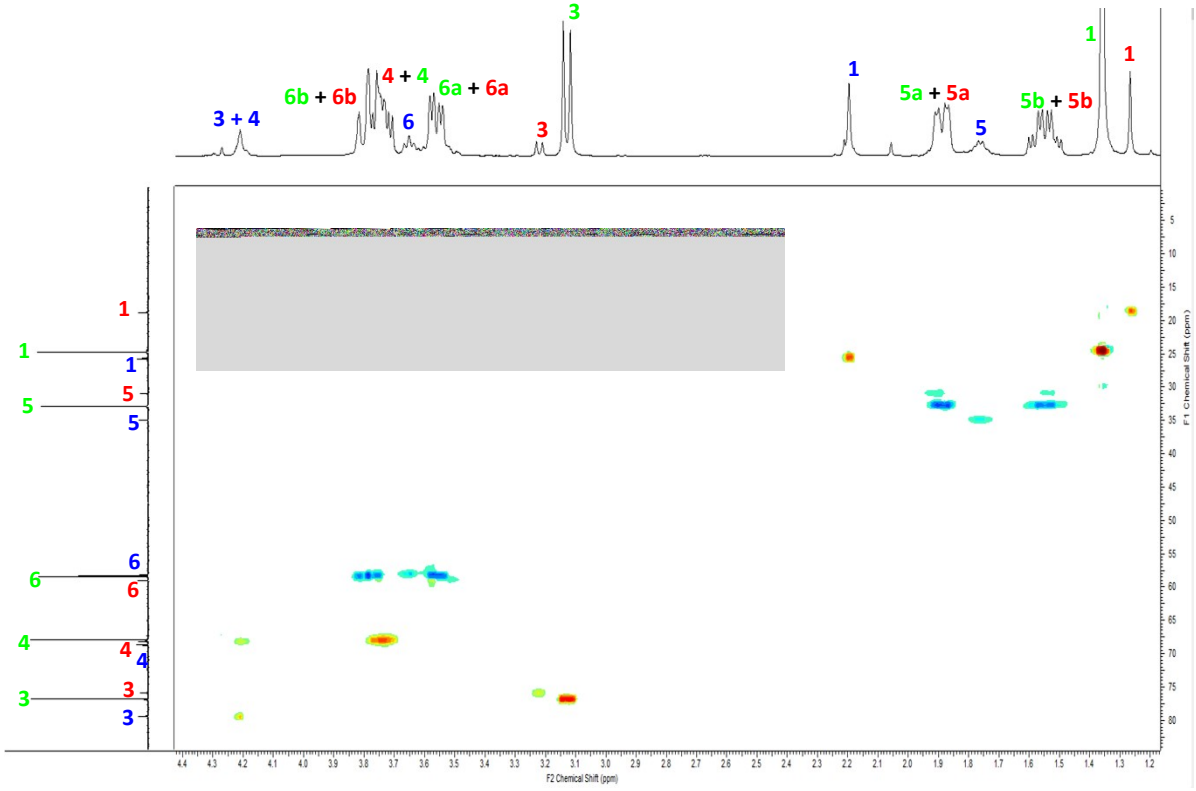
^{1}H and ^{13}C (identical to those obtained by Rale et al.[9]), HSQC and HMBC NMR spectra. However, thanks to the HSQC and HMBC NMR spectra, we were able to better identify each signal and so to give different assignments.

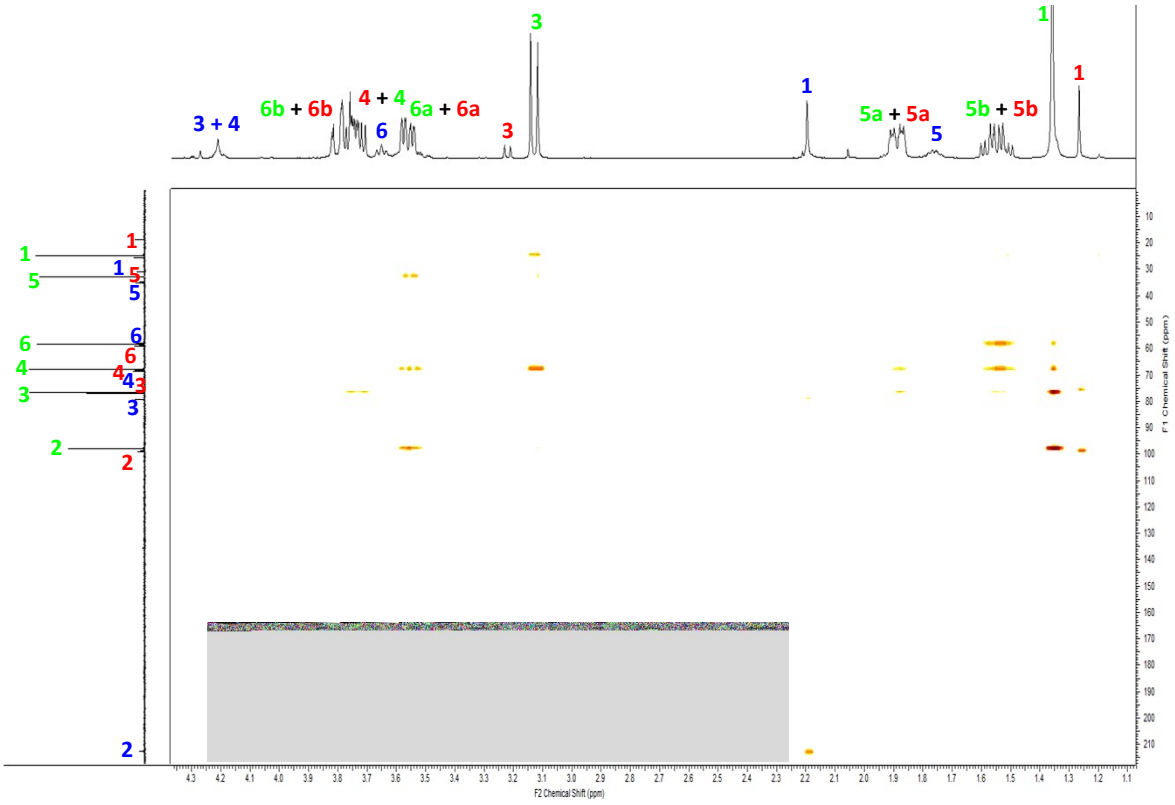
^{1}H NMR (400 MHz, D_2O): δ 4.21 (m, 2H, 3+4), 3.85-3.50 (8H, 6a + 6b + 6 + 6a + 6b + 4 + 4), 3.22 (d, 1H, *J*=9.5 Hz, 3), 3.13 (d, 1H, *J*=9.5 Hz, 3), 2.19 (s, 3H, 1), 1.95-1.85 (m, 2H, 5a + 5a), 1.77 (m, 2H, 5), 1.60-1.50 (m, 2H, 5b + 5b), 1.36 (s,3H, 1), 1.26 (s, 3H,1).



^{13}C NMR (100 MHz, D_2O): δ 213.04 (2), 99.14 (2), 98.17 (2), 79.59 (3), 76.95 (3), 76.04 (3), 68.85 (4), 68.07 (4), 68.02 (4), 59.14 (6), 58.49 (6), 58.24 (6), 35.05 (5), 32.96 (5), 31.05 (5), 25.79 (1), 24.82 (1), 18.85 (1)

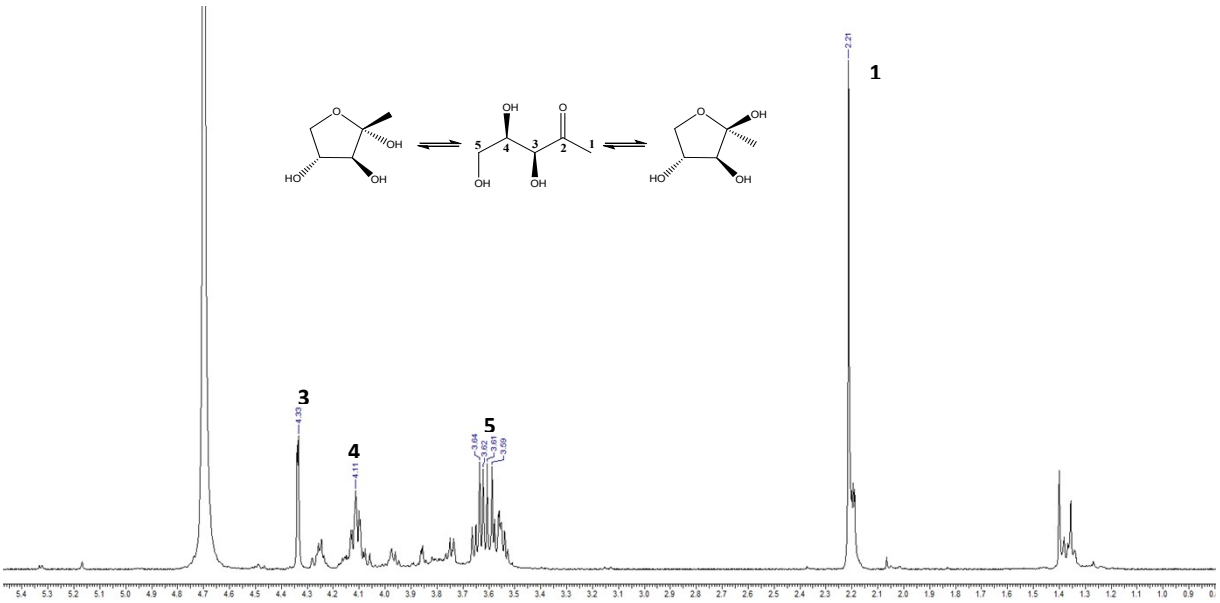



3 + 4
6b + 6b
4 + 4
6a + 6a
6
3
3
1
5a + 5a
5
5b + 5b
1
1
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)



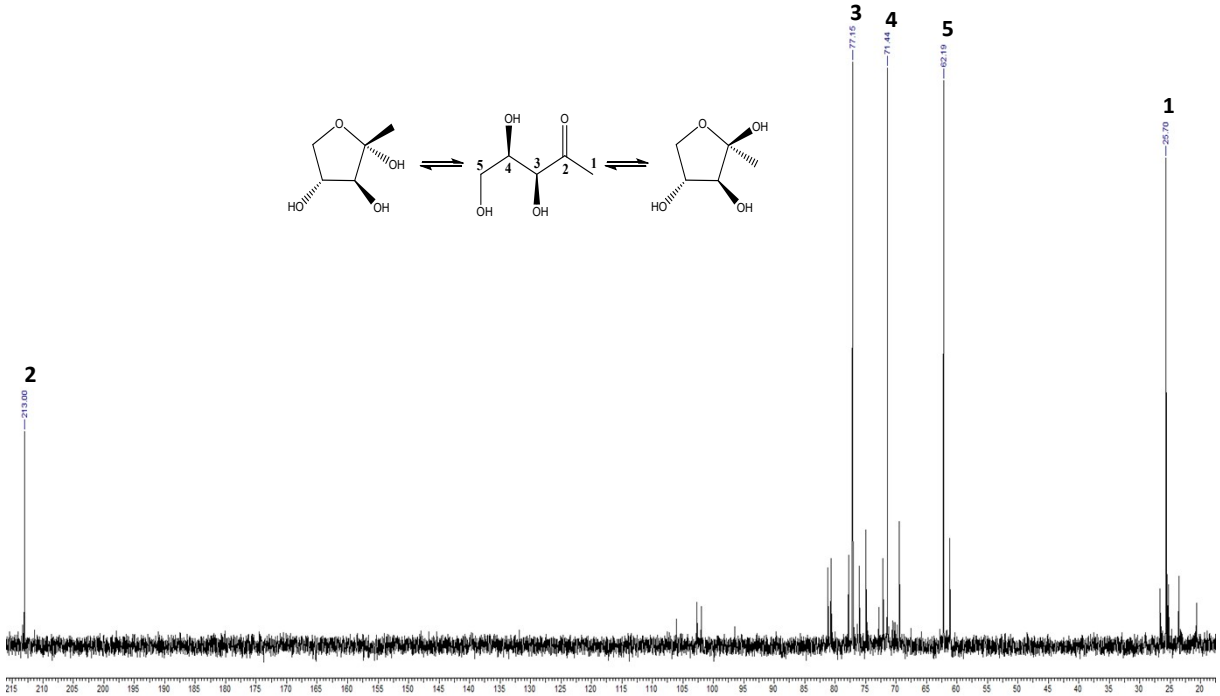
5.5. 1-deoxy-D-xylulose **15**

^{1}H NMR (400 MHz, D_2O): δ 4.33 (d, 1H, J=2 Hz, 3), 4.11 (m, 1H, 4), 3.60 (m, 2H, 5), 2.21 (s,



1H, 1). Only the signals of the linear product, the major one, are described

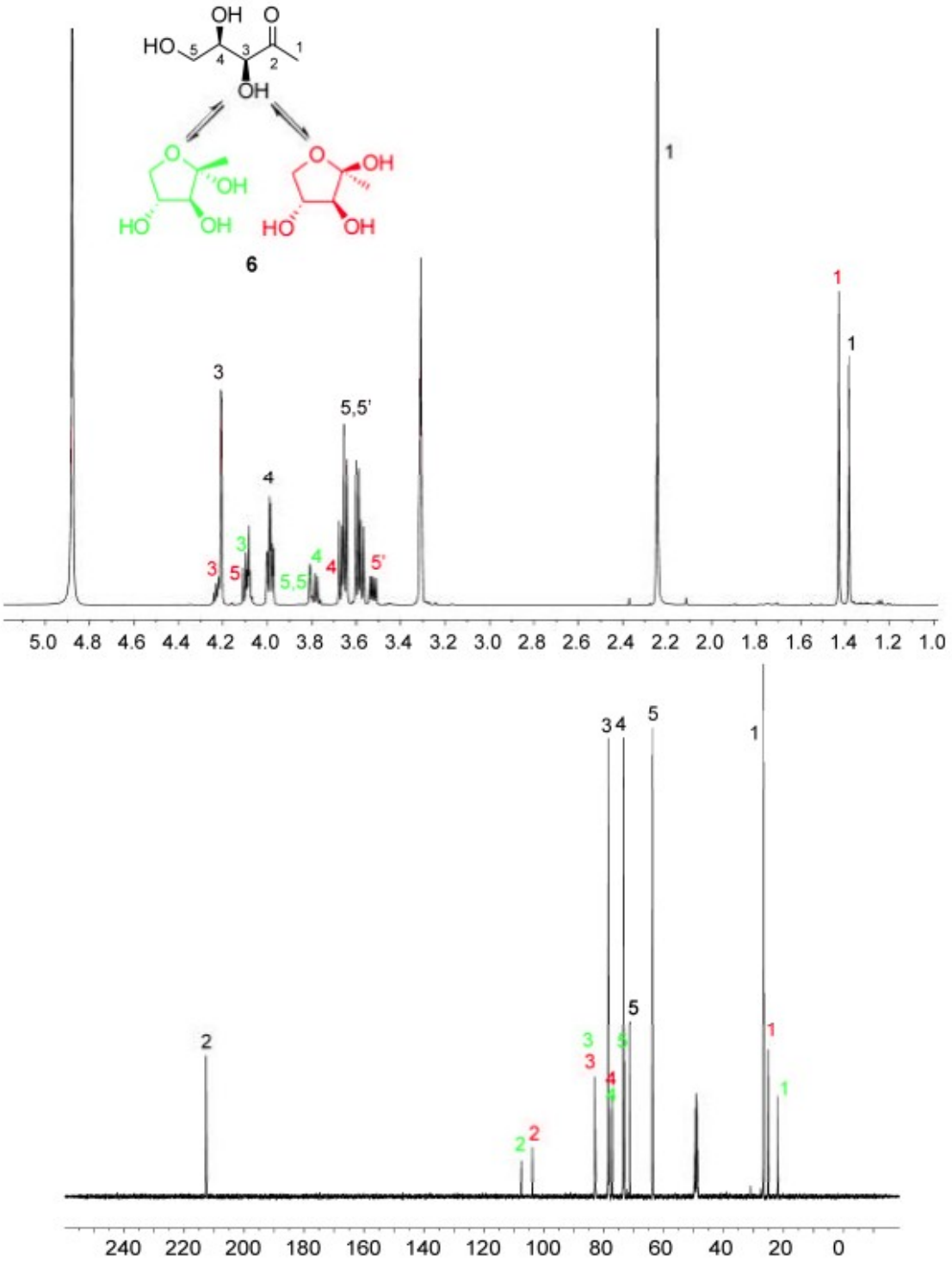
^{13}C NMR (100 MHz, D_2O): δ 213.00 (2), 77.15 (3), 71.44 (4), 62.19 (5), 25.70 (1). Only the



signals of the linear product, the major one, are described

As in Concia et al. paper (spectra presented below), we found similar spectra for this product, with a major linear form along with 2 cyclic forms and some threose.[11]

Observed ^{1}H-NMR and ^{13}C-NMR spectra of **1-deoxy-D-xylulose (6)** in CD_3OD.



6. E factor calculation

E factor is calculated as the mass of waste generated divided per the mass of product synthesized. In our following calculation we have omitted the amount of water and solvent used in the calculations for both our process and the comparable publications in the literature.

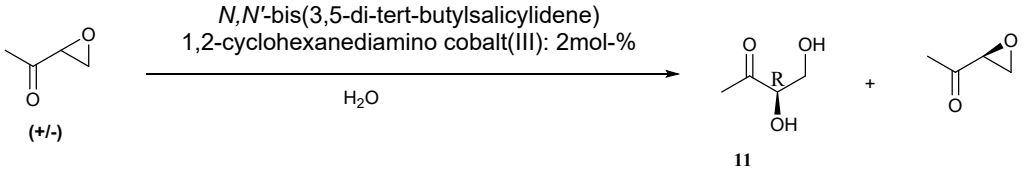
$$E = \frac{mass\ of\ the\ waste}{mass\ of\ the\ product}$$

We focused on the most efficient non enzymatic synthesises that we find in the literature and that includes detailed protocols. To note, when a chromatography was required for product purification, the silica gel waste has not been included in the calculations.

To note compound 14 was not found.

6.1. deoxy L-erythrulose **11**:

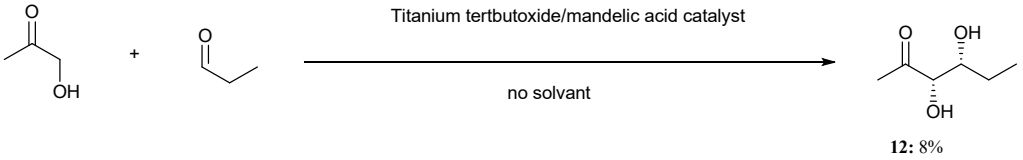
Jacobsen's synthesis[12] using hydrolytic kinetic resolution (HKR), for obtaining D-erythrulose.



E = 1.8

6.2. Compound **12** :

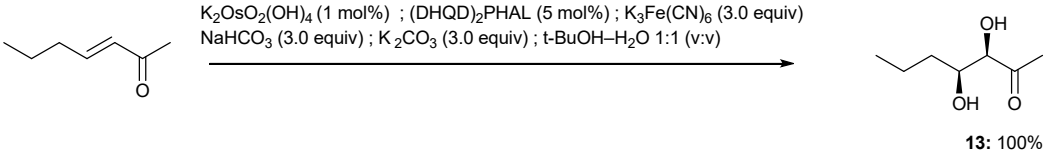
Mahrwald's synthesis[13] using titanium complex



E = 12.

6.3. Compound **13** :

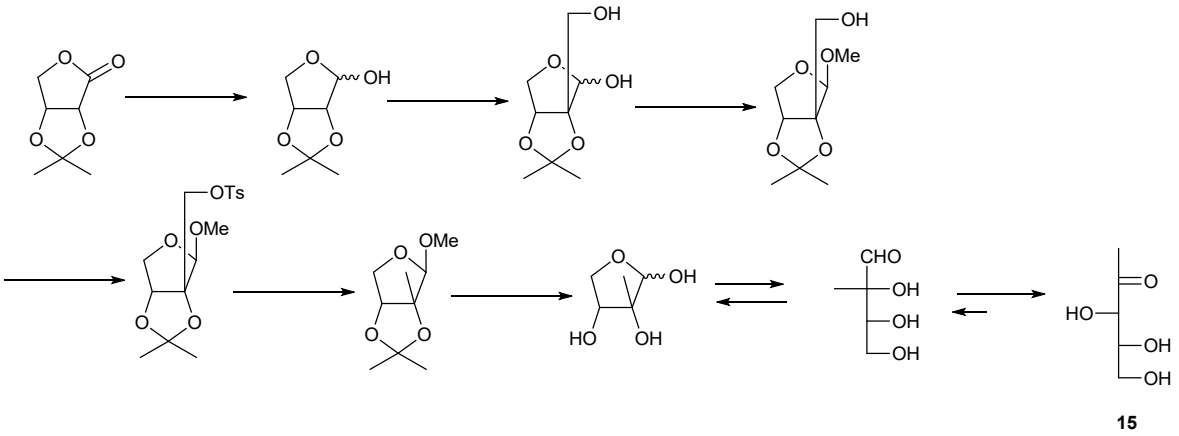
Bruckner synthesis[14] using a Sharpless di-hydroxylation, purification by flash column chromatography (no details reported).



E= 9.7

6.4. Deoxy-D-xylulose **15**

Serianni synthesis[15] in multi steps, from commercially available 2,3-O-isopropylidene-D-erythrono-1,4-lactone. The E factor was not calculated due to a large number of steps making this synthesis uncompetitive.



References

[1] R. Mahdi, C. Guérard-Hélaine, V. Prévot, V. de Berardinis, C. Forano, M. Lemaire, *ChemCatChem* **2015**, *7*, 3110–3115.

[2] S. Schneider, M. Gutiérrez, T. Sandalova, G. Schneider, P. Clapés, G. A. Sprenger, A. K. Samland, *Chem. Eur. J. of Chem. Bio.* **2010**, *11*, 681–690.

[3] G. F. Pauli, S.-N. Chen, C. Simmler, D. C. Lankin, T. Gödecke, B. U. Jaki, J. B. Friesen, J. B. McAlpine, J. G. Napolitano, *J. Med. Chem.* **2014**, *57*, 9220–9231.

[4] C. Fleckenstein, S. Roy, S. Leuthäußer, H. Plenio, *Chem. Commun.* **2007**, 2870–2872.

[5] R. Uson, A. Laguna, M. Laguna, D. A. Briggs, H. H. Murray, J. P. Fackler, in *Inorganic Syntheses* (Ed.: H.D. Kaesz), John Wiley & Sons, Inc., Hoboken, NJ, USA, **1989**, pp. 85–91.

[6] S. M. Kelly, N. C. Price, *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology* **1997**, *1338*, 161–185.

[7] J. A. Castillo, C. Guérard-Hélaine, M. Gutiérrez, X. Garrabou, M. Sancelme, M. Schürmann, T. Inoue, V. Hélaine, F. Charmantray, T. Gefflaut, L. Hecquet, J. Joglar, P. Clapés, G. A. Sprenger, M. Lemaire, *Advanced Synthesis & Catalysis* **2010**, *352*, 1039–1046.

[8] M. Schürmann, M. Schürmann, G. A. Sprenger, *Journal of Molecular Catalysis B: Enzymatic* **2002**, *19–20*, 247–252.

[9] M. Rale, S. Schneider, G. A. Sprenger, A. K. Samland, W.-D. Fessner, *Chemistry – A European Journal* **2011**, *17*, 2623–2632.

[10] C. Zang, Y. Liu, Z.-J. Xu, C.-W. Tse, X. Guan, J. Wei, J.-S. Huang, C.-M. Che, *Angew. Chem. Int. Ed.* **2016**, *55*, 10253–10257.

[11] A. L. Concia, C. Lozano, J. A. Castillo, T. Parella, J. Joglar, P. Clapés, *Chemistry* **2009**, *15*, 3808–3816.

[12] S. E. Schaus, B. D. Brandes, J. F. Larrow, M. Tokunaga, K. B. Hansen, A. E. Gould, M. E. Furrow, E. N. Jacobsen, *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.

[13] B. Schetter, C. Stosiek, B. Ziemer, R. Mahrwald, *Appl. Organometal. Chem.* **2007**, *21*, 139–145.
[14] K. Körber, P. Risch, R. Brückner, *Synlett* **2005**, *2005*, 2905–2910.
[15] S. Zhao, L. Petrus, A. S. Serianni, *Org. Lett.* **2001**, *3*, 3819–3822.