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Aldolase-Catalyzed Synthesis of Conformationally Constrained Iminocyclitols: Preparation of Polyhydroxylated Benzopyrrolizidines and Cyclohexapyrrolizidines

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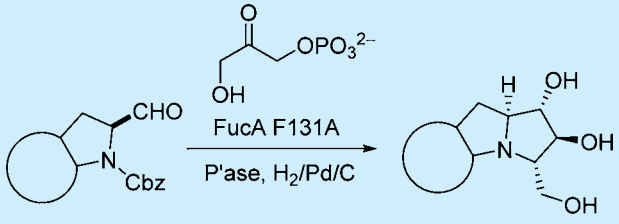
Supporting Information

ABSTRACT: A straightforward chemo-enzymatic synthesis of new polyhydroxylated benzopyrrolizidines and cyclohexapyrrolizidines is developed. The two-step strategy consists of L-fuculose-1-phosphate aldolase variant F131A-catalyzed aldol addition of dihydroxyacetone phosphate to *rac*-*N*-benzyloxycarbonylindoline-2-carbaldehyde as well as (2*S**,3a*S**,7a*S**)- and (2*S**,3a*R**,7a*R**)-*N*-benzyloxycarbonyloctahydroindole-2-carbaldehydes and a subsequent one-step catalytic deprotection−reductive amination.



Polyhydroxylated pyrrolizidine alkaloids are naturally occurring compounds receiving substantial interest as inhibitors of glycosidases and glycosyltransferases.[1] Modification of these compounds with fused aromatic or cyclohexane moieties may lead to benzopyrrolizidines (**1**) and cyclohexapyrrolizidines (**2**) (Figure 1) which constitute a new class of

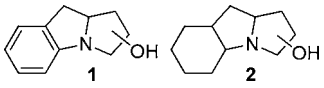


Figure 1. Polyhydroxylated benzopyrrolizidines (**1**) and cyclohexapyrrolizidines (**2**) as a new class of iminociclitols.

iminocyclitols with strong conformational restriction. This is notable because constrained iminocyclitol structures may improve their activity and selectivity by precisely directing the hydrogen bonds and hydrophobic contacts of ligand−protein, avoiding the multiple interactions that flexible structures can establish with different targets.[2]

The benzopyrrolizidine (**1**) contains the indoline unit which belongs to the "privileged structures" among natural products and pharmaceutically important compounds.[3] Furthermore, indoline derivatives constitute a structural class of compounds from which several drug candidates have emerged, including antineoplastic sulfonamides and muscarine receptor agonists and antagonists.[4] The tetrahydropyrrolo[1,2-*a*]indole (**1**) skeleton is also a common feature of the mitomycin family of antitumor antibiotics, and numerous approaches for the synthesis and functionalization of this system have been explored.[5]

We have reported that iminocyclitols of the pyrrolizidine, indolizidine, and quinolizidine type can be expediently prepared by a catalytic two-step synthesis, consisting of an enzymatic aldol addition of dihydroxyacetone phosphate (DHAP) to *N*-Cbz-pyrrolidine and *N*-Cbz-piperidine carbaldehyde derivatives and a reductive amination catalyzed by Pd/C. DHAP-dependent aldolases such as L-rhamnulose-1-phosphate aldolase (RhuA) and L-fuculose-1-phosphate aldolase (FucA) F131A variant were the aldol catalysts that provided adducts with high and complementary stereochemistry.[6] Therefore, diverse functional and structural polyhydroxylated molecules such as iminocyclitols can be easily accessed. Following this synthetic procedure, we herein report the preparation of the target polyhydroxylated benzopyrrolizidine (**1**) and cyclohexapyrrolizidine (**2**) type iminocyclitols. To this end, racemates of *N*-Cbz-indoline-2-carbaldehyde (**3**) and *N*-Cbz-octahydroindole-2-carbaldehydes **4** and **5** were selected as acceptor substrates (Figure 2). Owing to the low enantiomeric discrimination of the RhuA and FucA for *N*-Cbz-pyrrolidine

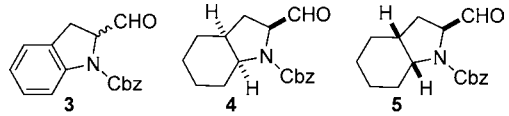


Figure 2. *rac*-*N*-Cbz-indoline-2-carbaldehyde (**3**), *rac*-*N*-Cbz-octahydroindole-2-carbaldehydes **4** and **5** used in this work (Cbz = benzyloxycarbonyl).

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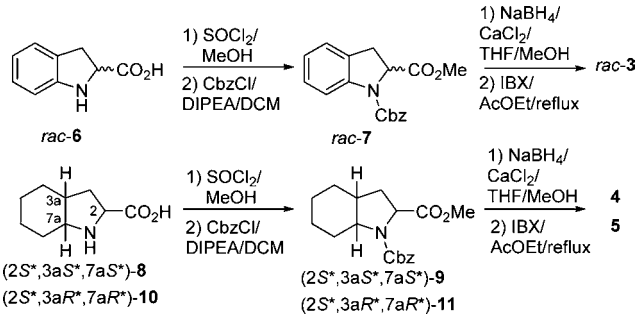
and *N*-Cbz-piperidine carbaldehyde derivatives,[6] it may be anticipated that the use of racemates will provide an additional source of product diversity by generating two diastereoisomeric aldol adducts for each racemate.

Aldehydes **3**-**5** were synthetized starting from commercially available indoline-2-carboxylic acid, *rac*-**6**, and from both (2*S**,3a*S**,7a*S**)- and (2*S**,3a*R**,7a*R**)-octahydroindole-2-carboxylic acids **8** and **10**, respectively (Scheme 1), in turn readily

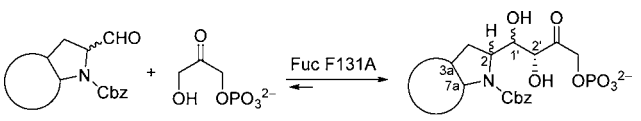
Scheme 1. Synthesis of Aldehydes 3−5



prepared from *rac*-**6**, using the procedures previously described in the literature.[7] Thus, the amino acids *rac*-**6**, **8**, and **10** were converted into the *N*-protected methyl esters *rac*-**7**, **9**, and **11** by esterification with thionyl chloride and methanol followed by protection with benzyl chloroformate (Scheme 1). These derivatives were then subjected to reduction with sodium borohydride in the presence of $CaCl_2$, and the corresponding alcohols thus obtained were oxidized to the aldehydes *rac*-**3**−**5** with 2-iodoxybenzoic acid (IBX).

Aldol additions of DHAP to *rac*-*N*-Cbz-indoline-2-carbaldehyde (**3**) and *rac*-*N*-Cbz-octahydroindole-2-carbaldehyde (**4** and **5**) derivatives were carried out. DHAP-dependent aldolases, such as RhuA, FucA F131A, and F206A/F131A variants, were selected as biocatalysts.[6b] These FucA variants have a hydrophobic pocket, created at the F131 position, that allow a better fit of bulky aldehyde substrates, e.g. prolinal derivatives, in the active site. Reactions were conducted at 4 °C to minimize the DHAP degradation and improve the aldol product formation.[8] Indeed, the reactions carried out at 25 °C rendered much lower product formation than those at 4 °C (Table 1).

Table 1. FucA F131A Catalyzed Aldol Addition of Dihydroxyacetone Phosphate (DHAP) to Indoline and Octahydroindole Carbaldehydes 3−5



aldehyde acceptor	aldol adduct formation[a] (%)
3	27[b] (22)[c] (18)[d]
4	33[b] (20)[c]
5	37[b] (27)[c] (7)[d]

[a]Product formation after 24 h of reaction measured by HPLC from the peak areas using an external standard method. FucA F131A (0.6 mg of protein) stirred at 1000 rpm was used in all cases. [b]DHAP 75 mM, 21 mM sodium borate buffer pH = 7.5, DMF (18% v/v), 4 °C. [c]DHAP 83 mM, DMF (20% v/v), 4 °C. [d]DHAP 83 mM, DMF (20% v/v), 25 °C.

The biocatalyst screening revealed that FucA F131A was the only one that tolerated aldehydes **3**−**5** at moderate to low conversion (Table 1). RhuA and FucA F206A/F131A did not catalyze the aldol reaction with any of the aldehydes assayed. As reported in previous works, FucA F131A was the biocatalyst of choice for the aldol additions of DHAP to pyrrolidine and piperidine-2-carbaldehyde derivatives.[6b] Herein, the versatility and broad synthetic applicability of the FucA F131A catalyst was fully demonstrated in the preparation of these complex conformationally constrained iminocyclitols.

The aldol additions of DHAP to aldehydes **3**−**5** were scaled up to 0.7−1 mmol (Scheme 2). The aldol adducts obtained from the enzymatic catalysis were submitted to the one-pot two-step process of *N*-Cbz-deprotection/reductive amination with H_2 in the presence of Pd/C. The cyclic compounds or the aldol adducts were purified and structurally characterized by NMR spectroscopy. From the structural analysis of the compounds obtained, the stereoselectivity of both the enzymatic aldol addition and the catalytic reductive amination were thus inferred. Based on mechanistic considerations on DHAP-dependent aldolase catalysis and on previous synthetic reports, it may safely be assumed that the absolute configuration at C2′ will be conserved throughout the reaction regardless of the acceptor substrate.[6b,9] Therefore, FucA F131A always delivers the C2′ *R* configured stereocenter (i.e., in the aldol adduct; see Table 1) during the catalysis with any acceptor electrophile.[6b,9d] The aldol addition of DHAP to *rac*-indoline **3** furnished two aldol adducts, which after dephosphorylation could not be separated by chromatography. After treatment with H_2/Pd/C the benzopyrrolizidines **13a** and **13b**, obtained as a 1:1 mixture, were separated and purified by flash chromatography. From the structural characterization of **13a** and **13b**, it can be inferred that the (*R*)-**3** indoline provided the aldol adduct **12a** with an *anti* (1′*R*,2′*R*) configuration while the (*S*)-**3** gave the *syn* (1′*S*,2′*R*) configured **12b**, which was consistent with the stereochemical outcome obtained using (*S*)- and (*R*)-*N*-Cbz-prolinal derivatives as acceptors, respectively.[6b] Thus, under the reaction conditions no kinetic discrimination by the aldolase was observed. The reductive amination was highly selective leading to only one of the two possible diastereoisomers. In this case, we observed that the hydrogenation took place from the face opposite to the C1 hydroxyl group, regardless of the relative stereochemistry of the other substituents (Figure 3a−b). As a result, the C1-OH/C3-CH_2OH adopts a relative *cis* configuration. This was consistent with previous results achieved for similar reactions with both six- and five-membered ring iminocyclitols.[9c,10]

The reaction with *rac*-octahydroindole (**4**) (i.e., with the proton at C2 in *cis* configuration with respect to the C3a and C7a protons) furnished also two aldol adducts, **14a** and **14b**, which could be separated by chromatography rendering equal amounts of each diastereoisomer (Scheme 2). Treatment of **14a** furnished the cyclohexapyrrolizidine **15** with a 1*S*,2*S*,3*R*,4a-*S*,8a*S*,9a*S* configuration arising from the (2*S*,3a*S*,7a*S*)-**4** aldehyde. In this case the reductive amination was stereoselective, but the stereochemistry at C3 was inverse to that found in the previous benzopyrrolizidines **13a** and **13b** (vide supra) (Scheme 2). It is likely that the addition of hydrogen took place from the face opposite to the one occupied by the six membered ring which actually controls the stereochemical outcome of the reaction (Figure 3c). On the other hand, treatment of **14b** with H_2/Pd/C did not render the expected tricyclic compound. The ^{1}H NMR spectrum of the recovered

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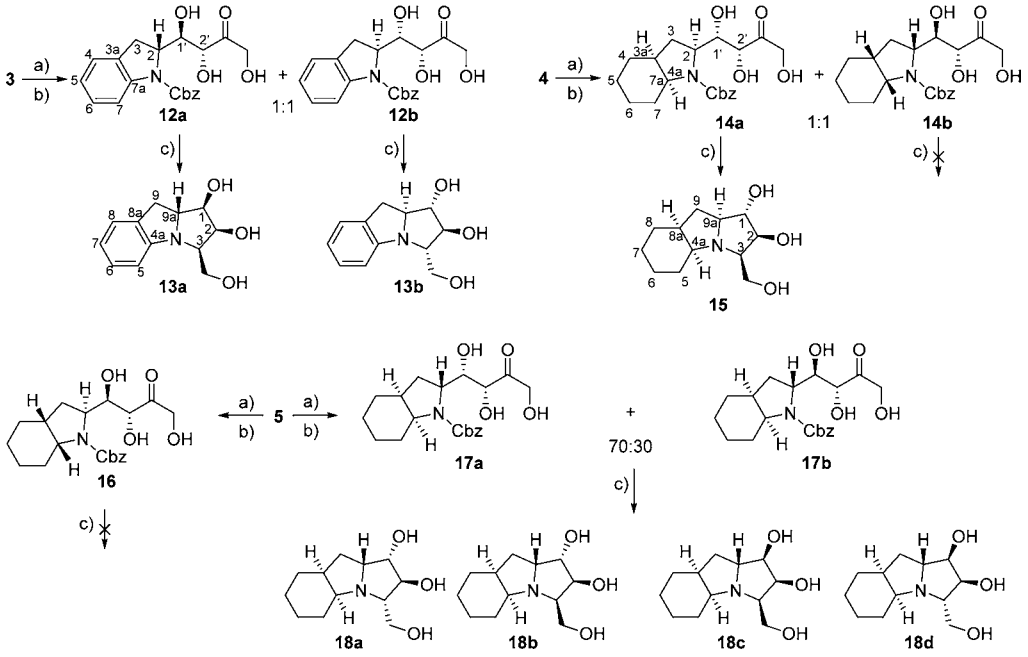
Scheme 2. Chemo-enzymatic Synthesis of Benzopyrrolizidines (13a–b) and Cyclohexapyrrolizidines (15, 18a–d) by a Two-Step Enzymatic Aldol Addition Reductive Amination[a]



[a]Reagents and conditions: (a) FucA F131A, DHAP, sodium borate buffer (20 mM, pH 7.5), DMF (17% v/v); (b) acid phosphatase (1 mg per mL of aqueous solution), sodium citrate buffer (0.4 M, pH 4.5); (c) H_2 (50 psi) Pd/C, MeOH.

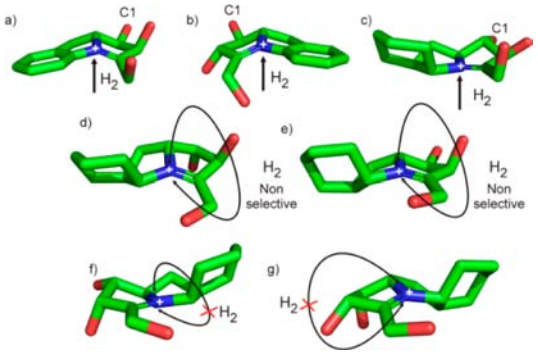


Figure 3. Minimum energy stereoisomers determined for the corresponding iminium cation intermediates before the reductive amination. Iminium cations from compounds (a) **12a**, (b) **12b**, (c) **14a**, (d) **17a**, (e) **17b**, (f) **14b**, and (g) **16**. Calculations were performed with DFT methodology (B3LYP hybrid functional with the 6-31G* basis set) with Spartan'10; Wave function, Inc.; Irvine, CA, 2010.[11]

material was complex with signals that presumably belong to a number of decomposition products since they can hardly be assigned to any single structure. Moreover, when the Cbz group was removed from **14b**, i.e. by treating a sample with H_2 (1 atm) in the presence of Pd/C, and immediately submitted to chemical reduction with $NaBH_3CN$, the expected product was not formed but again decomposition to a complex material was observed by ^1H NMR. A model of compound **14b** as an intramolecular iminium cation between the ketone and secondary amine revealed that both faces are sterically hindered by either the hydroxyl functions or the fused six-member ring (Figure 3f). Structural characterization of **14b** indicates a 2*R*,3a*R*,7a*R*,1′*R*,2′*R* configuration, thus arising from the corresponding addition of DHAP to the (2*R*,3a*R*,7a*R*)-**4** aldehyde acceptor. Hence, the stereochemical outcome of the enzymatic aldol additions (Scheme 2) was consistent with the previous example.[6b]

The *rac*-octahydroindole (**5**) (i.e., with the proton at C2 in *trans* configuration with respect to the C3a and C7a protons) furnished three aldol adducts **16**, **17a**, and **17b** (Scheme 2). Aldol adduct **16** could be separated, whereas **17a** and **17b** were obtained as a 70:30 mixture. The adduct **16** did not provide the corresponding cyclohexapyrrolizidine, decomposing upon treatment with H_2/Pd/C. This was similar to what was observed for **14b** (vide supra), with both compounds showing steric hindrance in each diastereotopic face of the iminium cation according to calculations (Figures 3f, g). Hence, the reductive amination appears to take place only when the fused ring with proline is in a relative *cis* configuration respect to the hydroxyl in position 2 (Figures 3c–e). Structural characterization of **16** indicates a 2*S*,3a*R*,7a*R*,1′*R*,2′*R* configuration arising from the (2*S*,3a*R*,7a*R*)-**5** acceptor which is consistent with the expected *anti* (1*R*,2*R*) stereochemistry of FucA F131A catalysis. The reductive amination of the **17a**:**17b** 7:3 mixture and subsequent purification gave a fraction of **18a** pure and another as a mixture of **18a**:**18b**:**18c**:**18d** in a 1:4:3:1 ratio. In this case, the reductive amination was not stereoselective. The minimum conformation of the cyclic iminium cation intermediate from **17b** (Figure 3e) showed no favored face for the addition of H_2, whereas in the case of **17a** (Figure 3d) one face is occupied by the six-member ring while the opposite was occupied by C1 resulting in none of the faces possessing any clear steric advantage for the addition of H_2. Moreover, as inferred from the structure of cyclohexapyrrolizidines **18a–d**, the enzymatic addition of DHAP to (2*R*,3a*S*,7a*S*)-**5** aldehyde was not stereoselective. This is because of the loss of diastereofacial discrimination in the approach of the aldehyde to the nucleophile (i.e., DHAP)–enzyme complex.

In summary, we have demonstrated the versatility of the enzymatic aldol addition reactions for the synthesis of new

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conformationally constrained iminocyclitols of the benzopyrrolidizine and cyclohexapyrrolizidine type. This strategy compares favorably with the chemical synthesis of bicyclic pyrrolidizines that started from more elaborate chiral polyhydroxylated pyrrolidine derivatives.[12] The compounds obtained in this work have not been previously described, and their biological activity against commercial glycosidases is currently in progress and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

General methodology and detailed experimental compound synthesis, analytical data, copies of ^{1}H, ^{13}C NMR and two-dimensional NMR experiments for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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