Aldolase and N-Heterocyclic Carbene Gold(I) Catalysts: Compartmentalization and Immobilization on Anionic Clays for Concurrent Hybrid Catalysis at Acidic pH

Cédric Gastaldi, Virgil Hélaine, Muriel Joly, Arnaud Gautier, Claude Forano*, Christine Guérard-Hélaine*

Université Clermont Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand, 63000 Clermont-Ferrand, France.

E-mail : christine.helaine@uca.fr; claude.forano@uca.fr

Supplementary Information

Table of contents

Material 2

Methods 3

1. LDH and FSA@LDH syntheses 3
   1.1. LDH synthesis 3
      1.1.1. Zn_2Cr phase 3
      1.1.2. $LiAl_2$ phase 3
   1.2. FSA immobilization on LDH 3
      1.2.1. FSA activity assay 4
      1.2.2. Effect of propargyl alcohol on FSA activity 4
      1.2.3. Effect of lithium on FSA activity 5
2. Study of a model reaction using *E. coli* cells harboring FSA. 5
   2.1. Cells storage conditions and FSA activity assay 5
   2.2. Effect of propargyl alcohol 6
   2.3. Effect of reactants on cell membrane 6
   2.4. Interactions between NHC-Au catalyst and *E. coli* cells 6
      2.4.1. Assessment of FSA activity in cells 6
      2.4.2. Assessment of NHC-Au catalyst activity in the presence of cells 7
   2.5. Cytometry studies 7
3. NHC-Au catalyst immobilization on LDH 8
   3.1. NHC-Au catalyst activity assay 8
   3.2. Immobilization on $LiAl_2$ LDH phase 8
   3.3. NHC-Au@LDH activity assay 9
4. Cells harboring FSA immobilization on $LiAl_2$ LDH 9
5. LDH characterization 10

5.1. X ray diffraction (XRD) and Infrared (FTIR) characterizations 10
5.2. Characterization of NHC-Au@LDH 11
5.3. Scanning Electron Microscopy (SEM) 12
6. Implementation of one-pot one-step reactions 12
6.1. Synthesis of (3*S*)-3,4-dihydroxy-2-butanone 12
6.2. Synthesis of (3*S*, 4*R*) 1,5-dideoxy-D-*threo*-2-hexulose 14
References 15

Material

Formaldehyde, propargyl alcohol, 3,3-diethoxy-1-propanol, D-fructose-6-phosphate dipotassium salt, β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH), Propidium iodide, Chrome nitrate ($Cr(NO_3)_3.9H_2O$), N,N-dimethylformamide (DMF) and glycyl-glycine were purchased from Sigma-Aldrich whereas hydroxyacetone was purchased from Fluka (purity 90%), formaldehyde and urea from Prolabo, aluminium chloride ($AlCl_3.9H_2O$) from Thermo Scientific and lithium chloride ($LiCl.H_2O$) as well as zinc nitrate ($Zn(NO_3)_2.6H_2O$) from Acros Organics. Resin QAE Sephadex™ A-25 was purchased from Cytiva and Dowex HCR-W2, strongly acidic, 16-40 mesh, was purchased from Sigma Aldrich. The cellulose membrane with a cut-off of 1 kDa was from the Pur-A-Lyzer™ Midi Dialysis Kit purchased from Sigma-Aldrich. The Coomassie protein assay reagent, used for Bradford tests, was purchased from Thermo Scientific. The SYBR Green I was purchased from Invitrogen. FSA powder, composed of FSA and glycylglycine buffer (1/1), was obtained from *E. coli* cells pellets following a purification method previously reported.[1] *E. coli* cells harbouring FSA, when used as is, were produced in the same manner. Glycerol dehydrogenase (GDH) was obtained as described by A. K. Samland.[2] A mix of glycerol-3-phosphate dehydrogenase/Triosephosphate isomerase (GDPH/TPI) was purchased from Sigma Aldrich. Gold-carbene complex was synthetized as previously published.[3]

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 cytometer used was a LSP Fortessa X-20.

Methods

1. LDH and FSA@LDH syntheses

1.1. LDH synthesis

1.1.1. Zn_2Cr phase

In a reactor containing 100 mL of water under a nitrogen atmosphere, 7 mL of a solution of 0.4 M of $Zn(NO_3)_2.6H_2O$ and 0.2 M of $Cr(NO_3)_3.9H_2O$ were added by means of a peristaltic pump set at 0.5 rpm, using a flow rate of 1.5 mL/min, i.e. 4.7 minutes of total addition. The pH was maintained to 6.5 by addition of a 0.6 M solution of sodium hydroxide. After the addition, the mixture was left under stirring for 1.5 hours. The solution was then washed 3 times with distilled water and centrifuged at 14000 rpm for 10 minutes for each wash. The solid was then dispersed in deionized water and the mass percentage was determined by drying a sample. 0.375 mg of LDH were obtained (75% yield).

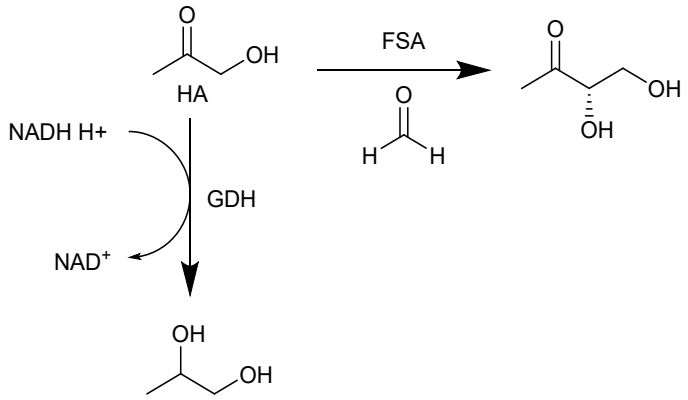
1.1.2. $LiAl_2$ phase

20 mL of a solution of 482 mg of $AlCl_3.9H_2O$ (2 mmol), 339 mg of $LiCl.H_2O$ (8 mmol) and 1.2 g of urea were placed into an autoclave heated at 120°C for 24 h. The suspension was centrifuged at 14000 rpm for 10 minutes and the pellets were washed 3 times with distilled water. The solid was then dried at 80°C for 8 h. 0.152 mg of a white powder was obtained (66% yield).

1.2. FSA immobilization on LDH

A 10 mL solution containing 10 mg of enzyme and 10 mg of LDH was stirred on a rotary shaker at 80 rpm for 2 h. After shaking, the suspension was centrifuged at 14000 rpm for 10 minutes. The precipitate was then washed with distilled water using three cycles of washing/centrifugation (14000 rpm for 10 min). The wet solid was then dispersed in water. The mass percentage was determined by drying a sample (40°C overnight). The quantity of protein was assayed using the Bradford assay in the initial suspension and in the supernatant after centrifugation, to determine the adsorption yield. For Zn_2Cr and $LiAl_2$, >99% of adsorption was obtained.

1.2.1. FSA activity assay



Scheme 1: FSA assay based on HA consumption monitoring by measuring the NADH consumed by GDH auxiliary enzyme over time.

FSA catalyzed model reaction: to a solution of hydroxyacetone (35 µL, 500 mM) in 1 mL of water, were added formaldehyde (42 µL, 500 mM) and 2 mg of FSA powder (50% glycylglycine) or the equivalent of 1 mg of FSA immobilized on LDH (around 2 mg). The pH was adjusted to the desired value, depending of the experiment, and the solution was stirred (Scheme 1). Aliquot were analyzed at various time using NMR or the FSA assay described below.

FSA assay: In 952 µL of a 50 mM glycyl-glycine buffer at pH 7.5 were added glycerol dehydrogenase GDH (20 µL), NADH (0.5 mM, 20 µL of a 12 mg/mL NADH solution in water) and 8 µL of the reaction medium (final volume of 1 mL). One mmol of NADH oxidized was equivalent to 1 mmol of HA used in the aldolisation reaction.

1.2.2. Effect of propargyl alcohol on FSA activity

To a solution of HA (35 µL, 500 mM) in 1 mL of water, were added formaldehyde (42 µL, 500 mM), propargyl alcohol (30 µL, 500 mM) and 2 mg of FSA powder (50% glycylglycine) or the equivalent of 1 mg of FSA immobilized on LDH (around 2 mg). The reaction was monitored by spectrophotometry at 340 nm following the disappearance of HA *via* NADH consumption (Figure 1), as described above.

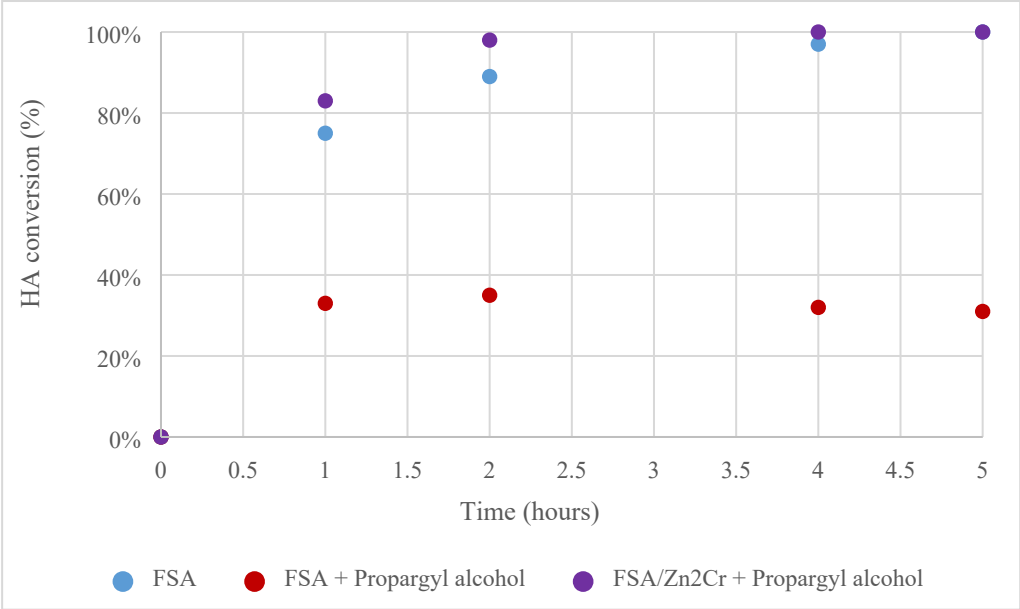


Figure 1: HA conversion in the presence or absence of propargyl alcohol, catalyzed by immobilized or free FSA

1.2.3. Effect of lithium on FSA activity

0.02 mg of FSA were incubated for 2 h in 1 mL of a 50 mM glycyl-glycine buffer pH 7.5 solution with 10 mM of LiCl (0.4 mg). After 2 h, FSA activity was assayed, based on the previously reported retro-aldolisation of F6P.[1] For this, NADH (0.5 mM, 20 µL of a 12 mg/mL NADH solution in water), auxiliary enzymes (commercial mixture of triose-phosphate isomerase and glycerol-3-phosphate dehydrogenase: 5 µL) and D-fructose-6-phosphate (30 mM, 30 µL of a solution in water at 430 mg/mL) were added in the previous solution. 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.

2. Study of a model reaction using *E. coli* cells harboring FSA.

2.1. Cells storage conditions and FSA activity assay

After production of the cells,[1] they were washed twice with distillated water and the pellets were suspended in a solution of 25% glycerol in water. Then aliquots of 1 mL, each containing 50 mg of cells, were conserved at -20°C. FSA activity was measured following the assay procedure reported in section 1.2.1 at pH 7, with the following modifications: the cells were washed with distillated water twice to remove glycerol, then 50 mg of cells were used instead of the FSA powder.

2.2. Effect of propargyl alcohol

In a vial, 0.5 M of both substrates (HA and formaldehyde) and 0.5 M of propargyl alcohol were added in a final volume of 1 mL of water. The pH of the solution was adjusted with sulphuric acid to 3. Then, 50 mg of cells were added (the cells were previously washed with distillated water twice to remove glycerol). The pH was again adjusted to 3 and the vial was shaken at 60°C. The progress of the reaction was monitored (Figure 2) by following the disappearance of HA using NMR spectroscopy or UV-visible spectrophotometry thanks to an auxiliary enzyme (see 1.2.1).

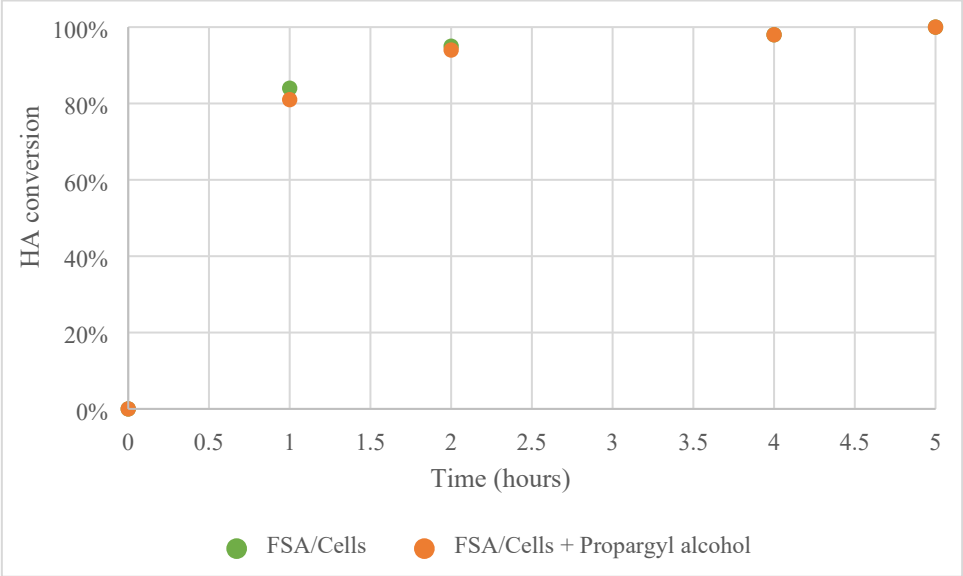


Figure 2: HA conversion with or without propargyl alcohol, catalyzed by FSA harbored in *E. coli* cells at pH 3 and 60°C

2.3. Effect of reactants on cell membrane

Two vials were prepared as follows: in a 1 mL solution containing 0.05 M of HA (35 µL) or propargyl alcohol (30 µL), 50 mg of cells were added. The suspensions were stirred for 2 h. The mixtures were then centrifuged and the supernatant was analysed by quantitative NMR using N,N-diméthylformamide (DMF) as an internal standard. The NMR sample tubes were prepared as follows: 50 µL of the suspension and 2 µL of DMF were added to 0.5 mL of D_2O.

2.4. Interactions between NHC-Au catalyst and *E. coli* cells

2.4.1.Assessment of FSA activity in cells

In a vial, 0.5 M of both substrates (HA and formaldehyde) and 3.0 mg/mL of NHC-Au were added in a final volume of 1 mL of water. The pH of the solution was adjusted with sulphuric acid to 3. 50 mg of cells were then added. The pH was adjusted again and the vial

was shaken at 60°C for 24 h. The progress of the reaction was monitored by following the disappearance of HA using NMR spectroscopy or UV-visible spectrophotometry thanks to an auxiliary enzyme (see 1.2.1).

2.4.2.Assessment of NHC-Au catalyst activity in the presence of cells

In a vial, 0.5 M of propargyl alcohol and 3.0 mg/mL of NHC-Au catalyst were added to a final volume of 1 mL of water. The pH of the solution was adjusted with sulfuric acid to 3. 50 mg of cells were then added. The pH was adjusted again and the vial was shaken at 60°C for 24 h. The progress of the reaction was monitored by following the disappearance of the propargyl alcohol using NMR spectroscopy.

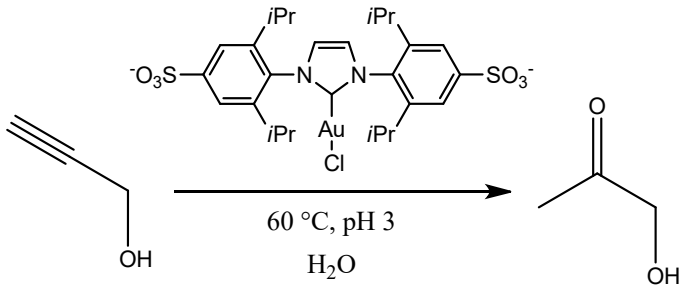
2.5. Cytometry studies

50 mg of cells in 25% glycerol were first washed twice with distillated water to remove the glycerol. The cells were then incubated in 1 mL of water under several conditions: pH 3 or 7, room temperature or 60°C, with or without 3 mg of NHC-Au catalyst, for 20 minutes. The cells suspensions were then diluted 1000 times in a phosphate buffered saline solution (137 mM NaCl, 2.7 mM KCl and 1.8 mM KH_2PO_4) at pH 7.4. The cells were then incubated for 10 min in the dark in the presence of propidium iodide (final concentration: 10 µg/mL) and Sybr Green I (final concentration: 1X). Viable and membrane-altered cells were counted with a LSR Fortessa X-20 cytometer (BD Biosciences). Results are reported in figure 2 in the main text.

To expand on this result, conversion of HA and formaldehyde (35 and 42 µL respectively) by 50 mg of cells harboring FSA in 1 mL of water in these conditions (pH 3 or 7, room temperature or 60°C, with or without 3 mg of NHC-Au catalyst) were monitored after 10 minutes (results are reported in figure 2 in the main text).

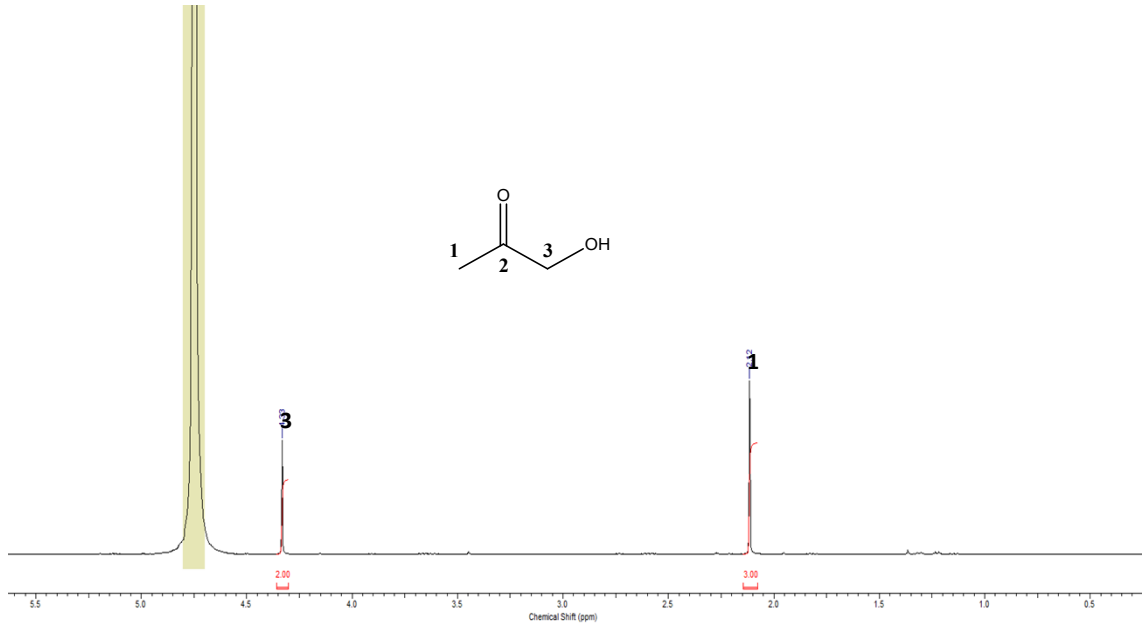
3. NHC-Au catalyst immobilization on LDH

3.1. NHC-Au catalyst activity assay



Scheme 2: Alcyne hydration catalyzed by NHC-Au complex

3.0 mg of free catalyst (0.75 mol%) was added to a 1 mL flask containing 500 µL of a 0.5 M propargyl alcohol solution (15 µL in 485 µL of water). The solution was stirred at 60 °C in a sand bath (Scheme 2). The reaction was monitored by ^{1}H NMR, 100% conversion was obtained after 2 h.



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

3.2. Immobilization on $LiAl_2$ LDH phase

The $LiAl_2$-NHC-Au(I) heterogeneous catalyst was prepared following the same protocol as described in part 1.1.2, but involving 407 mg of the NHC-Au(I) catalyst, initially into the

autoclave at the beginning of the synthesis reactor. $(N_2C_2H_2AuCl(C_6H_2(iPr)_2SO_3^-)_2)/LiAl_2$ molar ratio was set equal to 0.5. The immobilization yield was determined by quantification of the remaining NHC-Au(I) complex in the supernatant solutions, using UV spectrometry. For that purpose, two calibration lines were previously constructed at 270 and 278 nm using several concentrations of the NHC-Au catalyst. Using these calibration lines, NHC-Au catalyst concentration of the solution could be determined by its absorbance at these two wavelengths.

3.3. NHC-Au@LDH activity assay

The equivalent of 3.0 mg of free catalyst, i.e. around 10 mg of NHC-Au@LDH, were added to a 1 mL flask containing 500 µL of a 0.5 M propargyl alcohol solution (15 µL in 485 µL of water). The solution was stirred at 60°C in a sand bath. The reaction was monitored by ^{1}H NMR spectroscopy, 2% conversion was observed after 5 h.

4. Cells harboring FSA immobilization on $LiAl_2$ LDH

10 mL of a solution containing 500 mg of cells in water were placed in a 25 mL flask. Then 0, 100, 250 or 500 mg of $LiAl_2$ LDH (corresponding to a mass ratio LDH/cells of 0, 0.2, 0.5 and 1 respectively) were added. The mixtures were stirred at room temperature for 2 h. The pH of each solution was then adjusted to 3 with sulfuric acid (1M) and stirred at 60°C. Every 30 minutes, 1 mL was sampled, the substrates were added (35 µL of HA and 42 µL of formaldehyde) for a final concentration of 0.5 M, and the pH was adjusted to 3 with sulphuric acid (1M) again. The solution was stirred at 60°C and the pH was regularly controlled. After 1 h, the progress of the reaction was monitored by following the disappearance of HA by ^{1}H NMR spectroscopy (Figure 3).

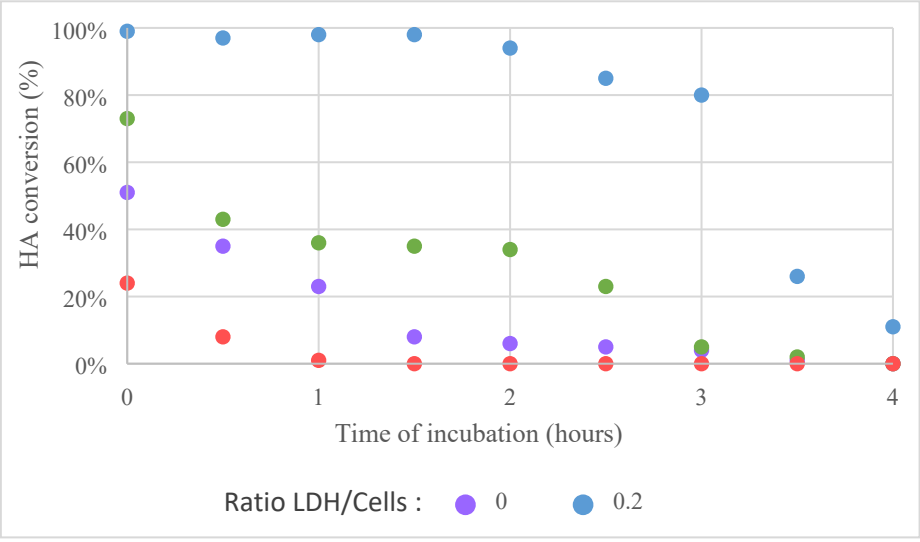


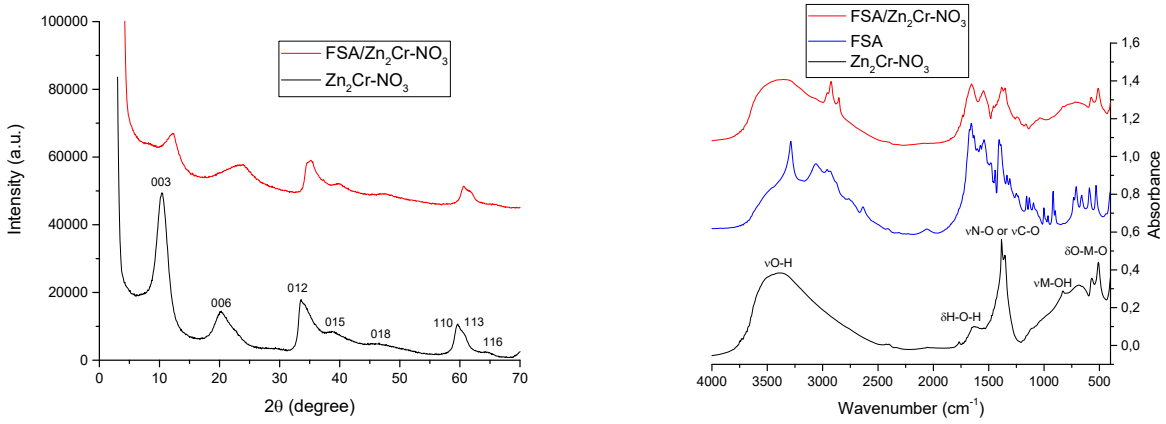
Figure 3: HA conversion rate after 1 h of reaction (HA + formaldehyde), after a variable period of preincubation of cells with various amounts of $LiAl_2$ LDH phase, at pH 3 and 60°C

5. LDH characterization

5.1. X ray diffraction (XRD) and Infrared (FTIR) characterizations

X-ray diffraction analyses (Figures 4A and B, left) were performed using a theta-theta Panalytical X'Pert Pro diffractometer equipped with Kα1/α2 Cu anticathode (1.5418 Å) and a X'Celeretor detector and using an acquisition program with counting step 0.0334° (2theta), counting time per step 50.8 ms and analysis angular range 2.5°-70°. Transmission mode FTIR analyses of KBr pellet samples (Figures 4A and B, right) were performed with a Nicolet 6700 FT-IR instrument. Vibrational range was set between 400 and 4000 cm^{-1}.

A)



B)

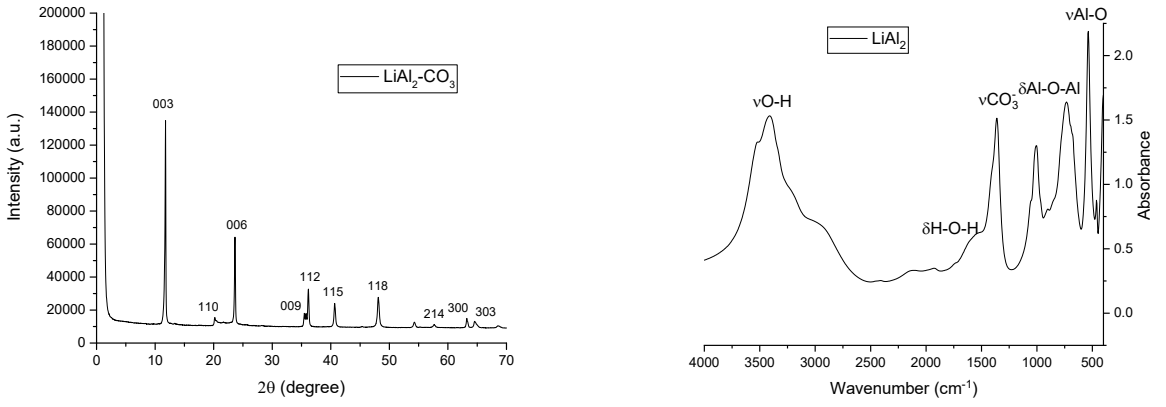


Figure 4: Powder X-ray diffractograms and IR spectra of LDH Zn_2Cr-NO_3 (A) and $LiAl_2$-CO_3 (B) phases

5.2. Characterization of NHC-Au@LDH

The NHC-Au catalyst was first built with ChemDraw software and, after minimization of its energy thanks to the MM2 force field method available in ChemBio 3D, a size of 15.3 Å in length and 6.1 Å in width could be estimated (figure 5A). Then, the hybrid material (NHC-Au@LDH) was analyzed by infrared and X-ray diffraction (Figure 6). LDH signature, either in the presence of a common anion like carbonate, or when the catalyst was intercalated, was found in both analyses, revealing no exfoliation due to the NHC-Au catalyst. Regarding XRD analysis, with the small anion carbonate, interlamellar distance could be calculated at 7,51 Å, reaching 21,35 Å after substitution of the carbonates by the NHC-Au catalyst (figure 6). This interlamellar size extension was in accordance with the estimated length of the catalyst, given the direction of intercalation of the catalyst (Figure 5B).

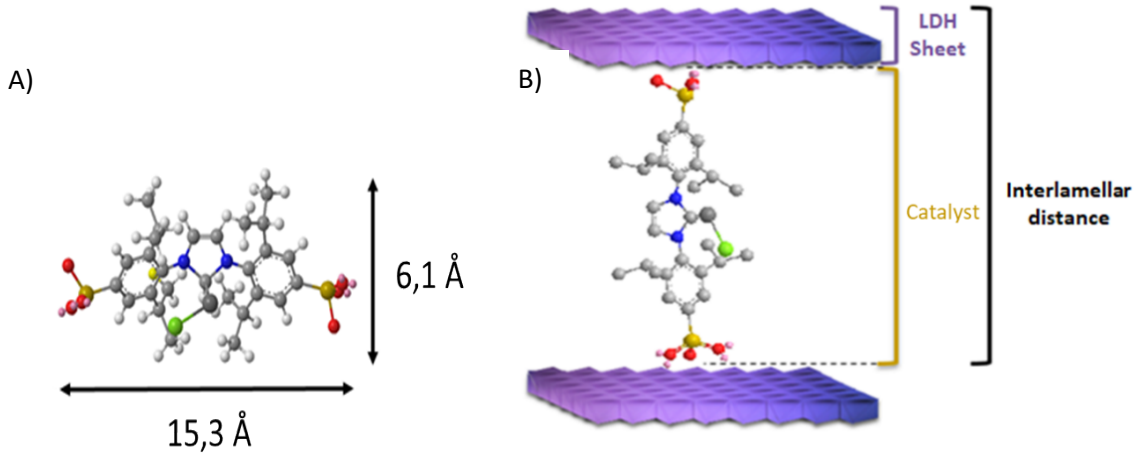


Figure 5: A) estimated size of the free NHC-Au catalyst; B) Schematic structure of $LiAl_2$ LDH intercalated by NHC-Au(I), directed by sulfonate interactions with the LDH positively charged layers.

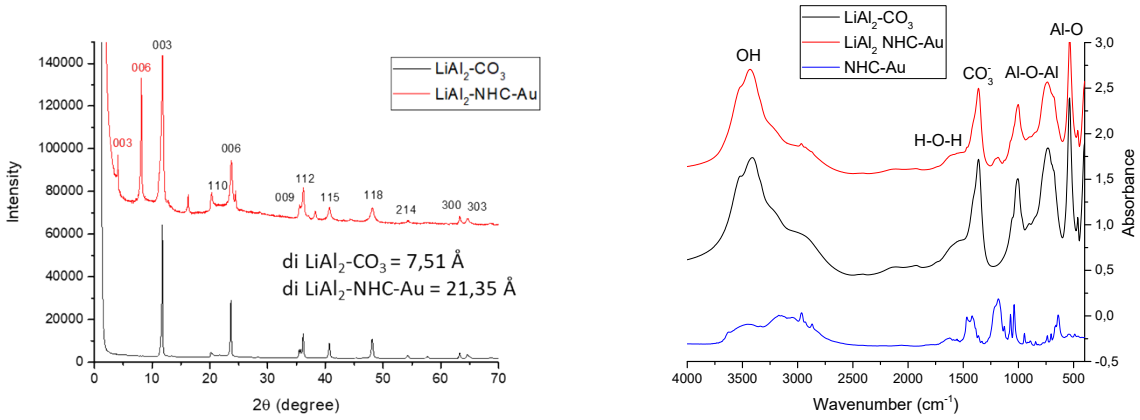


Figure 6: X-ray diffractograms and infrared spectra of $LiAl_2$-CO_3 and $LiAl_2$-NHC-Au(I) LDH

5.3. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) images were recorded using a JSM-7500F Field Emission Scanning Electron Microscope operating at an acceleration voltage of 3 kV (Figure 7).

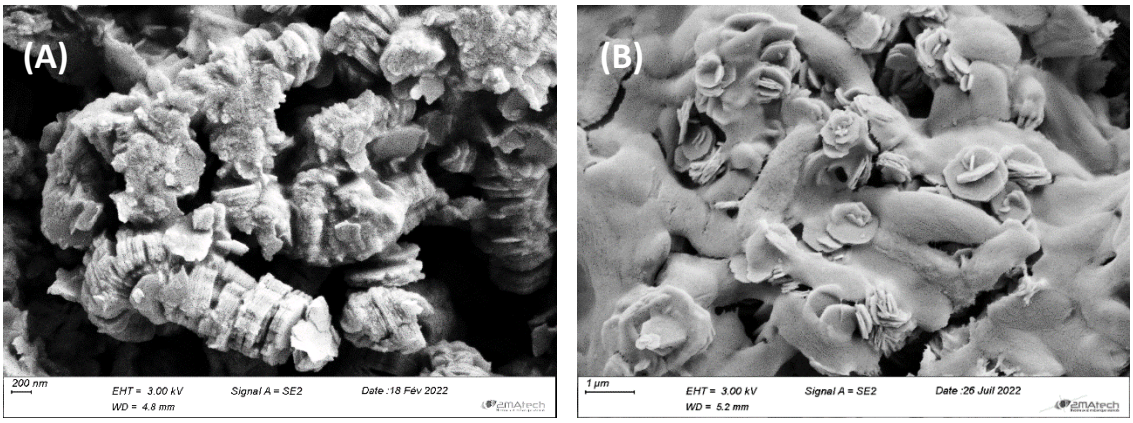


Figure 7 : SEM images of $LiAl_2$-CO_3 (A) and *E. coli* cells@$LiAl_2$-CO_3 (B)

6. Implementation of one-pot one-step reactions

To note, fructose-6-phosphate aldolase was proved to be stereoselective, always giving a (3*S*) aldol adduct, regardless of the substrate. This is due to the formation of a *cis*-enamine in the active site giving an accessible *si* face for the electrophile.[4–6] Generally, a subsequent *si* face attack of the aldehydic electrophile results in a 4*R* configuration in the aldol produced. Sometimes DHAP/DHA aldolase family could not be stereoselective in position 4, leading to a mixture of diastereoisomers due to a *re* face attack of the aldehyde.

6.1. Synthesis of (3*S*)-3,4-dihydroxy-2-butanone

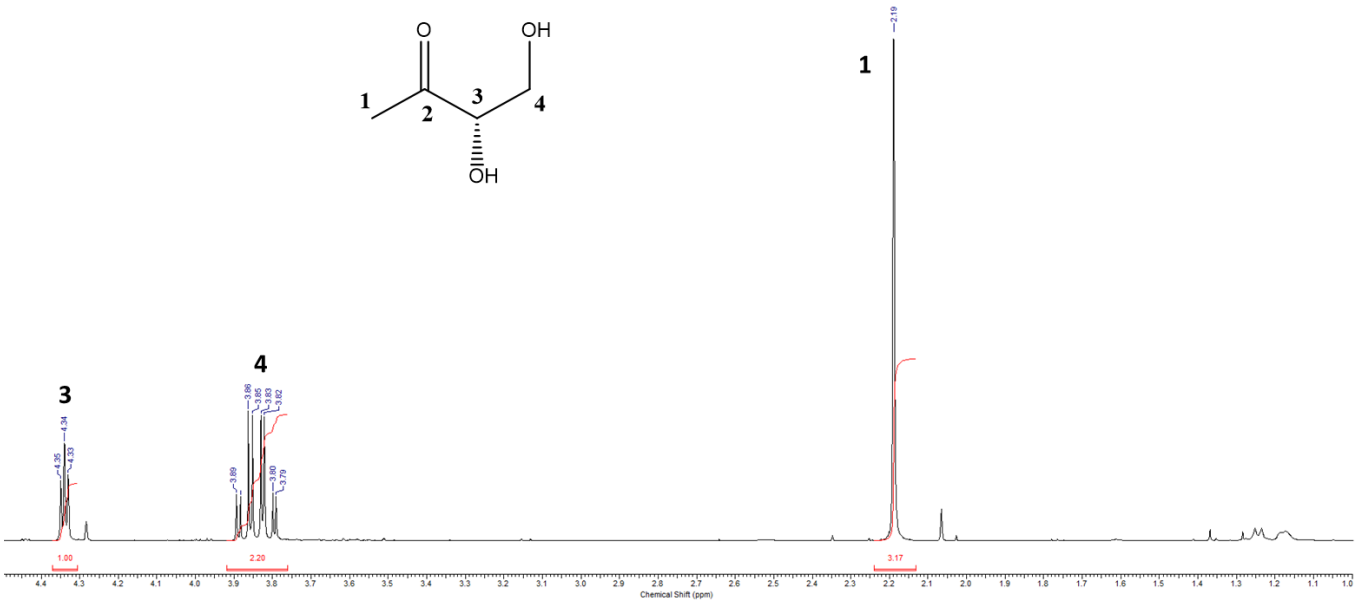
Procedure with no catalysts compartmentalization: In an Eppendorf, 3 mg of NHC-Au cata lyst, 0.5 mmol of propargyl alcohol and 0.5 mmol of formaldehyde were added to 1 mL of water. 1 mg of free FSA or 2 mg of FSA@Zn_2Cr or 50 mg of cells harboring FSA were added. The pH was adjusted to 3 and the mixture was then stirred at 60°C. Among the various options, only the mixture involving FSA@Zn_2Cr gave a HA conversion (12%) different from 0%.

Optimized procedure: In a cellulose membrane bag with a cut-off of 1 kDa, 18 mg of NHC-Au catalyst and 3.0 mmol of propargyl alcohol (0.5 M final) were added to a final volume of 0.5 mL. The bag was then sealed. In a beaker, 300 mg of cells (free or adsorbed on $LiAl_2$ with a ratio LDH/cells = 0.2, as previously described in paragraph 4) and 3.0 mmol of formaldehyde (0.5 M final) were added in 5.5 mL of distillated water, the pH of this solution being then adjusted to 2. Thereafter, the sealed membrane was placed into this solution. This was stirred, and the whole was placed at 60°C, the progress of the reaction being monitored by following the disappearance of the propargyl alcohol and the apparition of the final product, after 4 h and then 20 h reaction time, by ^{1}H NMR spectroscopy. At the end of the reaction, the pH 3.7 solution inside the membrane, containing NHC-Au catalyst, was filtered through a Sephadex-type anion exchange resin (bicarbonate form) to remove the catalyst. Elsewhere, the pH 3.7 solution inside the beaker containing the cells was centrifuged (14000 rpm for 5 min) and washed with water. Both solutions were mixed together, the pH adjusted to 7, and then evaporated under vacuum to recover the expected product as an oil. Without LDH, a HA conversion of 31% was obtained whereas it was increased to 45% in the presence of LDH.

When the same procedure, using cells adsorbed on $LiAl_2$ with a ratio LDH/cells = 0.2 was carried out, involving 0.2 M of substrates (1.2 mmol of propargyl alcohol and 1.2 mmol of formaldehyde), a HA conversion of 70% was reached. After purification, 85 mg of the aldol adduct were recovered (68% yield). $[\alpha]_D^{25}$: +63.0 (c 5.9, H_2O).[7,8]

^{1}H NMR spectrum was identical to the one obtained by Schürmann et al. and our group in previous works.[3,5]

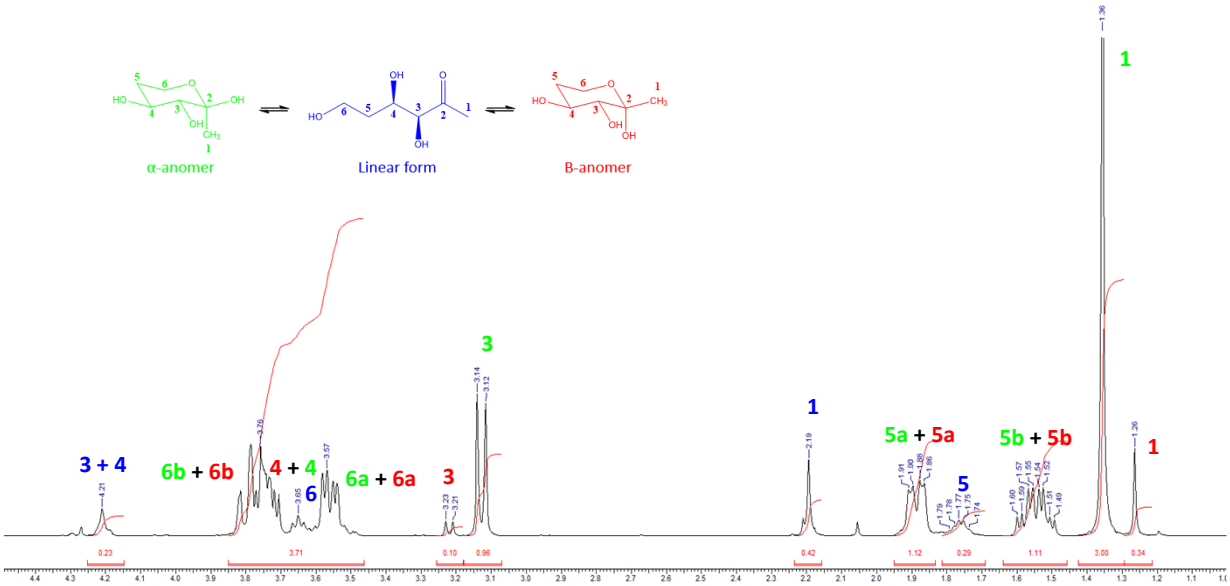
^{1}H NMR (400 MHz, D_2O): δ 4.33 (t, 1H, *J*=3.8 Hz, 3H), 3.83 (2dd, 2H, *J*=3.8 Hz and *J*=12.4 Hz, $H4_A$, $H4_B$), 2.18 (s, 3H, 1H).



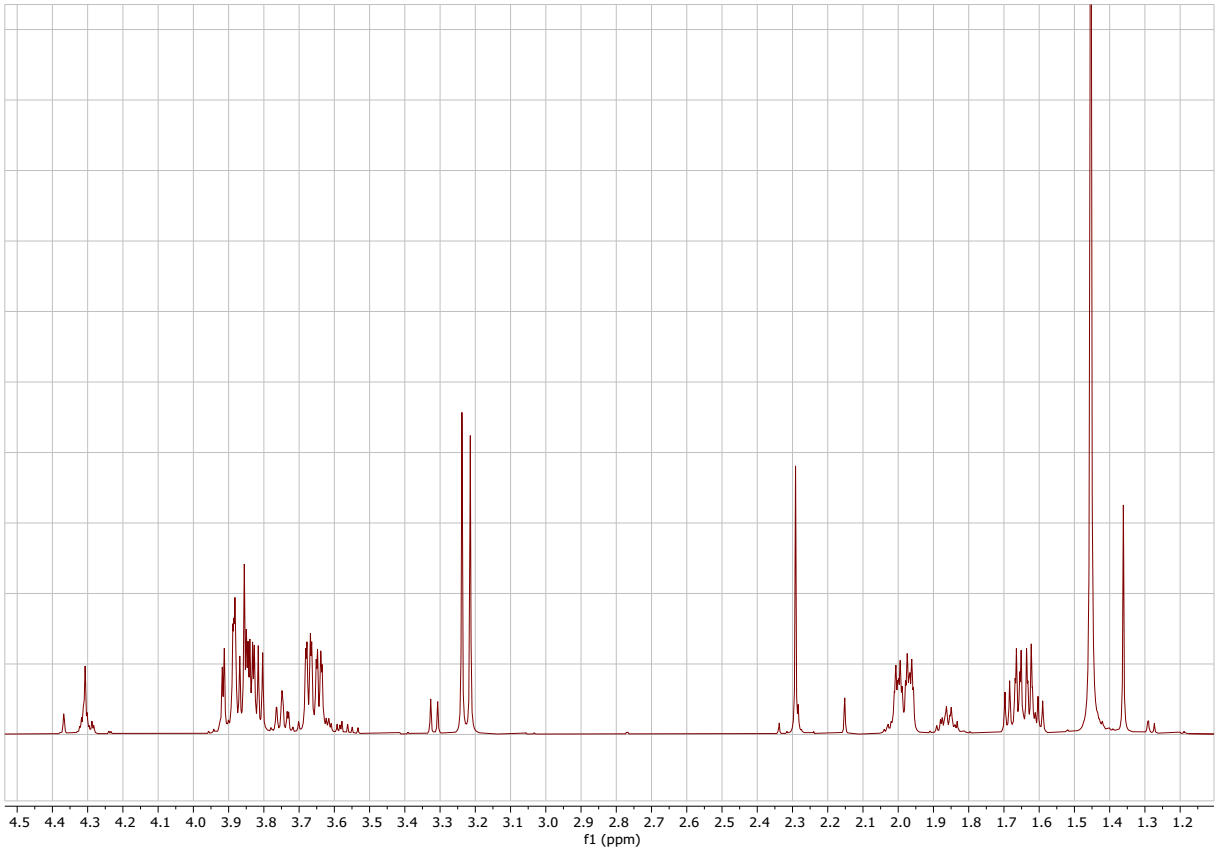
6.2. Synthesis of (3*S*, 4*R*) 1,5-dideoxy-D-*threo*-2-hexulose

Optimized procedure: to a solution of HA (200 mM, 15 µL) were added 3,3-diethoxy-1-propanol (160 mM, 25 µL), 50 mg of *E. coli* cells harboring FSA and 110 mg of an acidic resin (Dowex HCR-W2, strongly acidic, 16-40 mesh) in 1 mL of water. The resulting suspension was then stirred at room temperature. HA disappearance was monitored by spectrophotometry at 340 nm, following NADH consumption thanks to GDH auxiliary enzyme as previously mentioned. After 3 h, the solution was centrifuged (14000 rpm for 5 min) and the supernatant was evaporated under vacuum to give 23 mg of the product (97% yield). $[\alpha]_D^{25}$: -69.5 (c 4.1, H_2O).[10]

^{1}H NMR spectrum (identical to those obtained previously by our group[3]):



as a reminder, just below the ^{1}H NMR spectrum from the literature:



^{1}H NMR spectrum obtained in this study:

References

(1) Mahdi, R.; Guérard-Hélaine, C.; Prévot, V.; de Berardinis, V.; Forano, C.; Lemaire, M. Design of Artificial Metabolisms in Layered Nanomaterials for the Enzymatic Synthesis of Phosphorylated Sugars. *ChemCatChem* **2015**, *7* (19), 3110–3115.

(2) Schneider, S.; Gutiérrez, M.; Sandalova, T.; Schneider, G.; Clapés, P.; Sprenger, G. A.; Samland, A. K. Redesigning the Active Site of Transaldolase TalB from *Escherichia coli*: New Variants with Improved Affinity towards Nonphosphorylated Substrates. *ChemBioChem.* **2010**, *11* (5), 681–690.

(3) Gastaldi, C.; Mekhloufi, G.; Forano, C.; Gautier, A.; Guérard-Hélaine, C. Mixing Chemo- and Biocatalysis for Rare Monosaccharide Production by Combining Aldolase and N-Heterocyclic Carbene Gold Catalysts. *Green Chem.* **2022**, *24* (9), 3634–3639.

(4) Concia, A. L.; Lozano, C.; Castillo, J. A.; Parella, T.; Joglar, J.; Clapés, P. D-Fructose-6-Phosphate Aldolase in Organic Synthesis: Cascade Chemical-Enzymatic Preparation of Sugar-Related Polyhydroxylated Compounds. *Chemistry* **2009**, *15* (15), 3808–3816.

(5) Clapés, P.; Garrabou, X. Current Trends in Asymmetric Synthesis with Aldolases. *Adv. Synth. Catal.* **2011**, *353* (13), 2263–2283.

(6) Hélaine, V.; Gastaldi, C.; Lemaire, M.; Clapés, P.; Guérard-Hélaine, C. Recent Advances in the Substrate Selectivity of Aldolases. *ACS Catal.* **2022**, *12* (1), 733–761.

(7) Guérard-Hélaine, C.; Légeret, B.; Fernandes, C.; Prévot, V.; Forano, C.; Lemaire, M. Efficient Immobilization of Fructose-6-Phosphate Aldolase in Layered Double Hydroxide:

Improved Stereoselective Synthesis of Sugar Analogues. *New J. Chem.* **2011**, *35* (4), 776–779.
(8) Saravanan, T.; Junker, S.; Kickstein, M.; Hein, S.; Link, M.-K.; Ranglack, J.; Witt, S.; Lorillière, M.; Hecquet, L.; Fessner, W.-D. Donor Promiscuity of a Thermostable Transketolase by Directed Evolution: Efficient Complementation of 1-Deoxy- D -Xylulose-5-Phosphate Synthase Activity. *Angew. Chem. Int. Ed.* **2017**, *56* (19), 5358–5362.
(9) Schurmann, M.; Sprenger, G. A. Fructose-6-Phosphate Aldolase Is a Novel Class I Aldolase from *Escherichia coli* and Is Related to a Novel Group of Bacterial Transaldolases. *J Biol Chem* **2001**, *276* (14), 11055–11061.
(10) Rale, M.; Schneider, S.; Sprenger, G. A.; Samland, A. K.; Fessner, W.-D. Broadening Deoxysugar Glycodiversity: Natural and Engineered Transaldolases Unlock a Complementary Substrate Space. *Chem. Eur. J.* **2011**, *17* (9), 2623–2632.