DOI: 10.1002/adsc.201801530
Advanced Synthesis & Catalysis

# Aldolase-Catalyzed Asymmetric Synthesis of N-Heterocycles by Addition of Simple Aliphatic Nucleophiles to Aminoaldehydes

Raquel Roldán,[a] Karel Hernández,[a] Jesús Joglar,[a] Jordi Bujons,[a] Teodor Parella,[b] Wolf-Dieter Fessner,[c] and Pere Clapés[a,*]

[a] Dept. Biological Chemistry. Instituto de Química Avanzada de Cataluña IQAC-CSIC, Jordi Girona 18–26, 08034 Barcelona, Spain
Phone +34934006127
Fax: +34932045904
E-mail: pere.clapes@iqac.csic.es
[b] Servei de Ressonància Magnètica Nuclear. Universitat Autònoma de Barcelona, Bellaterra, Spain
[c] Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, Petersenstraße 22, D-64287 Darmstadt, Germany

Manuscript received: November 13, 2018; Revised manuscript received: January 22, 2019;
Version of record online: February 15, 2019

Supporting information for this article is available on the WWW under https://doi.org/10.1002/adsc.201801530



**Abstract:** Nitrogen heterocycles are structural motifs found in many bioactive natural products and of utmost importance in pharmaceutical drug development. In this work, a stereoselective synthesis of functionalized N-heterocycles was accomplished in two steps, comprising the biocatalytic aldol addition of ethanal and simple aliphatic ketones such as propanone, butanone, 3-pentanone, cyclobutanone, and cyclopentanone to *N*-Cbz-protected aminoaldehydes using engineered variants of D-fructose-6-phosphate aldolase from *Escherichia coli* (FSA) or 2-deoxy-D-ribose-5-phosphate aldolase from *Thermotoga maritima* ($DERA_{Tma}$) as catalysts. FSA catalyzed most of the additions of ketones while $DERA_{Tma}$ was restricted to ethanal and propanone. Subsequent treatment with hydrogen in the presence of palladium over charcoal, yielded low-level oxygenated N-heterocyclic derivatives of piperidine, pyrrolidine and N-bicyclic structures bearing fused cyclobutane and cyclopentane rings, with stereoselectivities of 96–98 *ee* and 97:3 dr in isolated yields ranging from 35 to 79%.

**Keywords:** Biocatalysis; Aldol reaction; Aldolases; Nitrogen heterocycles; Reductive amination

## Introduction

Nitrogen heterocycles are structural motifs frequently found in bioactive natural products and pharmaceuticals, being an area of constant development owing to their importance in drug discovery.[1] The ring type, e.g. piperidine, pyrrolidine, pyrrolizidine, N-bicyclics, and the structure, position and stereochemistry of the substituents, play a fundamental role in modulating their biological activity.[1b,2] Methods for the total synthesis of a broad structural diversity of N-heterocycles, either as building blocks or as final compounds for biological testing, are thus in high demand.[1a] Although the number of chemical routes available to prepare these compounds is extensive, developing new stereoselective synthetic methodology is essential yet challenging.[1a,3]

Biocatalytic C–C bond forming reactions by means of carboligases is highly attractive because of unparalleled high efficiency and stereoselectivity, avoidance of unnecessary functional group protection and use of mild reaction conditions. Among them, aldolases have proven their remarkable synthetic utility in the preparation of different types of highly oxygenated N-heterocycles, usually termed as iminosugars or azasugars.[4]

Recent synthetic developments of D-fructose-6-phosphate aldolase from *E. coli* (FSA, EC 4.1.2.-)[5] showed that FSA D6X variants (X = A, L, N, Q, S, T, E, and H), and double variants combined with A165G, L107A, L163A and T26X (X: I, A, L or V) mutations, are effective catalysts for homo and crossed aldol additions of simple aliphatic ketones and aldehydes leading to the preparation of chiral intermediates and deoxysugars.[6] Hence, these FSA variants can offer new synthetic options toward the preparation of iminosugar derivatives having a low degree of hydroxylation. Likewise, 2-deoxy-D-ribose-5-phosphate aldolase (DERA, EC 4.1.2.4) has been recognized as a powerful aldolase for the synthesis of deoxysugars and iminosugars.[7] Wild-type DERA was unique among the aldolases in that it uses simple aliphatic aldehydes both as nucleophilic and electrophilic aldol components and therefore is a qualified catalyst for the proposed synthesis.

We have investigated the systematic chemoenzymatic preparation of low-level functionalized *N*-heterocyclic compounds along a two-steps strategy: The first step consists of an aldol addition of ethanal (**1a**) and simple linear (**1b–d**) or cyclic aliphatic ketone (**1e–g**) to an *N*-Cbz-protected amino aldehyde **2a–c** (Figure 1) catalyzed by FSA variants and DERA from *Thermotoga maritima* ($DERA_{Tma}$). In a second step, the aldol adducts obtained are transformed into the corresponding N-heterocycles via intramolecular reductive amination by using $H_2$–Pd/C.

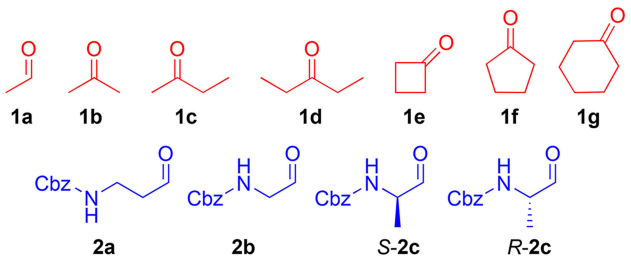



**Figure 1.** Panel of nucleophiles **1a–g** and electrophiles **2a–c** assayed for enzymatic cross-aldolization.

## Results and Discussion

### Screening Experiments

We studied the aldol addition of simple aliphatic nucleophiles **1a–g** (concentration range 100 mM to 2.6 M (15% v/v), see SI for optimization analysis) to electrophiles **2a–c** (80–100 mM) using FSA variants and wild-type $DERA_{Tma}$ as catalysts (Figure 2) in small-scale preparative synthetic conversions. FSA catalysts were selected among those found to be efficient toward the nucleophiles **1** and *N*-Cbz-aminoaldehydes **2**.[4c,6b,8] The screening was performed by direct HPLC monitoring of aldol adduct formation. Electrophilic components chosen were prochiral substrates **2a** and **2b**, which avoid complications arising from kinetic enantiomer selectivity, and pure enantiomers of chiral substrate **2c**. The latter contains a chiral center of defined absolute configuration that acts as a reference in the determination of the overall relative configuration of the products.

Aldol additions of nucleophiles **1a–f** to electrophiles *N*-Cbz-3-aminopropanal (**2a**) and *N*-Cbz-2-aminoethanal (**2b**) were catalyzed by several FSA variants (Figure 2). Variants with different amino acids substituting for D6 were found to be active for various nucleophile-electrophile combinations, some of them yielding good to excellent product formation. Interestingly, FSA A165G was also efficient for the additions of **1b–c**, **e–f** to **2a** and **2b**, and a quintuple FSA mutant (FSA qm: FSA L107Y/A129G/R134S/A165G/S166G) for addition of **1a** to **2a**. In contrast, FSA A165G and all variants that include the native D6 residue had been found inactive when hydroxyaldehyde derivatives were employed as electrophiles.[6b] Hence, *N*-Cbz-aminoaldehydes appear to establish different, and potentially stronger, interactions with the FSA substrate binding site through the carbamate and/or aromatic moieties, which likely render them electrophiles superior to hydroxyaldehydes.[4d,8a] This is also consistent with the fact that previously developed FSA A165G was more efficient than the wild-type in aldol additions of hydroxyacetone (HA) and dihydroxyacetone (DHA) to **2a** and, particularly, to **2b**.[8a] Likewise, wild-type FSA also converted **1a–c** and **1f** using D,L-glyceraldehyde-3-phosphate, the best electrophilic substrate known to date for wild-type FSA.[6a] None of the FSA variants assayed catalyzed the addition of **1a–g** to either enantiomer of *N*-Cbz-alaninal (**2c**). This was quite surprising since FSA A165G can catalyze the addition of HA and DHA to both *S*-**2c** and *R*-**2c**.[8a] Cyclohexanone (**1g**) was not accepted as a substrate, probably due to steric hindrance.

In comparison, $DERA_{Tma}$ was also active for the aldol addition of ethanal (**1a**) and propanone (**1b**) to **2a** and to *R*- and *S*-**2c**, whereas **2b** only reacted with **1b** (Figure 2). In stark contrast to all FSA catalysts, $DERA_{Tma}$ was highly active with both enantiomers of the α-substituted amino aldehydes **2c**. On the other hand, $DERA_{Tma}$ did not catalyze additions with any of the ketones **1c–g** to any of the electrophiles **2a–c**. This is consistent with the observation that the affinity of DERA for ketones is significantly lower than for ethanal (**1a**), which is its natural nucleophilic substrate.[9]

### Preparative Synthesis and Product Characterization

Addition of **1a** to *N*-Cbz-3-aminopropanal (**2a**) was catalyzed by FSA D6L, D6N, D6E/A165G, FSA qm

| | Electrophile | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2a | | | | | | | 2b | | | | | | | *S*-2c | | | | | | | *R*-2c | | | | | | |
| | Nucleophile | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1a | 1b | 1c | 1d | 1e | 1f | 1g | 1a | 1b | 1c | 1d | 1e | 1f | 1g |
| | Aldol adduct % | | | | | | | Aldol adduct % | | | | | | | Aldol adduct % | | | | | | | Aldol adduct % | | | | | | |
| **FSA** | **3a** | **3b** | **3c/4c** | **3d** | **3e/4e** | **3f/4f** | – | – | **15b** | **15b/16b** | **15d/16d** | **15e** | **15f/16f** | – | (4*S*,5S)-**29** | (4*S*,5*S*)-**29** | – | – | – | – | – | (4*R*,5*S*)-**29** | (4S,5R)-**29** (4*R*,5*R*)-**30** | – | – | – | – | – |
| wild-type | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 17 | 0 | 0 | 10 | 10 | 0 | No reaction | | | | | | | No reaction | | | | | | |
| D6A | 3 | 31 | 18 | 15 | 12 | 6 | 0 | 0 | 56 | 20 | 25 | 21 | 8 | 0 | | | | | | | | | | | | | | |
| D6L | 30 | 21 | 10 | 0 | 52 | 16 | 0 | 0 | 17 | 10 | 10 | 10 | 10 | 0 | | | | | | | | | | | | | | |
| D6N | 40 | 99 | 10 | 0 | 22 | 11 | 0 | 0 | 50 | 10 | 0 | 10 | 10 | 0 | | | | | | | | | | | | | | |
| D6Q | 10 | 18 | 30 | 19 | 18 | 11 | 0 | 0 | 20 | 21 | 10 | 10 | 46 | 0 | | | | | | | | | | | | | | |
| D6S | 8 | 69 | 61 | 30 | 20 | 9 | 0 | 0 | 33 | 22 | 34 | 10 | 5 | 0 | | | | | | | | | | | | | | |
| D6T | 13 | 97 | 71 | 42 | 30 | 26 | 0 | 0 | 67 | 65 | 47 | 17 | 12 | 0 | | | | | | | | | | | | | | |
| D6E | 10 | 24 | 53 | 71 | 20 | 10 | 0 | 0 | 10 | 10 | 35 | 10 | 10 | 0 | | | | | | | | | | | | | | |
| D6H | 10 | 47 | 10 | 0 | 29 | 11 | 0 | 0 | 29 | 0 | 10 | 16 | 12 | 0 | | | | | | | | | | | | | | |
| A165G | 0 | 68 | 18 | 10 | 52 | 40 | 0 | 0 | 76 | 70 | 10 | 80 | 50 | 0 | | | | | | | | | | | | | | |
| L107A | 0 | 21 | 10 | 0 | 17 | 10 | 0 | 0 | 40 | 10 | 0 | 0 | 20 | 0 | | | | | | | | | | | | | | |
| L163A | 0 | 21 | 10 | 0 | 10 | 10 | 0 | 0 | 26 | 10 | 0 | 0 | 19 | 0 | | | | | | | | | | | | | | |
| D6H/L107A | 10 | 75 | 10 | 0 | 23 | 10 | 0 | 0 | 18 | 0 | 0 | 0 | 10 | 0 | | | | | | | | | | | | | | |
| D6L/L107A | 10 | 40 | 10 | 0 | 15 | 10 | 0 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | | | | | | | | | | | | | | |
| D6E/A165G | 50 | 24 | 10 | 0 | 11 | 10 | 0 | 0 | 34 | 0 | 10 | 40 | 10 | 0 | | | | | | | | | | | | | | |
| D6E/L163A | 10 | 35 | 10 | 10 | 10 | 10 | 0 | 0 | 31 | 0 | 10 | 23 | 10 | 0 | | | | | | | | | | | | | | |
| FSA qm | 93 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | | | | | | | | | | | | |
| DERA$_{Tma}$ | 77 | 70 | No reaction | | | | | 0 | 85 | No reaction | | | | | 98 | 81 | No reaction | | | | | 80 | 91 | No reaction | | | | |

**Figure 2.** Results of conversion to aldol adduct from the screening of FSA variants and DERA$_{Tma}$ as catalysts for the aldol addition of nucleophiles **1a–g** to electrophiles **2a–c**. Conversions (mol %) at 24 h were determined by HPLC analysis using the external standard methodology. Conditions: [**1a**] = 100 mM; [**1b**] = 2.6 M (15% v/v), [**1c**] = 0.60 M, [**1d**] = 0.47 M, [**1e**] = 0.68 M, [**1f**] = 0.56 M, and [**1g**] = 0.48 M (5% v/v; in **1c–g**), [**2**] = 80 mM, except for the addition if **1a** to *R*-**2c** and *S*-**2c** catalyzed by DERA$_{Tma}$ where [**1a**] = [**2c**] = 100 mM. Reactions were conducted in aqueous 50 mM triethanolamine buffer pH 8 at 25 °C. FSA qm = FSA quintuple mutant = FSA L107Y/A129G/R134 S/A165G/S166G. A complete list of all FSA variants assayed can be found in SI.

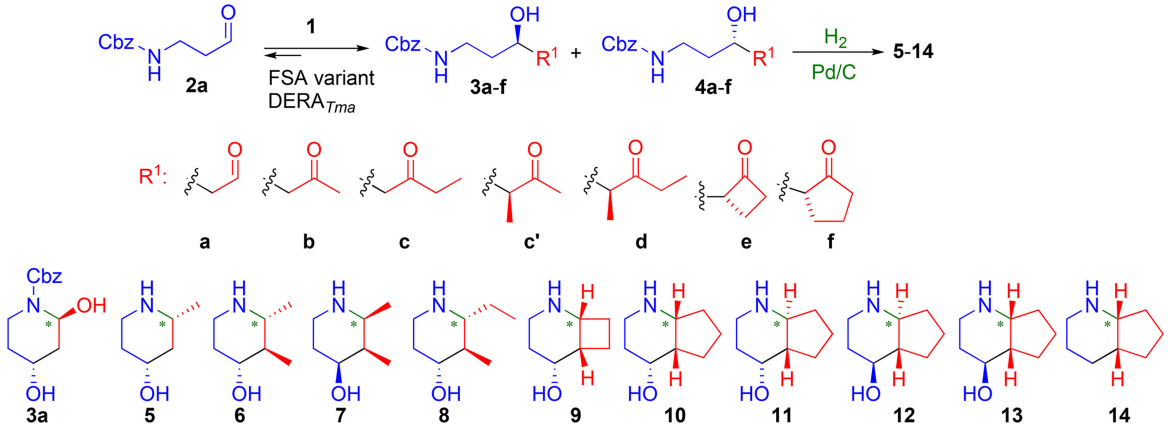
and DERA$_{Tma}$. FSA qm and DERA$_{Tma}$ catalysis yielded the cyclic *N*-Cbz-protected hemiaminal **3a** with *trans*-configured hydroxyl groups at C2:C4 positions in 45% and 25% isolated yields, respectively (Table 1, entry 1 and 2). The lower yield of **3a** obtained with DERA$_{Tma}$ is most likely due to consumption of **1a** in the competing enzymatic trimerization of **1a** leading to a stable trideoxyhexose (i.e., 3*R*,5*R*-dihydroxyhexanal; see Scheme 3).[10] On the other hand, neither trimerization reaction nor self-aldol addition of **1a** was detected with FSA qm variant.[6b] Single addition of **1a** to **2a** was exclusively obtained with both FSA qm and DERA$_{Tma}$ catalysts, and no consecutive double addition product was observed, neither upon application of an excess of ethanal nor upon continuous addition of **1a** using a syringe pump. In the self-addition of **1a** and the crossed addition of **1a** to **2a** DERA is reported to show strict *R*-stereoselectivity for the newly formed chiral centers.[10–11] Therefore, **3a** was assigned a (2*S*,4*R*)-configuration. Identical NMR spectra were recorded for the adduct obtained by catalysis of FSA qm, and their matching optical rotation (Table 1) verified that the identical product (2*S*,4*R*)-**3a** was formed in both cases, which is consistent with the stereochemical outcome expected for FSA catalysis.

FSA variants D6N and D6T were the most effective catalysts for the aldol addition of **1b** to **2a**, whereas FSA A165G was the best for the reaction between **1b** and **2b** (Figure 2), furnishing **3b** and **15b** in 85% and 35% isolated yields, respectively, and with high enantioselectivity (96%–98% *ee*) as determined by HPLC on chiral stationary phase (Table 1 entry 3 and Table 2 entry 1). DERA$_{Tma}$ catalyst gave identically configured aldol adducts in 47% and 30% isolated yields, with 84% and 90% *ee*, respectively (Table 1 entry 4 and Table 2 entry 2). No self-addition of **2b** was observed in the reactions catalyzed by DERA$_{Tma}$. Again, a single addition product was exclusively obtained in the aldol reaction of **1b** with **2a** and **2b** with FSA D6N, FSA A165G and DERA$_{Tma}$ catalysts. On the basis of the stereochemical outcome of both enzymes in examples with non-natural substrates,[4c,e,10] **3b** has (*R*)- and **15b** has (*S*)-configuration. Intramolecular reductive amination of (*R*)-**3b** was stereoselective, furnishing **5** with *cis*-stereochemistry as expected from previous works.[12] On the other hand, (*S*)-**15b** gave a mixture of both *cis*- and *trans*-configured pyrrolidines **17** and **18**, respectively.

The addition of butanone (**1c**) to **2a** could be catalyzed by FSA D6S, D6T and D6E, and addition to **2b** by D6T and A165G variants, generally with good conversions (Figure 2). The preparative reaction be-

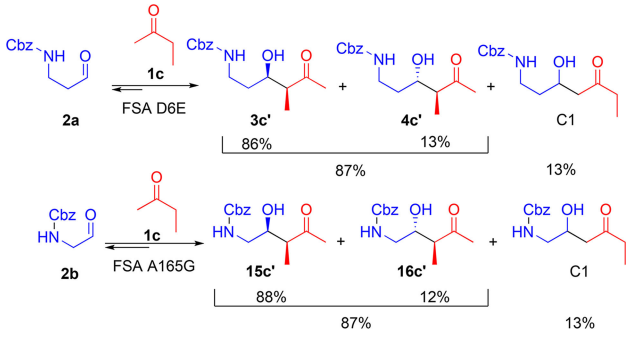
**Table 1.** Preparative scale FSA- and DERA-catalyzed aldol additions of **1a–f** to *N*-Cbz-3-aminopropanal (**2a**).




| Entry | Biocatalyst | Nu | Product | Aldol adduct formed,[a] % | Yield,[b] % | Aldol addition er or dr, % | Reductive amination product,[c] dr |
|---|---|---|---|---|---|---|---|
| 1 | FSA qm | **1a** | **3a**[d] | 85 | 45 | –[e] | – |
| 2 | DERA$_{Tma}$ | **1a** | **3a**[d] | 77 | 25 | –[e] | – |
| 3 | FSA D6 N | **1b** | **3b** | 94 | 85 | 98:2[f] | **5** |
| 4 | DERA$_{Tma}$ | **1b** | **3b** | 70 | 47 | 92:8[f] | – |
| 5 | FSA D6E | **1c** | **3c′/4c′** | 78 | 48 | 86:14[g] | **6**:**7** 86:14 |
| 6 | FSA D6E | **1d** | **3d** | 71 | 35 | >97:3[h,i] | **8** |
| 7 | FSA D6L | **1e** | **3e/4e** | 56 | 46 | 80:20[i] | **9** |
| 8 | FSA A165G | **1f** | **3f/4f** | 75 | 65 | 67:33[i] | **10**:**11**:**12**:**13**:**14** 15:11:15:22:37 |

[a] Values determined by HPLC analysis of preparative scale reactions.
[b] Isolated yield of aldol adduct.
[c] Diastereomer ratio determined by NMR.
[d] Aldol adduct isolated as cyclic hemiaminal in equilibrium with its open form.
[e] er not determined; optical rotation: FSA qm $[\alpha]_D^{20} = +8.5$ ($c = 2.1$ in MeOH); DERA$_{Tma}$ $[\alpha]_D^{20} = +4.6$ ($c = 0.69$ in MeOH).
[f] er determined by chiral HPLC, referenced to a racemic sample prepared by chemical synthesis (see SI).
[g] Inferred from the reductive amination products **6**:**7**.
[h] No other diastereomers were detected by NMR analysis.
[i] dr of the aldol adduct determined by NMR analysis. FSA qm = FSA L107Y/A129G/R134S/A165G/S166G.
Nu: Nucleophile. Green asterisk indicates the stereogenic center formed after reductive amination.

tween **1c** and **2a** using FSA D6E produced two regioisomeric adducts, one was arising from the attack of the α-methylene carbon of **1c** (i.e., C3 attack) and the other one from the attack of the α-methyl carbon (i.e., C1 attack), with a C1:C3 ratio of 13:87. Chromatographic separation of the product isomers from the reaction mixture proved difficult and only allowed enrichment, furnishing a major fraction (48%) containing a **3c′/4c′** diasteromer mixture in a 86:14 ratio, originating exclusively from C3 attack (Table 1, entry 5) as inferred from the NMR analysis of the derived N-heterocycles **6** and **7**, and a second fraction (1%) consisting of a mixture of aldol regioisomers from C1:C3 attack in a 56:43 molar ratio, which could not be further resolved (Scheme 1). On the other hand, D6T and A165G FSA variants were identified as the best catalysts for the addition of **1c** to **2b** (Figure 2). Similar as above, the preparative

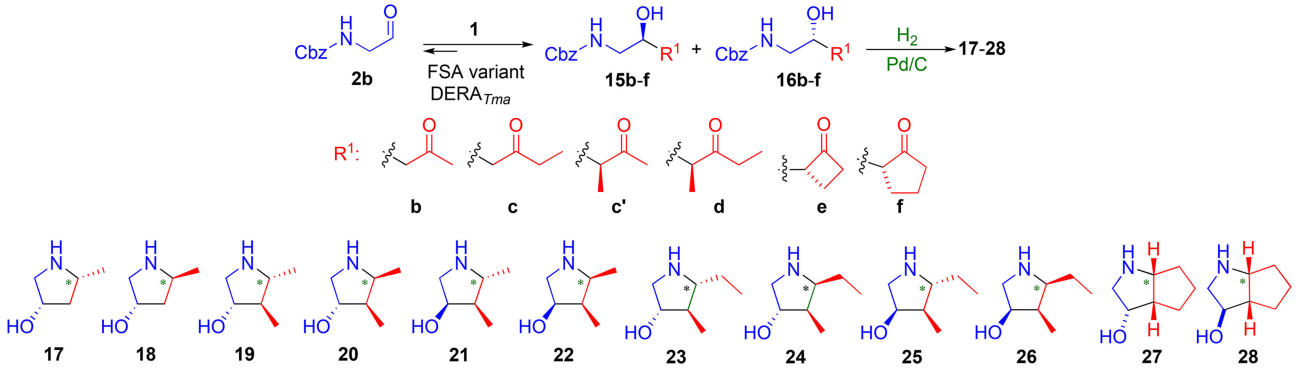



**Scheme 1.** Product distribution in the aldol addition of **1c** to **2a** and **2b** catalyzed by FSA D6E and A165G variants.

reaction using FSA A165G furnished a C1:C3 regioisomeric mixture in 13:87 ratio, which upon chromatographic separation yielded a major fraction (28%)

**Table 2.** Preparative scale FSA- and DERA-catalyzed aldol additions of **1b–f** to *N*-Cbz-2-aminoethanal (**2b**).




| Entry | Biocatalyst | Nu | Aldol adduct | Aldol adduct formed,[a] % | Isolated yield,[b] % | Aldol addition er or dr,% | Reductive amination products,[c] dr |
|---|---|---|---|---|---|---|---|
| 1 | FSA A165G | **1b** | **15b** | 95 | 35 | 99:1[d] | **17**:**18** 70:30 |
| 2 | DERA$_{Tma}$ | **1b** | **15b** | 85 | 30 | 95:5[d] | – |
| 3 | FSA A165G | **1c** | **15c′**/**16c′** | 60 | 28 | 88:12 | **19**:**20**:**21**:**22** 80:8:8:4 |
| 4 | FSA D6T | **1d** | **15d**:**16d** | 47 | 30 | 87:13 | **23**:**24**:**25**:**26** 79:8:5:8 |
| 5 | FSA A165G | **1e** | **15e** | 87 | 37 | >95:5[e] | –[g] |
| 6 | FSA A165G | **1f** | **15f**:**16f** | 50 | 38 | 50:50[e,f] | **27**:**28** 33:67 |

[a] Values obtained at preparative scale and determined by HPLC.
[b] Isolated yield of aldol adduct.
[c] dr: diastereomeric ratio determined by NMR.
[d] er determined by chiral HPLC, a racemic sample was obtained by non-stereoselective chemical methods (see SI).
[e] dr of the aldol adduct determined by NMR.
[f] The 50:50 diastereomeric mixture obtained in the aldol addition was purified by column chromatography. Two fractions were obtained: one of pure **15f** and another one of a **15f**:**16f** 33:67 mixture in order to characterize both N-heterocycles. The reductive amination was carried out on the **15f**:**16f** 33:67 mixture.
[g] The reductive amination produced a complex mixture of products, which could not be assigned to any structure.
Nu: Nucleophile. Green asterisk indicates the stereogenic center formed after reductive amination.

containing a 88:12 mixture of diastereomers **15c′**/**16c′** arising from C3 attack (Table 2, entry 3), and a second fraction (2.8%) as a 68:32 mixture of C1:C3 aldol regioisomers (Scheme 1). This is in contrast to results observed for the aldol addition of **1c** to L-G3P using FSA D6H as catalyst, where exclusively C1 attack was observed.[6a]

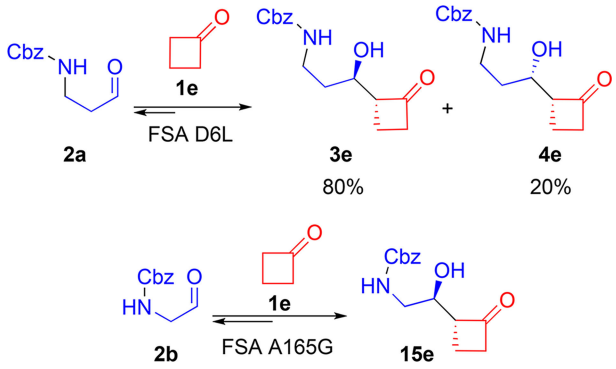
Consistent with observations for butanone addition to L-G3P, i.e. *si*-face attack of the FSA-nucleophile enamine complex to the C=O *si*-face of the electrophile, the major diastereomers **3c′**/**15c′** obtained with electrophiles **2a** and **2b** correspond to the expected D-*threo* (i.e. *syn*) configuration.[6a] The minor diastereomers **4c′**/**16c′** arise from an inverted orientation of the C=O electrophile exposing its *re*-face to the FSA–Nu complex.[4d,12b] Intramolecular reductive amination of the **3c′**/**4c′** mixture gave the corresponding N-heterocycles **6** and **7** stereoselectively as directed by the relative orientation of the C4 hydroxyl group (Table 1, entry 5).[13] From the **15c′**/**16c′** mixture four *N*-pyrrolidine heterocycles **19–22** were generated as a consequence of the lack of stereoselectivity in the reductive amination step with each of the diastereoisomers, as typical for imine reductions in five-membered ring systems (Table 2 entry 3).

3-Pentanone (**1d**) was also tolerated as a nucleophilic substrate by different FSA variants, with D6T and D6E being the most efficient catalysts (Figure 2). The D6E catalyzed addition of **1d** to **2a** furnished only the *syn* diastereomer **3d** (Table 1, entry 6), while FSA D6T catalyzed addition to **2b** yielded a *syn*:*anti* mixture of **15d**/**16d** in a 87:13 ratio (Table 2, entry 4). Considering the stereochemical outcome of FSA in previous reactions, **3d** has (4*S*,5*R*)-configuration, **15d** should be (4*S*,5*S*), and **16d** (4*R*,5*S*). Reductive amination of **3d** furnished the *N*-heterocycle **8** with good diastereoselectivity, while from **15d**/**16d** four diastereoisomers **23–26** were identified, among which the

major **23** (79%) and the minor **26** (8%) showed the C2 configuration predicted for Pd catalysis.[13–14]

Cyclobutanone (**1e**) and cyclopentanone (**1f**) were also converted in the presence of **2a** and **2b** electrophiles. FSA variants D6L, D6Q and, particularly, A165G were the most efficient (Figure 2). As reported previously,[6a,b] the cyclic ketones **1e** and **1f** imposed an *E*-configured FSA K85-enamine nucleophile complex, contrary to the *Z*-configuration invariably observed with acyclic nucleophiles such as hydroxyethanal, hydroxyacetone or dihydroxyacetone, and other aliphatic ketone nucleophiles.[4d,6c,15] The structural and mechanistic features invariably impose an attack of the nucleophile from its *si*-face to the electrophile, yielding the (*R*)-configuration at C-α next to the carbonyl group (Scheme 2).[6b,c] The addition of **1e** to **2b** (Table 2, entry 5), catalyzed by FSA A165G, furnished only diastereomer **15e** indicating a preferential attack at the *si*-face of the electrophilic carbonyl. On the contrary, the addition of **1e** to **2a** was less selective, furnishing an *anti*:*syn*-mixture of diastereomers **3e**/**4e** in a 80:20 ratio, which indicates an unexpectedly high 20% chance of unconventional attack at the *re*-face of the electrophilic carbonyl group. Nevertheless, upon reductive amination of this mixture, only product **9** was obtained from **3e** but no *N*-bicyclic product corresponding to **4e** was detectable by NMR analysis, possibly due to strain imposed upon cyclization (Table 1, entry 7) (Scheme 2).



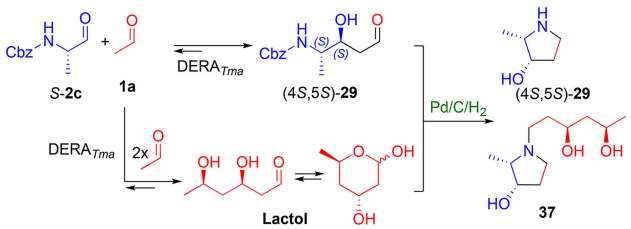

**Scheme 2.** Product distribution in the aldol addition of **1e** to **2a** and **2b** catalyzed by FSA D6E and A165G variants.

Additions of **1f** to **2a**–**b** gave mixtures of *anti*:*syn* adducts with dr of 66:34 for **3f**/**4f** and 50:50 for **15f**/**16f**, respectively (Table 1 entry 8 and Table 2 entry 6). The occurrence of diastereoisomers observed for some of the aldol additions studied is plausibly explained by an imperfect orientation of the electrophilic C=O relative to the enzyme-enamine complex, thereby generating epimers at C-β to the carbonyl group of the nucleophiles. However, it is even more conceivable that a spontaneous isomerization of the tertiary stereocenter at C-α next to the carbonyl in the cyclic aldol adduct may occur under the reaction conditions (aq buffer at pH 7.5–8). Isomerizations of this kind, which effectively lead to mirror image compounds of the imperfect aldol diastereomers, have been reported previously for aldol additions of cyclopentanone to L-G3P and upon addition of cyclopentanone enolate to Boc-*N*-phenylalaninal in aqueous buffered solutions.[6a,16]

Reductive amination of **15f**/**16f** furnished the N-bicyclic derivatives **27**/**28** (Table 2, entry 6), respectively, with identical configuration of the new stereogenic center formed. Reductive amination of mixture **3f**/**4f** was not stereoselective and yielded a mixture of four diastereoisomers **10**–**13** (Table 1, entry 8). In addition, an unexpected dehydroxylated product **14** was also detected by NMR analysis as the major component, which was probably formed during the Cbz removal/reductive amination step in the presence of the Pd catalyst by formal $H_2O$ elimination/hydrogenation.

Addition of ethanal (**1a**) and propanone (**1b**) to individual enantiomers of (*S*)-**2c** and (*R*)-**2c** by $DERA_{Tma}$ catalysis furnished the corresponding aldol adducts in 20%–79% isolated yields (Table 3). The lower isolated yields obtained for the addition products from **1a** (1 eq) were mainly caused by difficulties in separating (3*R*,5*R*)-dihydroxyhexanal (present in its lactol form), which simultaneously formed by enzymatic self-aldolization of **1a** as a side reaction. When a mild excess of **1a** was employed (1.25 eq) to achieve complete conversion of **2c** to **29**, no subsequent addition was observed (tandem addition to **2c**) but the formation of the lactol increased, thus complicating its removal.

Consequently, during the hydrogenation procedure the imino moiety of the formed **29** underwent a subsequent intermolecular reductive amination with the aldehyde group of the remaining lactol, yielding the corresponding conjugate **37** (Scheme 3).

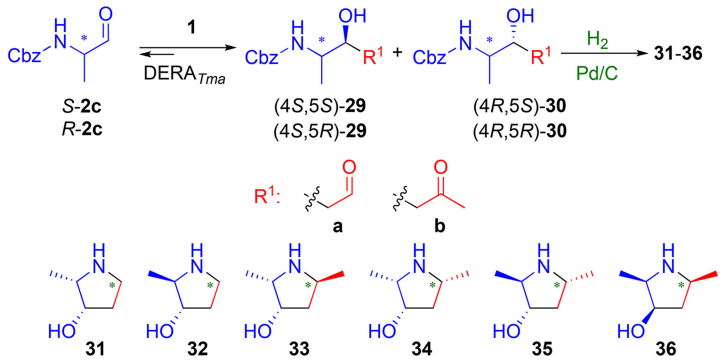



**Scheme 3.** $DERA_{Tma}$-catalyzed aldol addition of **1a** to *S*-**2c** with an excess of **1a** leading to the formation of 3*R*,5*R*-dihydroxyhexanal and formation of **37** during reductive amination.

The chiral center of (*S*)-**2c** and (*R*)-**2c** was used as reference to assign the absolute stereochemistry of the

**Table 3.** Preparative scale DERA$_{Tma}$-catalyzed aldol addition of **1a** and **1b** to (*S*)- and (*R*)-*N*-Cbz-2-alaninal (*S*-**2c** and *R*-**2c**).




| Nu | E | Aldol adduct formed,[a] % | Yield,[b]% | Aldol adduct | dr,[c] % | Reductive amination product,[d] dr |
|---|---|---|---|---|---|---|
| **1a** | *S*-**2c** | 85[e] | 34[f] | (4*S*,5*S*)-**29** | >95:5 | **31** |
| **1a** | *R*-**2c** | 80[e] | 20[f] | (4*R*,5*S*)-**29** | >95:5 | **32** |
| **1b** | *S*-**2c** | 81 | 79 | (4*S*,5*S*)-**29** | >95:5 | **33**:**34** 56:44 |
| **1b** | *R*-**2c** | 91 | 78 | (4*S*,5*R*)-**29**:(4*R*,5*R*)-**30** | 77:23 | **35**:**36** 77:23 |

[a] Determined by HPLC.
[b] Isolated yield of aldol adduct.
[c] dr: inferred from the reductive amination products.
[d] dr: diasteromeric ratio assessed by NMR.
[e] [**1a**] = [E] = 100 mM.
[f] Several purification column chromatography runs had to be performed to remove the trimerization product from enzymatic double addition of **1a**.
Nu = Nucleophile. E = Electrophile. Green asterisk indicates the stereogenic center formed after reductive amination.

pyrrolidine derivatives **31**–**36** and infer the stereochemical outcome of the biocatalytic aldol addition and reductive amination. The aldol addition of **1a** to (*S*)-**2c** and (*R*)-**2c** was fully stereoselective furnishing (4*S*,5*S*)-**29** and (4*S*,5*S*)-**29**, respectively, consistent with previous reports.[17] A similar situation was found upon addition of **1b** to (*S*)-**2c**. On the other hand, (*R*)-**2c** gave a mixture of diasteroisomers (4*S*,5*R*)-**29**:(4*R*,5*R*)-**30** in a 77:23 dr, indicative of a loss of enantiofacial selectivity during the electrophile approach. Reductive amination of (4*S*,5*S*)-**29** gave a mixture of the N-heterocycles **33**/**34**, epimeric at C2, while from the mixture of (4*S*,5*R*)-**29**:(4*R*,5*R*)-**30** the pyrolidines **35**/**36** resulted, in which the new configuration was controlled by the hydroxyl group at C4, in agreement with previous reports.[12c,13]

## Conclusions

FSA variants and DERA$_{Tma}$ are able to catalyze aldol additions of ethanal (**1a**) and simple linear (**1b**–**d**) and cyclic (**1e**–**f**) aliphatic ketones to *N*-protected aminoaldehydes (**2a**–**c**), affording diversely functionalized N-heterocycles. FSA variants with different residues at position D6 are active for various nucleophile-electrophile combinations, though the optimal catalyst depends on both the nucleophile and electrophile structure. Such unprecedented novel activity was also hidden in the previously reported variant FSA A165G, which was again particularly active towards *N*-Cbz-2-aminoethanal (**2b**). Hence, the effectivity of a FSA catalyst toward a nucleophile strongly depends upon the quality of the electrophilic component. Aldol additions of ethanal (**1a**) to **2a** and propanone (**1b**) to **2a** and **2b** were fully stereoselective, yielding (*R*)-configured aldol adducts identical to those obtained using DERA$_{Tma}$ catalysis. Butanone gave preferentially an addition at the methylene carbon with a C1:C3 regioisomer ratio of 17:83 and an 86:14 *syn*:*anti* dr for the C3-regiosomer. Cyclobutanone and cyclopentanone imposed an *E* configured FSA K85-enamine nucleophile complex, therefore under FSA catalysis the *anti* adducts were mainly formed. This was unequivocally established with cyclobutanone, though inconclusive for cyclopentanone presumably because of spontaneous isomerization at the tertiary C-α stereocenter to the carbonyl in aqueous media at pH 7.8. DERA$_{Tma}$ catalyzed the addition of ethanal and propanone to both enantiomers of *N*-Cbz-alaninal, reactions unattainable by any of the FSA variants screened. The additions were fully diastereoselective except for addition of propanone to *S*-**2c**. Thus, FSA

and DERA catalysts seem to be highly complementary tools, where FSA variants offer greater flexibility with structural variations in the nucleophile reactants, and DERA is superior for the conversion of sterically more demanding electrophiles.

Stereochemical outcome of reductive amination reactions followed a general pattern identical to that observed previously in the synthesis of other iminosugars.[4c,13] The straightforward two-step synthetic approach comprising biocatalytic carboligation and chemical reductive amination described in this work foresees new avenues in the synthesis of N-heterocycles, allowing the preparation of new derivatives that otherwise are difficult to prepare by the use of existing chemical methodologies.

## Experimental Section

**Materials**. Synthetic oligonucleotides were purchased from Eurofins MWG Operon. Phosphoglucose isomerase from baker's yeast (*S. cerevisiae*), glucose-6-phosphate dehydrogenase from baker's yeast (*S. cerevisiae*), D,L-glyceraldehyde 3-phosphate (G3P), nicotinamide adenine dinucleotide phosphate ($NADP^+$), antibiotics, acrylamide-bisacrylamide, buffer components, propanolamine, aminoethanal dimethyl acetal, *S*-2-amino-1-propanol, *R*-2-amino-1-propanol, *N*-(benzyloxycarbonyloxy)succinimide, ethanal, acetone, butanone, 3-pentanone, cyclobutanone, cyclopentanone, cyclohexanone were purchased from Sigma-Aldrich. Culture media components for bacteria were from Pronadisa. Milli-Q grade water was used for analytical and preparative HPLC, buffer preparations and other assay solutions were obtained from an Arium™ Pro Ultrapure Water Purification System (Sartorius Stedim Biotech). All the other solvents used were of analytical grade. 2-Deoxy-D-ribose-5-phosphate aldolase from *Thermotoga maritima* ($DERA_{Tma}$) as cell free crude extract was a generous gift from Prozomix Ltd (activity 0.056 U $mg^{-1}$ lyophilized powder); 1 U is defined as the amount of protein that catalyzes the cleavage of 1 μmol of 2-deoxy-D-ribose-5-phosphate (Dr5P) per minute at [Dr5P] = 0.7 μM, 50 mM triethanolamine buffer pH 7.5 and 30 °C.

***FSA catalysts***. The single FSA variants A165G, FSA L107A, FSA L163A, D6A, D6L, D6N, D6Q, D6S, D6T, D6E, D6H, the double variants FSA D6H/A165G, FSA D6H/L107A, FSA D6H/L163A, FSA D6E/A165G, FSA D6E/L107A, FSA D6E/L163A, FSA D6L/A165G, FSA D6L/L107A, FSA D6L/L163A were obtained using the procedure described.[8] The FSA multiple variants FSA L107Y/A129G/A165G/S166G, L107Y/A129G/R134S/A165G/S166G, FSA L107Y/A129G/R134E/A165G/S166G, FSA L107Y/A129G/R134H/A165G/S166G, FSA L107Y/A129G/R134P/A165G/S166G and FSA L107Y/A129G/R134V/A165G/S166G were obtained following the mutagenesis and protein expression and purification procedures described in our previous publications.[4c] Activity values are shown in Table S1.[8]

**Methods**. ***HPLC analyses***. HPLC analyses were performed on an X-Bridge™ C18, 5 μm, 4.6 × 250 mm column from Waters (Milford, USA). Samples (30 μL) were injected and eluted with the following conditions: solvent system (A) aqueous trifluoroacetic acid (TFA) (0.1% (v/v) and (B): TFA (0.095% (v/v)) in $CH_3CN/H_2O$ (4:1), gradient elution from 10–100% B in 30 min, flow rate 1 mL·$min^{-1}$, detection at 215 nm, column temperature 30 °C. The amount of aldol adduct product was quantified from the peak areas using and external standard methodology. Samples were withdrawn (50 μL) from the reaction medium, centrifuged, diluted with methanol (450 μL) and analyzed with HPLC.

***NMR analysis***. High field $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) analyses were carried out using an AVANCE 500 BRUKER spectrometer equipped with a high-sensitive CryoProbe for [D2]$H_2O$ and [D4]MeOH solutions. Full characterization of the described compounds was performed using typical gradient-enhanced 2D experiments: COSY, NOESY, HSQC and HMBC, recorded under routine conditions. When possible, NOE data were obtained from selective 1D NOESY versions using a single pulsed-field-gradient echo as a selective excitation method and a mixing time of 500 ms. Routine, $^1H$ (400–500 MHz) and $^{13}C$ (101 MHz) NMR spectra of compounds were recorded with Varian Mercury-400 and Varian Anova-500 spectrometers.

***Determination of the specific optical rotations*** ($[\alpha]_D^{25}$). Specific optical rotations were measured with a Perkin Elmer Model 341 (Überlingen, Germany) polarimeter (Na lamp, 589 nm). The products (5–20 mg) were dissolved in methanol (1 mL) and the samples were measured at 25 °C with a 0.1 dm cell.

***Chiral HPLC analyses***. Chiral HPLC analyses were performed on a CHIRALPACK® IC, 5 μm, 4.6 × 250 mm column from Daicel (Illkirch, France). Samples (10 μL) were injected and eluted with hexane:isopropanol (85:15 (v/v)) for 40 min, flow rate 1 mL·$min^{-1}$, detection at 209 nm.

***Preparation of N-Cbz-aminoaldehydes***. The *N*-Cbz-aminoaldehydes were synthesized from the corresponding aminoalcohols using the procedures described in previous publications.[13] The amino group of the aminoalcohols was first protected with benzyloxycarbonyl (Cbz) group, followed by oxidation of the alcohol function using 2-iodoxybenzoic acid (IBX) with a yield between 90–95%. In most instances, a simple workup followed by a rough crystallization or precipitation provided the *N*-Cbz-aminoaldehydes in a form pure enough to be used in the enzymatic aldol reactions.

### Screening of the Enzymatic Reactions

*General procedure for aldol addition of ethanal and ketones to N-Cbz-aminoaldehydes.*

Reactions were conducted in 96 well plates of 1 mL of capacity. Total reaction volume was 300 μL. *N*-Cbz-aminoaldehydes **2a–c** (80 mM final concentration) was dissolved in propanone (**1b**) (45 μL). For the other ketones (**1c–g**), **2a–c** (80 mM final concentration) was dissolved in the corresponding ketone (15 μL). For ethanal (**1a**): to **2a–c** (80 mM final concentration), **1a** (30 μL of a 1 M stock solution) was added. For all of them: 50 mM triethanolamine buffer pH 8.0 (15 μL of a 1 M stock solution) was then added. The reaction was started by adding the lyophilized FSA variant prepara-

tion (3 mg·mL$^{-1}$, 1 mg dissolved in 240 μL, 270 μL or 255 μL of plain water) or DERA$_{Tma}$ (1.5 mg, 0.084 U dissolved in 240 μL, 270 μL or 255 μL of plain water). Reaction mixtures were shaken (1500 rpm) at 25 °C. Reaction monitoring by HPLC: samples (50 μL) were diluted in MeOH (450 μL) and after centrifugation were injected to the HPLC system.

### Scale up of Aldol Addition of Ketones (1b–g) to *N*-Cbz-aminoaldehydes using FSA Catalysis

*General procedure*: The reaction was conducted in a 50 mL screw capped conical-bottom polypropylene tubes (Falcon tubes). To an FSA catalyst solution (3 mg mL$^{-1}$) in plain water (16 mL or 18 mL), 1 M triethanolamine buffer pH 8 (1 mL) was added. To this solution, *N*-Cbz-aminoaldehydes **2a–b** (80 mM) dissolved in the ketones (3 mL of **1b** or 1 mL of **1c–g**) was added. Total reaction volume was 20 mL. Reaction mixtures were shaken (1000 rpm) at 25 °C for 24 h. After that, the reaction was stopped with MeOH (30 mL) to precipitate the enzyme. Then, the mixture was filtered through Celite®, the MeOH was evaporated and the aqueous residue was lyophilized, purified characterized and submitted to reductive amination.

### Intramolecular Reductive Amination

General procedure: The aldol adduct (3 mM final concentration) was dissolved in $H_2O$:MeOH 1:1 (70 mL), then Pd/C (60 mg) was added. The mixture was shaken under $H_2$ (2.5 atm) overnight at room temperature. After that, the reaction was filtered and the solvent was evaporated. Products were characterized without any further purification.

### Product Description

**(*R*)-*N*-Cbz-6-amino-4-hydroxyhexan-2-one** (**3b**): This compound was obtained following the procedure described above. *N*-Cbz-3-aminopropanal (**2a**) (331.2 mg, 1.6 mmol) and FSA D6N were used. After 24 h an additional amount of FSA D6N (3 mg ml$^{-1}$) was added and the reaction was shaken (1000 rpm) at 25 °C for 24 h. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 266.2 mg (85%) of **3b**. $[\alpha]_D^{20} = +10.74$ ($c = 2.1$ in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R = 16$ min; Chiral HPCL: $t_R = 25$ min (racemic sample: $t_R(R) = 25$ min, $t_R(S) = 30$ min); $^1$H NMR (400 MHz, $CD_3OD$) δ 7.45–7.18 (m, 5H), 5.07 (s, 2H), 4.19–3.99 (m, 1H), 3.23 (t, $J = 7.0$ Hz, 2H), 2.59 (d, $J = 6.4$ Hz, 2H), 2.15 (s, 3H), 1.76–1.47 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 210.4, 159.0, 138.4, 129.4, 128.9, 128.8, 67.4, 66.5, 51.7, 38.6, 38.2, 30.6. The intramolecular reductive amination reaction produced: **(2*R*,4*R*)-2-methylpiperidin-4-ol** (**5**): $^1$H NMR (400 MHz, $CD_3OD$) δ 3.77 (tt, $J = 11.0$, 4.5 Hz, 1H), 3.41–3.33 (m, 1H), 3.25–3.14 (m, 1H), 2.97 (td, $J = 13.4$, 3.0 Hz, 1H), 2.09 (tdd, $J = 13.3$, 4.7, 2.5 Hz, 2H), 1.55 (tdd, $J = 13.7$, 11.0, 4.5 Hz, 1H), 1.39 (dd, $J = 13.1$, 11.3 Hz, 1H), 1.32 (d, $J = 6.6$ Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 65.2, 51.4, 42.3, 39.5, 30.9, 18.0. ESI-TOF: calculated for $[M+H]^+$ $C_6H_{14}NO$: 116.1075, found 116.1077.

**(*S*)-*N*-Cbz-5-amino-4-hydroxypentan-2-one** (**15b**): This compound was obtained following the procedure described above. *N*-Cbz-glycinal (**2b**) (310.4 mg, 1.6 mmol) and FSA A165G were used. The product was purified by silica gel column chromatography and eluted with a step gradient of Hexane:AcOEt from 1:0 to 3:7, yielding 114.8 mg (29%) of **15b**. $[\alpha]_D^{20} = +3.4$ ($c = 1.9$ in MeOH); HPLC:10 to 100% of B in 30 min, $t_R = 15$ min; Chiral HPCL: $t_R = 31$ min (racemic sample: $t_R(R) = 31$ min, $t_R(S) = 28$ min); $^1$H NMR (400 MHz, $CD_3OD$) δ 7.34 (d, $J = 6.0$ Hz, 5H), 5.08 (s, 2H), 4.18–4.04 (m, 1H), 3.16 (dd, $J = 5.8$, 1.4 Hz, 2H), 2.64–2.52 (m, 2H), 2.15 (s, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 209.9, 159.1, 138.3, 129.4, 129.0, 128.8, 126.3, 68.0, 67.5, 48.4, 47.4, 30.6. The intramolecular reductive amination reaction produced two diastereoisomers in a 70:30 ratio. Major: **(3*S*,5*R*)-5-methylpyrrolidin-3-ol** (**17**). $^1$H NMR (400 MHz, $CD_3OD$) δ 4.41–4.31 (m, 1H), 3.22 (p, $J = 7.0$, 7.0, 6.9, 6.9 Hz, 1H), 2.92 (dd, $J = 12.2$, 1.5 Hz, 1H), 2.87 (dd, $J = 12.2$, 4.9 Hz, 1H), 2.27 (ddd, $J = 13.5$, 7.8, 6.5 Hz, 1H), 1.32 (dddd, $J = 13.6$, 7.8, 3.7, 1.2 Hz, 1H), 1.27 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 71.8, 54.5, 54.0, 42.1, 19.3. Minor: **(3*S*,5*S*)-5-methylpyrrolidin-3-ol** (**18**). $^1$H NMR (400 MHz, $CD_3OD$) δ 4.41–4.29 (m, 1H), 3.45 (dp, $J = 10.0$, 6.5, 6.5, 6.4, 6.4 Hz, 1H), 3.27 (dd, $J = 12.1$, 5.2 Hz, 1H), 2.83–2.74 (m, 1H), 1.92 (ddt, $J = 13.4$, 6.2, 1.4, 1.4 Hz, 1H), 1.48 (ddd, $J = 13.4$, 10.0, 5.8 Hz, 1H), 1.20 (d, $J = 6.5$ Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 71.5, 54.0, 52.9, 42.8, 18.3. ESI-TOF: calculated for $[M+H]^+$ $C_5H_{12}NO$: 102.0919, found 102.0210.

Aldol addition of **1c** to **2a. Regioisomer I** (arising from the attack of the methylene carbon, C3-attack): ***N*-Cbz-6-Amino-4-hydroxy-3-methylhexan-2-one** (mixture of diasteromers **3c′**:**4c′**). This mixture was obtained following the procedure described above. *N*-Cbz-3-aminopropanal (**2a**) (1.6 mmol), butanone (**1c**) and FSA D6E were used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 213 mg (48%) of product. $[\alpha]_D^{20} = +33.5$ ($c = 2.1$ in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R = 18$ min. Only one diasteromer was detected by NMR. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.50–7.19 (m, 5H), 5.05 (s, 2H), 3.90 (dt, $J = 9.2$, 4.5 Hz, 1H), 3.26–3.15 (m, 2H), 2.67–2.53 (m, 1H), 2.14 (s, 3H), 1.57 (tdd, $J = 15.2$, 7.7, 5.1 Hz, 2H), 1.06 (d, $J = 7.0$ Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 214.0, 158.2, 137.1, 128.7, 128.3, 128.1, 69.0, 65.9, 52.3, 37.3, 34.7, 28.2, 9.8. **Regioisomer II** (C1 attack): **(*R*)-*N*-Cbz-7-amino-5-hydroxy-heptan-3-one**: The product was purified by preparative HPLC and eluted with a step gradient of $H_2O$:$CH_3CN$ from 1:0 to 1:1, flow rate 1 mL min$^{-1}$ and detection at 215 nm, yielding 25 mg of a mixture 44:56 of regioisomers I:II. $[\alpha]_D^{20} = +18.5$ ($c = 0.9$ in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R = 19$ min; **Regioisomer II**: $^1$H NMR (400 MHz, $CD_3OD$) δ 7.59–7.17 (m, 5H), 5.07 (s, 2H), 4.09 (dt, $J = 8.2$, 4.0 Hz, 1H), 3.24 (q, $J = 7.0$, 6.4 Hz, 2H), 2.59–2.54 (m, 2H), 2.49 (qd, $J = 7.3$, 1.8 Hz, 2H), 1.76–1.60 (m, 2H), 1.01 (t, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 212.7, 159.0, 138.5, 129.2, 128.9, 128.8, 66.2, 65.3, 49.1, 36.8, 36.1, 34.6, 6.43. The intramolecular reductive amination reaction of **regioisomer I** produced two diastereoisomers in a 87:13 rate. Major: **(2*R*,3*R*,4*R*)-2,3-dimethylpiperidin-4-ol** (**6**): $^1$H NMR (400 MHz, $CD_3OD$) δ 3.36–3.22 (m, 2H), 2.96 (td, $J = 13.3$,

3.1 Hz, 1H), 2.81 (dq, $J$=10.7, 6.5 Hz, 1H), 2.05 (ddt, $J$=13.7, 5.2, 2.8 Hz, 1H), 1.71–1.56 (m, 1H), 1.44–1.32 (m, 1H), 1.30 (d, $J$=6.5 Hz, 3H), 1.06 (d, $J$=6.5 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 70.6, 56.3, 43.6, 42.3, 31.6, 16.3, 12.4. Minor. **(2*S*,3*R*,4*S*)-2,3-dimethylpiperidin-4-ol** (**7**): $^1$H NMR (500 MHz, $CD_3OD$) δ 3.83 (dt, $J$=10.2, 4.4 Hz, 1H), 3.32–3.28 (m, 2H), 3.24 (d, $J$=12.0 Hz, 1H), 2.91–2.85 (m, 1H), 2.05–2.02 (m, 1H), 1.77–1.66 (m, 2H), 1.25 (d, $J$=6.8 Hz, 3H), 0.94 (d, $J$=7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 68.3, 53.8, 42.7, 37.7, 26.6, 15.1, 4.8. ESI-TOF: calculated for $[M+H]^+$ $C_7H_{16}NO$: 130.1231, found 130.1236.

Aldol addition of **1c** to **2b. Regioisomer I** (arising from the attack of the methylene carbon, C3-attack): **(3*S*,4*S*)-*N*-Cbz-5-amino-4-hydroxy-3-methylpentan-2-one** (mixture of diasteromers **15c′**:**16c′**). This mixture was obtained following the procedure described above. *N*-Cbz-glycinal (**2b**) (1.6 mmol), butanone (**1c**) and FSA A165G were used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 119.2 mg (28%) of product. $[\alpha]_D^{20}$ = +13.0 ($c$ = 0.78 in MeOH); HPLC:10 to 100% of B in 30 min, $t_R$ = 17 min; Only one diasteromer was detected by NMR. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.38–7.25 (m, 5H), 5.08 (s, 2H), 3.99 (q, $J$=5.8 Hz, 1H), 3.16 (qd, $J$=13.9, 6.1 Hz, 2H), 2.65 (dd, $J$=7.3, 5.6 Hz, 1H), 2.16 (s, 3H), 1.10 (d, $J$=7.0 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 213.6, (C=O carbamate and *Cipso* not detected), 129.4, 129.0, 128.9, 71.6, 67.5, 51.2, 45.8, 29.0, 11.0. **Regioisomer II** (C1-attack): ***N*-Cbz-6-amino-5-hydroxyhexan-3-one**. The product was purified by preparative HPLC and eluted with a step gradient of $H_2O$:$CH_3CN$ from 100:0 to 50:50, flow rate 1 mL min$^{-1}$ and detection at 215 nm, yielding 12 mg of a 68:32 mixture of regioisomers I:II. $[\alpha]_D^{20}$ = +0.15 ($c$ = 0.8 in MeOH); HPLC:10 to 100% of B in 30 min, $t_R$ = 18 min; **Regioisomer II**: $^1$H NMR (400 MHz, $CD_3OD$) δ 7.45–7.09 (m, 5H), 5.05 (s, 2H), 4.09 (dt, $J$=11.6, 5.9 Hz, 1H), 3.17–3.10 (m, 2H), 2.54–2.50 (m, 2H), 2.46 (q, $J$=7.3 Hz, 2H), 0.98 (t, $J$=7.3 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ (C=O carbamate and carbonyl and C*ipso* not detected), 128.0, 127.6, 127.4, 66.6, 66.1, 46.3, 46.0, 36.0, 6.4. The intramolecular reductive amination reaction of **regioisomer I** produced four diasteroisomers in 80:8:8:4 ratio. Major (80%). **(3*S*,4*R*,5*R*)-4,5-dimethylpyrrolidin-3-ol** (**19**): $^1$H NMR (500 MHz, $CD_3OD$) δ 3.99 (td, $J$=6.6, 4.9 Hz, 1H), 3.40 (dd, $J$=12.1, 6.9 Hz, 1H), 3.14 (dq, $J$=9.7, 6.6 Hz, 1H), 3.06–2.99 (m, 1H), 1.79–1.69 (m, 1H), 1.39 (d, $J$=6.6 Hz, 3H), 1.11 (d, $J$=6.9 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 75.2, 60.4, 50.1, 47.5, 15.3, 13.3. Minor (8%) **(3*S*,4*R*,5*S*)-4,5-dimethylpyrrolidin-3-ol** (**20**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.15 (dt, $J$=4.5, 2.0 Hz, 1H), 3.98–3.90 (m, 1H), 3.53–3.46 (m, 1H), 3.11–3.06 (m, 1H), 2.26–2.19 (m, 1H), 1.31 (d, $J$=7.0 Hz, 3H), 0.92 (d, $J$=7.5 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 75.6, 56.7, 50.5, 44.1, 11.6, 9.9. Minor (8%) **(3*R*,4*R*,5*R*)-4,5-dimethylpyrrolidin-3-ol** (**21**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.24 (t, $J$=4.1 Hz, 1H), 3.43–3.40 (m, 1H), 3.38–3.33 (m, 1H), 3.02–2.97 (m, 1H), 1.88–1.77 (m, 1H), 1.35 (d, $J$=7.1 Hz, 3H), 1.05 (d, $J$=2.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 71.8, 58.6, 52.1, 45.2, 13.7, 8.8. Minor (4%) **(3*R*,4*R*,5*S*)-4,5-dimethylpyrrolidin-3-ol** (**22**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.30 (td, $J$=4.7, 2.6 Hz, 1H), 3.84–3.75 (m, 1H), 3.35–3.27 (m, 1H), 3.19–3.14 (m, 1H), 2.35–2.30 (m, 1H), 1.33 (d, $J$=10.8 Hz, 3H), 1.05 (d, $J$=2.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 71.8, 57.2, 51.5, 40.0, 13.8, 7.1. ESI-TOF: calculated for $[M+H]^+$ $C_6H_{14}NO$: 116.1075, found 116.1078.

**(4*S*,5*R*)-*N*-Cbz-7-amino-5-hydroxy-4-methylheptan-3-one** (**3d**). This compound was obtained following the procedure described above. *N*-Cbz-3-aminopropanal (**2a**) (1.6 mmol), 3-pentanone (**1d**) and FSA D6E were used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 164.1 mg (35%) of **3d**. $[\alpha]_D^{20}$ = +36.5 ($c$=2.3 in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R$=20 min; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.45–7.17 (m, 5H), 5.05 (s, 2H), 3.83 (ddd, $J$=9.4, 5.6, 3.7 Hz, 1H), 3.20 (hept, $J$=7.2 Hz, 2H), 2.62 (p, $J$=6.8 Hz, 1H), 2.52 (qd, $J$=7.2, 2.0 Hz, 2H), 1.67–1.39 (m, 2H), 1.08 (d, $J$=7.0 Hz, 3H), 0.99 (t, $J$=7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 215.2, 157.6, 137.0, 128.0, 127.5, 127.3, 69.4, 65.9, 51.5, 37.5, 34.7, 34.5, 10.4, 6.5. The intramolecular reductive amination reaction produced **(2*R*,3*R*,4*R*)-2-ethyl-3-methylpiperidin-4-ol** (**8**). $^1$H NMR (400 MHz, $CD_3OD$) δ 3.27–3.25 (m, 2H), 2.87 (td, $J$=13.1, 2.9 Hz, 1H), 2.56 (ddd, $J$=10.6, 7.4, 3.3 Hz, 1H), 2.02 (ddt, $J$=13.3, 5.1, 2.8 Hz, 1H), 1.85 (dqd, $J$=15.2, 7.6, 2.8 Hz, 1H), 1.66–1.49 (m, 2H), 1.38 (tq, $J$=10.2, 6.5 Hz, 1H), 1.05 (d, $J$=6.5 Hz, 3H), 1.00 (t, $J$=7.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 73.1, 62.9, 44.5, 43.3, 34.0, 25.3, 13.8, 9.3. ESI-TOF: calculated for $[M+H]^+$ $C_8H_{18}NO$: 144.1388, found 144.1385.

***N*-Cbz-6-amino-5-hydroxy-4-methyl-hexan-3-one** (mixture of diasteromers **15d** (4*S*,5*S*):**16d** (4*R*,5*S*). This mixture was obtained following the procedure described above. *N*-Cbz-glycinal (**2b**) (1.6 mmol), 3-pentanone and FSA D6T were used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 134.4 mg (30%) of product. $[\alpha]_D^{20}$ = +12.3 ($c$=1.26 in MeOH); HPLC:10 to 100% of B in 30 min, $t_R$=19 min. Only one diasteromer was detected by NMR. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.44–7.16 (m, 5H), 5.19–5.02 (m, 2H), 3.92 (q, $J$=5.6 Hz, 1H), 3.16–3.06 (m, 2H), 2.64 (dt, $J$=12.9, 6.8 Hz, 1H), 2.51 (q, $J$=7.2 Hz, 2H), 1.08 (d, $J$=7.0 Hz, 3H), 0.97 (t, $J$=7.2 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 214.6, 157.6, 136.9, 128.0, 127.5, 127.4, 70.5, 66.1, 49.0, 44.4, 34.3, 10.3, 6.5. The intramolecular reductive amination reaction produced four diasteoisomers in ≈79:8:5:8 rate. Major (79%): **(3*S*,4*R*,5*R*)-5-ethyl-4-methylpyrrolidin-3-ol** (**23**). $^1$H NMR (500 MHz, $CD_3OD$) δ 3.97 (q, $J$=5.3 Hz, 1H), 3.36–3.30 (m, 1H), 3.02 (dd, $J$=12.0, 4.5 Hz, 1H), 2.91 (tdd, $J$=8.7, 5.1, 1.5 Hz, 1H), 1.91–1.75 (m, 2H), 1.72–1.61 (m, 1H), 1.10 (d, $J$=7.0 Hz, 3H), 1.03 (t, $J$=7.5 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 75.7, 66.7, 50.3, 46.3, 25.1, 14.5, 9.9. Minor (8%): **(3*S*,4*R*,5*S*)-5-ethyl-4-methylpyrrolidin-3-ol** (**24**). $^1$H NMR (400 MHz, $CD_3OD$) $^1$H NMR (500 MHz, $CD_3OD$) δ 4.24 (t, $J$=4.1 Hz, 1H), 3.41–3.34 (m, 1H), 3.17–3.12 (m, 2H), 1.89–1.82 (m, 2H), 1.54–1.43 (m, 1H), 1.10–1.09 (m, 3H), 1.02–0.98 (m, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 72.0, 64.8, 52.0, 43.6, 23.6, 15.5, 10.1. Minor (5%): **(3*R*,4*R*,5*S*)-5-ethyl-4-methylpyrrolidin-3-ol** (**25**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.14 (d, $J$=4.5 Hz, 1H), 3.73–3.66 (m, 3H), 3.49–3.44 (m, 2H), 3.11–3.07 (m, 1H), 2.32–2.24 (m, 1H), 1.71–1.65 (m,

2H), 1.04 (d, $J$=7.5 Hz, 3H), 0.88 (d, $J$=7.5 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 75.7, 62.7, 50.6, 43.0, 22.1, 9.9, 9.5. Minor (8%): **(*3R,4R,5R*)-5-ethyl-4-methylpyrrolidin-3-ol** (**26**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.35 (q, $J$=5.7 Hz, 1H), 3.47–3.43 (m, 1H), 3.27–3.23 (m, 1H), 3.08–3.04 (m, 1H), 2.38 (td, $J$=7.4, 5.0 Hz, 1H), 1.74–1.66 (m, 1H), 1.06 (d, $J$=5.1 Hz, 19H), 0.97 (d, $J$=5.2 Hz, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 71.3, 63.1, 49.7, 39.2, 25.9, 9.9, 6.7. ESI-TOF: calculated for $[M+H]^+$ $C_7H_{16}NO$: 130.1231, found 130.1234.

**(*R*)-2-((*R*)-*N*-Cbz-3-amino-1-hydroxypropyl)cyclobutan-1-one** (**3e**) **and (*R*)-2-((*S*)-*N*-Cbz-3-amino-1-hydroxypropyl) cyclobutan-1-one** (**4e**): These compounds were obtained following the procedure described above as a mixture of **3e**:**4e** in a 80:20 ratio. *N*-Cbz-3-aminopropanal (**2a**) (1.6 mmol), cyclobutanone (**1e**) and FSA D6L were used. The mixture was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 201.8 mg (46%) of the mixture. $[\alpha]_D^{20}$ = + 36.49 ($c$=1.82 in MeOH); HPLC:10 to 100% of B in 30 min, $t_R$=17 min. Major **3e** (80%): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.41–7.25 (m, 5H), 5.06 (s, 2H), 3.73 (dt, $J$=9.0, 4.4 Hz, 1H), 3.47–3.35 (m, 1H), 3.23 (t, $J$=6.9 Hz, 2H), 3.03–2.78 (m, 2H), 2.09 (qd, $J$=10.3, 5.9 Hz, 1H), 1.96 (td, $J$=10.6, 5.2 Hz, 1H), 1.86–1.63 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 212.3, 159.0, 138.4, 129.4, 129.0, 128.8, 69.1, 67.4, 66.9, 45.7, 38.7, 36.1, 14.4. Minor **4e** (20%): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.41–7.25 (m, 5H), 5.06 (s, 2H), 3.94–3.87 (m, 1H), 3.21–3.10 (m, 1H), 3.22–3.15 (m, 2H), 3.01–2.80 (m, 2H), 2.14–1.90 (m, 2H), 1.71–1.52 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 212.3, 159.0, 138.4, 129.4, 129.0, 128.8, 67.2, 67.1, 66.8, 45.9, 38.5, 36.5, 13.5. The intramolecular reductive amination reaction produced **(1*R*,2*R*,6*R*)-1,2,6-trimethylbicyclo[4.2.0]octane** (**9**) as the solely product. $^1$H NMR (400 MHz, $CD_3OD$) δ 3.77 (dt, $J$=10.7, 5.7 Hz, 1H), 3.63–3.55 (m, 1H), 3.17 (dt, $J$=13.2, 4.2 Hz, 1H), 2.74 (t, $J$=7.1 Hz, 1H), 2.62 (ddd, $J$=13.7, 12.0, 2.9 Hz, 1H), 2.34–2.19 (m, 2H), 1.96–1.78 (m, 2H), 1.78–1.63 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 64.2, 52.9, 40.3, 37.3, 28.1, 24.4, 18.4. ESI-TOF: calculated for $[M+H]^+$ $C_7H_{14}NO$: 128.1075, found 128.1070.

**(*R*)-2-((*S*)-*N*-Cbz-2-amino-1-hydroxyethyl)cyclobutan-1-one** (**15e**). This compound was obtained following the procedure described above. *N*-Cbz-glycinal (**2b**) (1.6 mmol), cyclobutanone (**1e**) and FSA A165G were used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 157.8 mg (37%) of the title compound. HPLC: 10 to 100% of B in 30 min, $t_R$=16 min; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.39–7.21 (m, 5H), 5.05 (s, 2H), 3.77 (dt, $J$=7.2, 4.9 Hz, 1H), 3.48–3.36 (m, 1H), 3.25 (dd, $J$=10.6, 6.0 Hz, 2H), 2.88–2.82 (m, 2H), 2.13–1.94 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 210.4, 157.6, 139.9, 128.0, 127.5, 127.4, 69.1, 66.1, 63.1, 44.3, 12.8. The reductive amination gave a complex NMR spectrum, which could not be resolved and that probably indicate a mixture of side products.

**(*R*)-2-((*R*)-*N*-Cbz-3-amino-1-hydroxypropyl)cyclopentan-1-one** (**3f**) **and (*R*)-2-((*S*)-*N*-Cbz-3-amino-1-hydroxypropyl) cyclopentan-1-one** (**4f**). These compounds were obtained following the procedure described above as a mixture of **3f**:**4f** in a 67:33 ratio. *N*-Cbz-3-aminopropanal (**2a**) (1.6 mmol), cyclopentanone (**1f**) and FSA A165G were used. The mixturet was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 303.5 mg (65%) of a mixture **3f**:**4f** in a 67:33 ratio. HPLC: 10 to 100% of B in 30 min, $t_R$=18 and 19 min. Major (67%) (**3f**): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.41–7.17 (m, 5H), 5.06 (s, 2H), 3.78 (ddd, $J$=9.1, 5.4, 3.4 Hz, 1H), 3.27–3.12 (m, 2H), 2.35–2.21 (m, 2H), 2.18–2.09 (m, 3H), 1.83–1.71 (m, 3H), 1.70–1.61 (m, 1H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 221.4, 157.5, 137.0, 128.0, 127.5, 127.3, 69.1, 66.1, 53.7, 38.7, 37.4, 25.8, 22.6, 20.2. Minor (33%) (**4f**): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.41–7.17 (m, 5H), 5.06 (s, 2H), 4.06 (ddd, $J$=8.4, 4.7, 3.2 Hz, 1H), 3.28–3.16 (m, 2H), 2.28–2.18 (m, 1H), 2.18–2.09 (m, 5H), 1.83–1.71 (m, 1H), 1.70–1.61 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 220.9, 157.5, 137.0, 128.0, 127.5, 127.3, 66.1, 66.8, 55.2, 38.9, 38.5, 36.3, 24.0, 21.5. The intramolecular reductive amination reaction produced four diasteroisomers including a dehydroxylated analogue (**14**) in a 15:11:15:22:37 ratio. Owing to the signal overlapping, some chemical shifts identified as multiplets are given as the middle point rather than as range. Selected signals of the NMR spectra. Detailed assignment in Figure S15. Minor **10** (15%): **(4*R*,4a*S*,7a*R*)-octahydro-1*H*-cyclopenta[*b*]pyridin-4-ol** (**10**). $^1$H NMR (500 MHz, MeOD) δ 4.19 (dt, $J$=10.6, 5.2 Hz, 1H), 3.64 (m, 1H), 3.36 (m, 1H), 3.02 (m, 1H), 2.49 (m, 1H), 2.19–1.59 (m, 6H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 65.5, 59.0, 44.6, 42.3. Minor **11** (11%): **(4*R*,4a*S*,7a*S*)-octahydro-1*H*-cyclopenta[*b*]pyridin-4-ol** (**11**). $^1$H NMR (500 MHz, MeOD) δ 3.99 (td, $J$=4.7, 3.1 Hz, 1H), 3.77 (m, 1H), 3.32 (m, 1H), 3.17 (dt, $J$=12.8, 4.4 Hz, 1H), 2.22 (m, 1H), 2.19–1.59 (m, 7H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 63.2, 55.4, 45.4, 37.9. Minor **12** (15%): **(4*S*,4a*S*,7a*S*)-octahydro-1*H*-cyclopenta[*b*]pyridin-4-ol** (**12**). $^1$H NMR (500 MHz, MeOD) δ 3.62 (m, 1H), 3.50 (m, 1H), 3.08 (dd, $J$=13.6, 3.5 Hz, 1H), 2.90 (td, $J$=11.3, 7.1 Hz, 1H), 2.17 (m, 1H), 2.19–1.59 (m, 8H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 70.5, 59.9, 49.7, 45.0. Minor **13** (22%): **(4*S*,4a*S*,7a*R*)-octahydro-1*H*-cyclopenta[*b*]pyridin-4-ol** (**13**). $^1$H NMR (500 MHz, MeOD) δ 3.48 (m, 1H), 3.47 (m, 1H), 3.01 (m, 1H), 2.78 (td, $J$=11.2, 7.1 Hz, 2H), 2.14 (m, 1H), 2.19–1.59 (m, 8H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 61.4, 57.9, 43.9, 42.1. Major **14** (37%) dehydroxylated analogue: **(4a*R*,7a*R*)-octahydro-1*H*-cyclopenta[*b*]pyridine** (**14**): $^1$H NMR (500 MHz, MeOD) δ 3.58 (ddd, $J$=7.9, 5.4, 2.9 Hz, 4H), 3.27 (t, $J$=3.7 Hz, 2H), 3.01 (m, 1H), 2.35–2.25 (m, 1H), 2.11 (m, 1H), 1.79 (m, 2H), 1.85 (m, 2H), 1.71 (m, 1H), 1.76 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 57.8, 42.3, 37.1, 28.2, 26.2, 22.4, 20.5, 17.8. ESI-TOF: calculated for $[M+H]^+$ $C_8H_{16}NO$: 142.1231, found 142.1238.

**(*R*)-2-((*S*)-*N*-Cbz-2-amino-1-hydroxyethyl)cyclopentan-1-one** (**15f**) **and (*R*)-2-((*R*)-*N*-Cbz-2-amino-1-hydroxyethyl) cyclopentan-1-one** (**16f**). These compounds were obtained following the procedure described above as a mixture of **15f**:**16f** in a 50:50 ratio; HPLC: 10 to 100% of B in 30 min, $t_R$=17 and 18 min, respectively. *N*-Cbz-glycinal (**2b**) (1.6 mmol), cyclopentanone (**1f**) and FSA A165G were used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from

1:0 to 3:7, yielding two fraction pools: fraction pool 1 containing **15f** pure (53 mg) and a fraction pool 2 as a mixture of **15f**:**16f** in a 1:2 ratio (117.2 mg), total 170.2 mg (38%) of product. **Fraction pool 1 (*R*)-2-((*S*)-*N*-Cbz-2-amino-1-hydroxyethyl)cyclopentan-1-one** (**15f**). $^1$H NMR (400 MHz, $CD_3OD$) δ 7.38–7.24 (m, 5H), 5.07 (s, 2H), 4.09 (td, $J$ = 6.9, 3.0 Hz, 1H), 3.23 (dd, $J$ = 13.7, 6.2 Hz, 1H), 3.14 (dd, $J$ = 13.7, 7.2 Hz, 1H), 2.33–2.15 (m, 2H), 2.15–1.92 (m, 4H), 1.83–1.70 (m, 1H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 222.2, 159.0, 138.4, 129.4, 129.0, 128.9, 69.6, 67.5, 53.2, 46.3, 39.8, 23.7, 21.7. **Fraction pool 2 15f**:**16f** 33:67 ratio. NMR of **16f**: **(*R*)-2-((*R*)-*N*-Cbz-2-amino-1-hydroxyethyl)cyclopentan-1-one** (**16f**): $^1$H NMR (400 MHz, $CD_3OD$) δ 7.42-7.16 (m, 5H), 5.07 (s, 2H), 3.83 (q, $J$ = 5.5 Hz, 1H), 3.38–3.29 (m, 2H), 2.32–1.98 (m, 5H), 1.91–1.71 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 222.5, 138.5, 129.4, 128.8, 70.8, 67.5, 52.6, 45.7, 40.1, 27.5, 21.8. The intramolecular reductive amination reaction of the fraction pool 2 (33:67 **15f**:**16f** ratio) produced two diastereoisomers the corresponding to the aldol adducts **27** and **28**, respectively. From aldol adduct **15f**: **(3*S*,3a*S*,6a*R*)-octahydrocyclopenta[*b*]pyrrol-3-ol** (**27**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.35 (dt, $J$ = 6.7, 3.5 Hz, 1H), 3.99 (ddd, $J$ = 9.0, 7.2, 3.3 Hz, 1H), 3.15 (d, $J$ = 3.5 Hz, 2H), 2.81 (tdd, $J$ = 9.3, 6.4, 3.6 Hz, 1H), 2.00–1.92 (m, 1H), 1.93–1.86 (m, 2H), 1.89–1.75 (m, 2H), 1.67–1.62 (m, 1H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 70.6, 64.6, 53.9, 47.1, 30.8, 26.0, 24.9. From aldol adduct (**16f**): **(3*R*,3a*S*,6a*R*)-octahydrocyclopenta[*b*]pyrrol-3-ol** (**28**): $^1$H NMR (500 MHz, $CD_3OD$) δ 4.19–4.12 (m, 2H), 3.20–3.15 (m, 1H), 3.09 (dt, $J$ = 12.1, 1.6 Hz, 1H), 2.65 (ddd, $J$ = 9.6, 7.9, 6.3 Hz, 1H), 1.99–1.92 (m, 1H), 1.93–1.87 (m, 1H), 1.69–1.67 (m, 1H), 1.68–1.61 (m, 1H), 1.57–1.51 (m, 1H), 1.40 (dq, $J$ = 13.3, 6.7 Hz, 1H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 75.6, 63.0, 51.7, 51.6, 31.7, 29.1, 24.9. ESI-TOF: calculated for $[M+H]^+$ $C_7H_{14}NO$: 128.1075, found 128.1079.

## Scale up of Aldol Addition of Ethanal (1a) to Cbz-3-aminopropanal (2a)

**(2*S*,4*R*)-*N*-Cbz-piperidine-2,4-diol** (**3a**): The reaction was conducted in a 50 mL screw capped conical-bottom polypropylene tube. To a FSA L107Y/A129G/R134S/A165G/S166G (3 mg·mL$^{-1}$) solution in plain water (18.9 mL), 1 M triethanolamine buffer, pH 8 (1 mL) was added. To this solution, *N*-Cbz-3-aminopropanal (331.2 mg, 80 mM) and ethanal (100 mM, 112 µL) were added. Total reaction volume was 20 mL. Reaction mixtures were shaken (1000 rpm) at 25 °C for 24 h. After that, the reaction was stopped with MeOH (30 mL) to precipitate the enzyme, and the mixture was filtered through Celite®. MeOH was evaporated and the reaction was lyophilized. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 191.3 mg (47%) of **3a**. $[\alpha]_D^{20} = +8.51$ ($c$ = 2.1 in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R$ = 14.5 min; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.59–7.24 (m, 5H), 5.58–5.47 (m, 1H), 5.22–4.99 (m, 2H), 4.11–4.06 (m, 1H), 4.03 (tt, $J$ = 11.3, 4.5 Hz, 1H), 3.12–2.98 (m, 1H), 2.24–2.13 (m, 1H), 2.02–1.92 (m, 1H), 1.94–1.84 (m, 1H), 1.49–1.42 (m, 1H), 1.42–1.33 (m, 1H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 155.7, 136.5, 128.2, 127.8, 127.5, 83.0, 67.1, 63.4, 38.7, 37.0, 35.1, 31.2. Rotamer: $^1$H NMR (400 MHz, $CD_3OD$) δ 7.59–7.24 (m, 5H), 5.49–5.43 (m, 1H), 5.16–5.08 (m, 2H), 4.03–3.99 (m, 1H), 3.97–3.91 (m, 1H), 3.45–3.38 (m, 1H), 2.12–2.04 (m, 1H), 1.86 (dt, $J$ = 14.7, 3.9 Hz, 1H), 1.81–1.70 (m, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 155.4, 136.5, 128.2, 127.8, 127.5, 82.5, 67.1, 60.0, 35.2, 33.0, 31.3. ESI-TOF: calculated for $[M+H]^+$ $C_{13}H_{18}NO_4$: 252.1235, found 252.1231.

## Scale up of Aldol Additions of Propanone (1b) to 2a–c Catalyzed by DERA$_{Tma}$

General procedure: The reactions were conducted in 50 mL screw capped conical-bottom polypropylene tubes. To a DERA$_{Tma}$ solution (3 mg·mL$^{-1}$) in plain water (17 mL), 1 M triethanolamine buffer, pH 8 (1 mL) was added. To this solution, the aldehyde (**2a–c**) (80 mM) dissolved in propanone (**1b**) (2 mL) was added. Total reaction volume was 20 mL. Reaction mixtures were shaken (1000 rpm) at 25 °C for 24 h. After that, the reaction was stopped with MeOH (30 mL) to precipitate the enzyme. Then, the mixture was filtered through Celite®, the MeOH was evaporated and the aqueous residue was lyophilized ad submitted to reductive amination (identical procedure as described above).

**(*R*)-*N*-Cbz-6-amino-4-hydroxyhexan-2-one** (**3b**): This compound was obtained following the procedure described above. *N*-Cbz-3-aminopropanal (**2a**) (331.2 mg, 1.6 mmol) was used. After 24 h additional DERA$_{Tma}$ (3 mg·ml$^{-1}$) was added and the reaction was shaken (1000 rpm) at 25 °C for 24 h (70% product formed by HPLC). The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 198.6 mg (47%) of product. $[\alpha]_D^{20} = +15.8$ ($c$ = 0.39 in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R$ = 16 min; Chiral HPCL: $t_R$ = 23 min (racemic sample: $t_R(R)$ = 23 min, $t_R(S)$ = 27 min). NMR spectral data was identical to that obtained by FSA D6N catalysis (see above).

**(*S*)-*N*-Cbz-5-amino-4-hydroxypentan-2-one** (**15b**): This compound was obtained following the procedure described above. *N*-Cbz-glycinal (**2b**) (310.4 mg, 1.6 mmol) was used (85% **15b** formed). The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 120.2 mg (30%) of product. $[\alpha]_D^{20} = +2.2$ ($c$ = 0.92 in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R$ = 15 min; Chiral HPCL: $t_R$ = 25 min (racemic sample: $t_R(R)$ = 25 min, $t_R(S)$ = 23 min). NMR spectra were identical to that obtained by FSA A165G catalysis (see above).

**(4*S*,5*S*)-*N*-Cbz-5-amino-4-hydroxyhexan-2-one** (**4*S*,5*S*-29**): This compound was obtained following the procedure described above. (*S*)-*N*-Cbz-Alaninal (*S*-**2c**) (331.2 mg, 1.6 mmol), propanone (**2b**) (2 mL) and DERA$_{Tma}$ (in 17 mL of plain water) were used (81% (4*S*,5*S*)-**29** formed). The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 336.9 mg (79%) of product. $[\alpha]_D^{20} = -23.8$ ($c$ = 2.6 in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R$ = 16 min; $^1$H NMR (400 MHz, $CD_3OD$) δ 7.46–7.14 (m, 5H), 5.05 (d, $J$ = 4.8 Hz, 2H), 4.00 (td, $J$ = 6.5, 3.2 Hz, 1H), 3.66 (qd, $J$ = 6.8, 3.1 Hz, 1H), 2.56–2.50 (m, 2H), 2.10 (s, 3H), 1.13 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 208.7,

157.1, 137.0, 128.0, 127.5, 127.4, 69.4, 66.0, 50.3, 46.5, 29.2, 15.8. The intramolecular reductive amination reaction produced two diasteroisomers in a 56:44 ratio. **(2*S*,3*S*,5*S*)-2,5-dimethylpyrrolidin-3-ol** (**33**) (56%) $^1$H NMR (400 MHz, $CD_3OD$) δ 4.09 (td, $J$ = 4.0, 2.0 Hz, 1H), 3.20 (dt, $J$ = 8.7, 6.6 Hz, 1H), 2.98 (qd, $J$ = 6.7, 3.9 Hz, 2H), 2.41–2.27 (m, 2H), 1.39 (ddd, $J$ = 14.1, 6.8, 2.2 Hz, 2H), 1.27 (d, $J$ = 6.5 Hz, 4H), 1.21 (d, $J$ = 5.4 Hz, 9H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 73.1, 59.9, 53.0, 42.4, 19.6, 11.7. **(2*S*,3*S*,5*R*)-2,5-dimethylpyrrolidin-3-ol** (**34**) (44%) $^1$H NMR (400 MHz, $CD_3OD$) δ 4.14–4.10 (m, 1H), 3.66 (dp, $J$ = 9.1, 6.8 Hz, 1H), 2.07 (ddd, $J$ = 13.6, 7.1, 1.3 Hz, 1H), 1.65 (ddd, $J$ = 13.6, 9.0, 4.5 Hz, 1H), 1.21 (d, $J$ = 5.7 Hz, 7H), 1.20 (d, $J$ = 5.4 Hz, 6H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 73.1, 58.1, 52.0, 42.3, 19.5, 11.8. ESI-TOF: calculated for $[M+H]^+$ $C_6H_{14}NO$: 116.1075, found 116.1076.

***N*-Cbz-5-amino-4-hydroxyhexan-2-one** (mixture of diasteromers (4*S*,5*R*)-**29**:(4*R*,5*R*)-**30** in a 77:23 ratio) This mixture was obtained following the procedure described above. (*R*)-*N*-Cbz-alaninal (*R*-**2c**) (331.2 mg, 1.6 mmol), acetone (2 mL) and $DERA_{Tma}$ (in 17 mL of plain water) were used (91% mixture formed). The mixture was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 330.1 mg (78%) of product. $[\alpha]_D^{20} = +2.8$ ($c$ = 0.7 in MeOH). HPLC: 10 to 100% of B in 30 min, $t_R$ = 16 min. Only one diasteromer was detected by NMR. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.35–7.19 (m, 5H), 5.05 (s, 2H), 3.94 (ddd, $J$ = 7.9, 6.1, 4.8 Hz, 1H), 3.56 (p, $J$ = 6.7 Hz, 1H), 2.55 (dd, $J$ = 7.8, 3.9 Hz, 2H), 2.12 (s, 3H), 1.12 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 208.8, 156.9, 136.9, 128.0, 127.5, 127.4, 70.2, 66.0, 51.1, 46.9, 29.2, 14.8. Another diasteromer was identified after the reduction amination reaction. The intramolecular reductive amination reaction produced two diastereoisomers in a 77:23 ratio. **(2*R*,3*S*,5*R*)-2,5-Dimethylpyrrolidin-3-ol** (**35**) (77%).$^1$H NMR (400 MHz, $CD_3OD$) δ 3.80 (td, $J$ = 6.5, 5.2 Hz, 1H), 3.35 (dt, $J$ = 14.1, 7.1 Hz, 1H), 3.04 (qd, $J$ = 6.6, 5.1 Hz, 1H), 2.31 (dt, $J$ = 13.0, 7.1 Hz, 1H), 1.35 (ddd, $J$ = 13.1, 7.7, 6.3 Hz, 1H), 1.21 (d, $J$ = 6.5 Hz, 4H), 1.12 (d, $J$ = 6.7 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 77.8, 60.1, 50.8, 41.4, 20.5, 16.3. **(2*R*,3*R*,5*S*)-2,5-dimethylpyrrolidin-3-ol** (**36**) (23%). $^1$H NMR (400 MHz, $CD_3OD$) δ 4.09 (ddd, $J$ = 6.1, 3.9, 2.2 Hz, 1H), 3.20 (dt, $J$ = 8.7, 6.7, 6.7 Hz, 1H), 2.98 (tt, $J$ = 6.7, 6.7, 3.3, 3.3 Hz, 1H), 2.41–2.31 (m, 4H), 1.44–1.35 (m, 1H), 1.27 (d, $J$ = 6.6 Hz, 3H), 1.20 (d, $J$ = 5.6 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 73.1, 59.9, 42.4, 19.9, 11.8. ESI-TOF: calculated for $[M+H]^+$ $C_6H_{14}NO$: 116.1075, found 116.1072.

## Scale up of Aldol Additions of Ethanal (1a) to 2a–c Catalyzed by $DERA_{Tma}$

**(2*S*,4*R*)-*N*-Cbz-piperidine-2,4-diol** (**3a**). This compound was synthetized following the procedure described above catalyzed by FSA variant. In this case $DERA_{Tma}$ (3 mg·mL$^{-1}$) was used and 77% aldol product formation by HPLC was obtained, yielding 99.3 mg (25%) of pure product. $[\alpha]_D^{20} = +4.6$ ($c$ = 0.69 in MeOH); HPLC: 10 to 100% of B in 30 min, $t_R$ = 14.5 min. NMR spectra was identical to that obtained by FSA L107Y/A129G/R134S/A165G/S166G catalysis (see above).

**(2*S*,3*S*)-2-methylpyrrolidin-3-ol** (**31**). The reaction was conducted in a 20 mL screw capped conical-bottom polypropylene tube. To a $DERA_{Tma}$ (60 mg, 3 mg·mL$^{-1}$) solution in plain water (18.0 mL), 1 M triethanolamine buffer, pH 8 (1.0 mL) was added. To this solution, (*S*)-*N*-Cbz-alaninal (*S*-**2c**) (414.0 mg, 2.0 mmol, 100 mM in the reaction) and ethanal (**1a**) (120 μL, 2 mmol, 100 mM in the reaction) were added. The reaction mixture was shaken (1000 rpm) at 25 °C for 24 h (98% aldol product formed as determined by HPLC). After that, the reaction was stopped with MeOH (30 mL) to precipitate the enzyme, and the mixture was filtered through Celite®. MeOH was evaporated and the reaction was lyophilized. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt: 100:0 (200 mL) 80:20 (200 mL), 70:30 (200 mL), 60:40 (200 mL) and 50:50 (700 mL), yielding 171.2 mg (34%) of aldol adduct. The reductive amination was conducted as follows: the aldol adduct (171.2 mg) was dissolved in MeOH (400 mL), then Pd (10%)/C with 50% humidity (200 mg) was added. The mixture was shaken under $H_2$ (2.5 atm) overnight at room temperature. After that, the reaction was filtered and the solvent was evaporated. Product (51.2 mg) was characterized without any further purification. $^1$H NMR (400 MHz, $CD_3OD$) δ 4.25 (t, $J$ = 3.1 Hz, 1H), 3.48 (tt, $J$ = 2 × 6.7, 2 × 3.4 Hz, 1H), 3.46–3.34 (m, 1H), 2.26–2.13 (m, 1H), 2.09–2.00 (m, 1H), 1.38 (d, $J$ = 6.7 Hz, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 72.1, 61.6, 44.0, 34.1, 11.7.

**(2*R*,3*S*)-2-methylpyrrolidin-3-ol** (**32**). This compound was synthetized following the procedure described for **(2*S*,3*S*)-2-methylpyrrolidin-3-ol** (**31**). In this case the 80% aldol product formation was obtained as determined by HPLC. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt: 100:0 (200 mL) 80:20 (200 mL), 70:30 (200 mL), 60:40 (200 mL) and 50:50 (750 mL). To remove the product from enzymatic double addition of **1a**, two purification runs were needed to obtain 101.0 mg (20%) of aldol adduct. The reductive amination was conducted as follows: the aldol adduct (40.0 mg) was dissolved in MeOH (100 mL), then Pd (10%)/C with 50% humidity (40 mg) was added. The mixture was shaken under $H_2$ (2.5 atm) overnight at room temperature. After that, the reaction was filtered and the solvent was evaporated. Product (51.2 mg) was characterized without any further purification. $^1$H NMR (400 MHz, $CD_3OD$) δ 4.13 (dt, $J$ = 5.4, 3.4, 3.4 Hz, 1H), 3.53 (ddt, $J$ = 10.3, 7.0, 2 × 3.2 Hz, 1H), 3.45–3.37 (m, 2H), 2.27 (dtd, $J$ = 14.1, 2 × 9.0, 5.4 Hz, 2H), 2.03–1.93 (m, 2H), 1.31 (d, $J$ = 7.0 Hz, 2H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 76.1, 63.4, 43.7, 32.3, 15.6.

## Organocatalytic Reactions:

***N*-Benzyloxycarbonyl-(*E*)-6-aminohex-4-en-2-one**: *N*-Cbz-3-aminopropanal (0.4 mmol, 82.8 mg) was dissolved in DMF (4 mL). Acetone (20%, 1 mL) and (D,L)-proline (0.08 mmol, 9.21 mg) were added. Total reaction volume was 5 mL. Reaction mixture was stirred at 25 °C for 4 hours. The solvent was evaporated and the product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 1:1, yielding 80 mg (80%) of

product. $^{1}$H NMR (400 MHz, $CD_3OD$) δ 7.43–7.23 (m, 5H), 6.85 (dt, $J$ = 16.0, 7.1 Hz, 1H), 6.06 (dt, $J$ = 16.2, 1.5 Hz, 2H), 5.04 (s, 2H), 3.36–3.05 (m, 2H), 2.41 (qd, $J$ = 6.7, 1.5 Hz, 1H), 2.19 (s, 3H).$^{13}$C NMR (101 MHz, $CD_3OD$) δ 199.8, 157.4, 146.1, 132.1, 128.0, 127.5, 127.3, 65.9, 38.9, 32.8, 25.2.

### Preparation of Racemic Aldol Adducts

*Lithium diisopropylamine reactions*: General procedure.[18] Lithium diisopropylamine (700 µL of a 2 M stock solution in THF) was added to anhydrous THF (8 mL) and cooled down to −78 °C. Then, propanone (**1b**) (100 µL) was added and stirred for 20 minutes. After that, the aldehyde, dissolved in anhydrous THF (2 mL), was added. The reaction was stirred for 2 hours at −78 °C, and then left to warm up to room temperature. When the reaction mixture was at room temperature, $NaHCO_3$ (20 mL of 10% aqueous solution) was added. The product was extracted by AcOEt (3 × 15 mL), the organic layers pooled, dried over anhydrous $Na_2SO_4$, and the solvent was evaporated to dryness.

***rac*-*N*-Cbz-6-amino-4-hydroxyhexan-2-one** (***rac*-3b**): *N*-Cbz-3-aminopropanal (**2a**) (209 mg, 1 mmol) was used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 130.6 mg (49%) of the title compound. The NMR spectra was indistinguishable from the one obtained enzymatically (see above).

***rac*-*N*-Cbz-6-amino-4-hydroxypentan-2-one** (***rac*-15b**): *N*-Cbz-glycinal (**2b**) (196 mg, 1 mmol) was used. The product was purified by silica gel column chromatography and eluted with a step gradient of hexane:AcOEt from 1:0 to 3:7, yielding 88.2 mg (35%) of the title compound. The NMR spectra was indistinguishable from the one obtained enzymatically (see above).

## Acknowledgements

*This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 635595* (*CarbaZymes*), *the Ministerio de Economía y Competitividad* (*MINECO*), *the Fondo Europeo de Desarrollo Regional* (*FEDER*) (*grant CTQ2015-63563-R to P.C. and CTQ2015-64436-P to T.P.*), *Acciones de Programación Conjunta Internacional* (*Tralaminol*) *PCI2018-092937.*

## References

[1] a) E. Marcantoni, M. Petrini, *Adv. Synth. Catal.* **2016**, *358*, 3657–3682; b) P. R. Lazzara, K. P. Fitzpatrick, C. C. Eichman, *Chem. Eur. J.* **2016**, *22*, 16779–16782; c) F. Lovering, J. Bikker, C. Humblet, *J. Med. Chem.* **2009**, *52*, 6752–6756; d) M. Rubiralta, E. Giralt, A. Diez, *Piperidine. Structure*, *Preparation*, *Reactivity*, *and Synthetic Applications of Piperidine and its Derivatives*, *Vol. 40*, Wiley-VCH Verlag GmbH & Co. KGaA, Amsterdam **1992**; e) M. J. Schneider, in *Alkaloids*: *Chemical and Biological Perspectives*, *Vol. Volume 10* (Ed.: S. W. Pelletier), Pergamon, **1996**, pp. 155–299.

[2] a) C. Saotome, Y. Kanie, O. Kanie, C.-H. Wong, *Bioorg. Med. Chem.* **2000**, *8*, 2249–2261; b) J. Bolleddula, K. DeMent, J. P. Driscoll, P. Worboys, P. J. Brassil, D. L. Bourdet, *Drug Metab. Rev.* **2014**, *46*, 379–419; c) O. O. Grygorenko, M. Kurkunov, I. A. Levandovskiy, A. V. Tymtsunik, *Synthesis* **2018**, *50*, 1973–1978; d) C. Wang, Z. Lu, *Org. Lett.* **2017**, *19*, 5888–5891.

[3] a) F. Zhang, Y. Peng, Y. Gong, *Chirality* **2008**, *20*, 805–811; b) S. R. Chemler, D. A. Copeland, *Top. Heterocycl. Chem.* **2013**, *32*, 39–75; c) S. Rougnon Glasson, J.-L. Canet, Y. Troin, *Tetrahedron Lett.* **2000**, *41*, 9797–9802; d) H. Peng, E. Wei, J. Wang, Y. Zhang, L. Cheng, H. Ma, Z. Deng, X. Qu, *ACS Chem. Biol.* **2016**, *11*, 3278–3283.

[4] a) S. Takayama, G. J. McGarvey, C.-H. Wong, *Annu. Rev. Microbiol.* **1997**, *51*, 285–310; b) L. J. Whalen, C.-H. Wong, *Aldrichimica Acta* **2006**, *39*, 63–71; c) A. Soler, M. L. Gutiérrez, J. Bujons, T. Parella, C. Minguillon, J. Joglar, P. Clapés, *Adv. Synth. Catal.* **2015**, *357*, 1787–1807; d) A. Szekrenyi, A. Soler, X. Garrabou, C. Guerard-Helaine, T. Parella, J. Joglar, M. Lemaire, J. Bujons, P. Clapés, *Chem. Eur. J.* **2014**, *20*, 12572–12583; e) A. Soler, X. Garrabou, K. Hernández, M. L. Gutiérrez, E. Busto, J. Bujons, T. Parella, J. Joglar, P. Clapés, *Adv. Synth. Catal.* **2014**, *356*, 3007–3024; f) P. Laborda, F. J. Sayago, C. Cativiela, T. Parella, J. Joglar, P. Clapés, *Org. Lett.* **2014**, *16*, 1422–1425; g) A. L. Concia, L. Gómez, T. Parella, J. Joglar, P. Clapés, *J. Org. Chem.* **2014**, *79* 5386–5389.

[5] M. Schürmann, G. A. Sprenger, *J. Biol. Chem.* **2001**, *276*, 11055–11061.

[6] a) R. Roldán, I. Sanchez-Moreno, T. Scheidt, V. Hélaine, M. Lemaire, T. Parella, P. Clapés, W.-D. Fessner, C. Guérard-Hélaine, *Chem. Eur. J.* **2017**, *23*, 5005–5009; b) R. Roldán, K. Hernandez, J. Joglar, J. Bujons, T. Parella, I. Sánchez-Moreno, V. Hélaine, M. Lemaire, C. Guérard-Hélaine, W.-D. Fessner, P. Clapés, *ACS Catal.* **2018**, *8*, 8804–8809; c) S. Junker, R. Roldan, H.-J. Joosten, P. Clapés, W.-D. Fessner, *Angew. Chem.* 2018, 130, 10310–10314; *Angew. Chem. Int. Ed.* **2018**, *57*, 10153–10157.

[7] H. J. M. Gijsen, L. Qiao, W. Fitz, C.-H. Wong, *Chem. Rev.* **1996**, *96*, 443–473.

[8] a) M. Gutierrez, T. Parella, J. Joglar, J. Bujons, P. Clapés, *Chem. Commun.* **2011**, *47*, 5762–5764; b) D. Güclü, A. Szekrenyi, X. Garrabou, M. Kickstein, S. Junker, P. Clapés, W.-D. Fessner, *ACS Catal.* **2016**, *6*, 1848–1852.

[9] L. Chen, D. P. Dumas, C.-H. Wong, *J. Am. Chem. Soc.* **1992**, *114*, 741–748.

[10] H. Sakuraba, K. Yoneda, K. Yoshihara, K. Satoh, R. Kawakami, Y. Uto, H. Tsuge, K. Takahashi, H. Hori, T. Ohshima, *Appl. Environ. Microbiol.* **2007**, *73*, 7427–7434.

[11] a) N. Ran, L. Zhao, Z. Chen, J. Tao, *Green Chem.* **2008**, *10*, 361–372; b) S. Hu, J. Tao, Z. Xie, WO2006134482 A1, **2006**.

[12] a) A. L. Concia, C. Lozano, J. A. Castillo, T. Parella, J. Joglar, P. Clapés, *Chem. Eur. J.* **2009**, *15*, 3808–3816;

b) J. A. Castillo, J. Calveras, J. Casas, M. Mitjans, M. P. Vinardell, T. Parella, T. Inoue, G. A. Sprenger, J. Joglar, P. Clapés, *Org. Lett.* **2006**, *8*, 6067–6070; c) L. Espelt, J. Bujons, T. Parella, J. Calveras, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.* **2005**, *11*, 1392–1401; d) L. Espelt, T. Parella, J. Bujons, C. Solans, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.* **2003**, *9*, 4887–4899.

[13] J. Calveras, M. Egido-Gabás, L. Gómez, J. Casas, T. Parella, J. Joglar, J. Bujons, P. Clapés, *Chem. Eur. J.* **2009**, *15*, 7310–7328.

[14] T. Kajimoto, L. Chen, K. K. C. Liu, C.-H. Wong, *J. Am. Chem. Soc.* **1991**, *113*, 6678–6680.

[15] A. Szekrenyi, X. Garrabou, T. Parella, J. Joglar, J. Bujons, P. Clapés, *Nat. Chem.* **2015**, *7*, 724–729.

[16] M. T. Konieczny, P. H. Toma, M. Cushman, *J. Org. Chem.* **1993**, *58*, 4619–4624.

[17] C.-H. Wong, R. L. Halcomb, Y. Ichikawa, T. Kajimoto, *Angew. Chem.* **1995**, *107*, 453-474; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 412–432.

[18] P. A. Wender, M. F. T. Koehler, M. Sendzik, *Org. Lett.* **2003**, *5*, 4549–4552.