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Chemo-enzymatic synthesis and glycosidase inhibitory properties of DAB and LAB derivatives†

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Alda Lisa Concia,[a] Livia Gómez,[a] Jordi Bujons,[a] Teodor Parella,[b] Cristina Vilaplana,[c] Pere Joan Cardona,[c] Jesús Joglar[a] and Pere Clapés*[a]

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A chemo-enzymatic strategy for the preparation of 2-aminomethyl derivatives of (2*R*,3*R*,4*R*)-2-(hydroxymethyl)pyrrolidine-3,4-diol (also called 1,4-dideoxy-1,4-imino-D-arabinitol, DAB) and its enantiomer LAB is presented. The synthesis is based on the enzymatic preparation of DAB and LAB followed by the chemical modification of their hydroxymethyl functionality to afford diverse 2-aminomethyl derivatives. This strategy leads to novel aromatic, aminoalcohol and 2-oxopiperazine DAB and LAB derivatives. The compounds were preliminarily explored as inhibitors of a panel of commercial glycosidases, rat intestinal disaccharidases and against *Mycobacterium tuberculosis*, the causative agent of tuberculosis. It was found that the inhibitory profile of the new products differed considerably from the parent DAB and LAB. Furthermore, some of them were active inhibiting the growth of *M. tuberculosis*.

1. Introduction

Polyhydroxylated pyrrolizidine 2-aminomethyl derivatives of (2*R*,3*R*,4*R*)-2-(hydroxymethyl)pyrrolidine-3,4-diol (also called 1,4-dideoxy-1,4-imino-D-arabinitol, DAB) (**1**),[1] isolated from *Arachniodes standishii* and *Angylocalyx boutiqueanus*,[2] as well as from marine sponges,[3] and its corresponding enantiomer LAB (**2**), are interesting iminocyclitols for their structural simplicity and exceptional biological activity.[4] They show a strong inhibitory effect on α-glucosidases from different sources[5–7] as well as on mice and rat intestinal glycosidases.[7,8] DAB is also a potent inhibitor of glycogen phosphorylase[9] and mulberry leaf extracts containing DAB have also been shown to possess an antihyperglycemic effect on streptozocin (STZ)-induced diabetic mice.[10] The activity regulation of glycogen-degrading enzymes in addition to the reduction of the postprandial glycaemia in blood makes DAB and LAB promising therapeutic agents for the prevention and treatment of type-II diabetes,[11,12] and the metabolic syndrome associated to hypercaloric diets.[12–14]

Structural modification of DAB and LAB has led to novel derivatives with unprecedented activities and selectivities. For instance, preliminary experiments suggest that 1,4-dideoxy-2-hydroxymethyl-1,4-imino-L-threitol (**3**, Fig. 1) may have a role in the mechanism of chaperoning the folding of the cystic fibrosis transmembrane conductance regulator (CFTR).[15] 2-Acetamido-1,4-imino-1,2,4-trideoxy-L-arabinitol (**4**) and its *N*-benzyl derivative (**5**) were found to be potent non-competitive inhibitors of D-hexosaminidase, that may lead to new strategies for the treatment of diseases such as cancer, arteriosclerosis and some lysosomal storage diseases, such as Tay–Sachs or Sandhoff diseases.[16] Moreover, it has been reported that modification of the hydroxymethyl moiety to generate 3,4-dihydroxypyrrolidin-2-yl (**6**) derivatives with different stereochemistries at positions 2, 3 and 4 has been performed, furnishing potent inhibitors of α-mannosidases.[17–19]

It was found that (2*R*,3*R*,4*S*)- and (2*S*,3*R*,4*S*)-2-aminomethylpyrrolidine-3,4-diol derivatives (**7**, **8**, Fig. 1) with aromatic

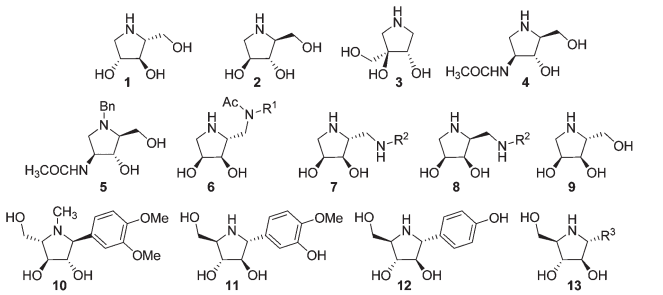

Fig. 1 Structures of biologically active pyrrolidine derivatives.

[a]*Dept Química Biológica y Modelización Molecular, Instituto de Química Avanzada de Cataluña, IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain. E-mail: pere.clapes@iqac.csic.es*
[b]*Servei de Ressonància Magnètica Nuclear, Departament de Química, Universitat Autònoma de Barcelona, Bellaterra, Spain*
[c]*Unitat de Tuberculosi Experimental, Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Universitat Autònoma de Barcelona, CIBERES, Edifici Laboratoris de Recerca, Crtra. de Can Ruti, Camí de les Escoles, s/n, 08916, Badalona, Spain*

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moieties lead to potent and selective competitive inhibitors of α-D-mannosidase from jack beans and from almonds.[18] The parent iminocyclitol, 1,4-dideoxy-1,4-imino-D-ribitol (**9**), with the two vicinal *cis*-oriented hydroxyl groups mandatory for effective inhibition, already possessed inhibitory activity against mannosidase.[20] Furthermore, a class of polyhydroxylated pyrrolidine derivatives were uncovered, namely codonopsinol[21] (**10**) and radicamines A (**11**) and B (**12**) isolated from *Codonopsis clematidea* and *Lobelia chinensis* Lour, respectively,[22] in which an aryl moiety is directly attached to the C-2 position of the pyrrolidine ring (**13**) (Fig. 1). These compounds were found to be inhibitors of α-glucosidase at the micromolar range.[23]

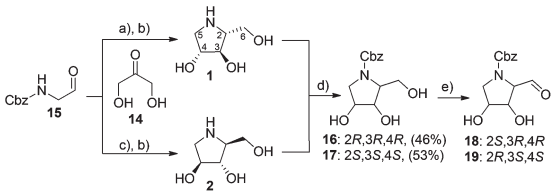
Inspired by these results we envisaged that modifications of DAB and LAB iminocyclitols may improve their inhibitory profiles in terms of potency and/or selectivity. Following our ongoing research program to develop new chemo-enzymatic methods for the synthesis of iminocyclitols as potential inhibitors of digestive glycosidases that decrease the post-prandial glycaemia,[7,14,24] we explored new derivatives with potential application in this field. Hence, it was regarded as interesting and significant to investigate on the synthesis and preliminary inhibitory properties of new 2-aminomethyl derivatives of DAB and LAB. Towards this end, a chemo-enzymatic strategy was envisaged to modify the hydroxymethyl functionality on DAB and LAB iminocyclitols with different aminomethyl moieties from aromatic amines, aminoalcohols or amino acid derivatives. Moreover, piperazines and their keto analogues, such as 2-oxopiperazines, have been recognized amongst the most important scaffolds in medicinal chemistry and drug discovery industries.[25–27] This prompted us to study the conjugation of these two moieties to obtain iminocyclitol fused 2-oxopiperazine derivatives, namely hexahydropyrrolo[1,2-*a*]pyrazin-4(1*H*)-one derivatives.

Preliminary exploration of the inhibitory properties of these compounds was directed towards commercial glycosidases and rat intestinal glycosidases. Moreover, the iminocyclitol derivatives active against α-L-rhamnosidase from *Penicillium decumbens* were assayed as inhibitors of the growth of the H37Rv Pasteur *Mycobacterium tuberculosis* strain, the causative agent of tuberculosis.[28]

2. Results and discussion

2.1 Synthesis

We envisaged that aldehydes **18** and **19** could be the most straightforward key intermediates to generate a collection of (2*R*,3*R*,4*R*)- and (2*S*,3*S*,4*S*)-3,4-dihydroxypyrrolidin-2-yl derivatives (Scheme 1). We have recently developed a straightforward chemo-enzymatic asymmetric stereodivergent methodology for the expedient synthesis of **1** and **2** starting from simple and achiral materials such as dihydroxyacetone (**14**) and *N*-Cbz-glycinal (**15**) (Scheme 1).[29,30] In this work, we optimized the conditions to obtain, after the imino protection with the Cbz, the highly valuable **16** and **17** intermediates in 46% and 53%



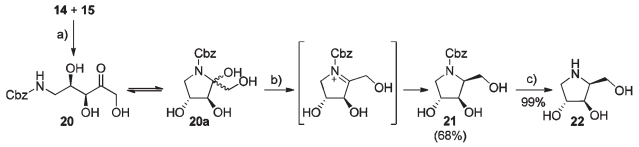
Scheme 1 Chemo-enzymatic synthesis of DAB (**1**) and LAB (**2**) derivatives. (a) D-Fructose-6-phosphate aldolase (FSA) mutant A129S/A165G, (b) H_2 (50 psi) Pd/C, (c) L-rhamnulose-1-phosphate aldolase from *E. coli* (RhuA), 200 mM aqueous borate buffer, (d) Cbz-OSu, 1 : 1 dioxane–water, (e) iodoxybenzoic acid (IBX), AcOEt reflux. Isolated yields in parentheses. Compounds **18** and **19** were not isolated.

isolated yields, respectively, which compare favorably with other reported procedures (*e.g.*, between 18–30% for the underivatized DAB and LAB).[5,31]

At this point we pursued a more efficient route that avoids removing and reintroducing the Cbz group. Therefore, we sought a potential new method to convert directly the aldol adduct into *N*-Cbz-protected DAB or LAB derivatives. In previous works we have observed that the products formed from the aldol addition of DHAP, DHA or glycolaldehyde to *N*-Cbz-aminoaldehydes consist of a mixture of acyclic and cyclic hemiaminal compounds in equilibrium (Scheme 2, **20–20a**).[32,33] The percentage of the hemiaminal was particularly significant for the five membered ring, owing to the minimal transition state strain energy for its formation as compared with other ring sizes.[34] Thus, we intended to perform a reductive dehydroxylation of the cyclic hemiaminal using the silane procedure in the presence of $BF_3{\cdot}OEt_2$ (Scheme 2).[35] The aldol adduct from the FSA catalyst was taken as an example. Interestingly, the reaction proceeded in 68% isolated yield; however, as ascertained by high field NMR data of the resulting iminocyclitol, the major configuration at C2 was opposite to that obtained with Pd/C with a 4 : 1 2*S* : 2*R* diastereomeric ratio. Hence, (2*S*,3*R*,4*R*)-2-(hydroxymethyl)pyrrolidine-3,4-diol (**22**) was obtained instead of **1**.

Remarkably, this result encompasses more general applicability in the chemo-enzymatic strategy for the synthesis of iminocyclitols, providing a useful potential stereocomplementary method for the reductive amination.

Following the synthetic plan, the hydroxymethyl group of **16** and **17** was selectively oxidized with iodoxybenzoic acid (IBX) to furnish aldehydes **18** and **19**, respectively (Scheme 1). With the aim to develop a straightforward methodology, these



Scheme 2 Chemo-enzymatic synthesis of (2*S*,3*R*,4*R*)-2-(hydroxymethyl)pyrrolidine-3,4-diol: (a) D-fructose-6-phosphate aldolase from *E. coli* mutant A129S/A165G, (b) $BF_3{\cdot}OEt_2$, and Et_3SiH in CH_2Cl_2 −78 °C to 0 °C, (c) H_2 (50 psi) Pd/C. Isolated yield in parentheses.

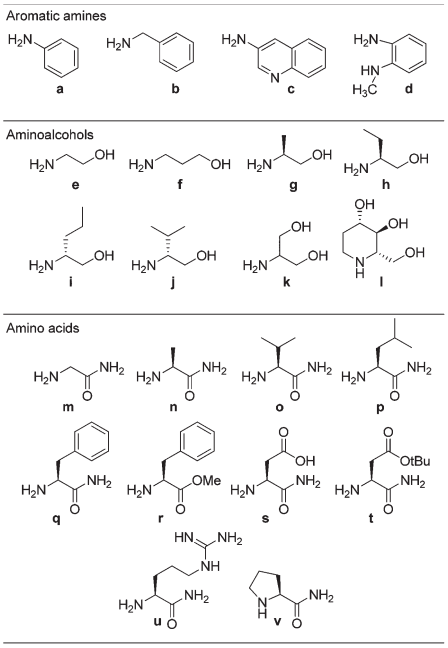


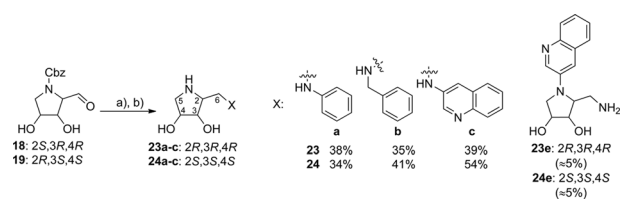
Fig. 2 Amines used for the preparation of the 2-aminomethyl derivatives of compounds **1** and **2**.

aldehydes were readily used after evaporation of the solvent without the need for isolation and purification. Indeed, the aldehydes reacted with primary amines ($R–NH_2$, Fig. 2) in anhydrous methanol at 20 °C under acidic conditions (acetic acid, pH = 5.0), furnishing the corresponding imines that were reduced *in situ* with $NaBH_3CN$. Deprotection of the Cbz group by hydrogenolysis in the presence of Pd/C gave the corresponding 2-aminomethyl derivatives of compounds **1** and **2** (see the next sections).

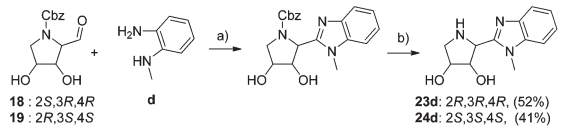
DAB and LAB derivatives with aromatic amines. The strategy depicted above worked for the selected aromatic amines **a**, **b** and **c**, which furnished derivatives **23a–c** and **24a–c** (Scheme 3) in 38–44% and 34–54% isolated yields, respectively.

Furthermore, unexpectedly (2*R*,3*R*,4*R*)- and (2*S*,3*S*,4*S*)-2-(aminomethyl)-1-(quinolin-3-yl)pyrrolidine-3,4-diol (**23e** and **24e**, respectively) were isolated (5%) after treatment of *N*-Cbz derivatives of **23c** and **24c** with H_2 in the presence of Pd/C. These products were characterized and are described in the Experimental section and ESI.†

Expectedly, the reaction of the aldehydes **18** and **19** with *N*-methyl-1,2-phenylenediamine (**d**), in the presence of acetic



Scheme 3 Synthesis and isolated yields of 2-aminomethyl aromatic derivatives **23** and **24**: (a) amines **a–c**, $NaBH_3CN$, AcOH, (b) H_2 (22 psi) Pd/C. Structures of compounds **23e** and **24e**.



Scheme 4 Synthesis of benzimidazole derivatives **23d** and **24d**. (a) AcOH at 25 °C, spontaneous oxidation; (b) H_2 (22 psi), Pd/C. Isolated yields in parentheses.

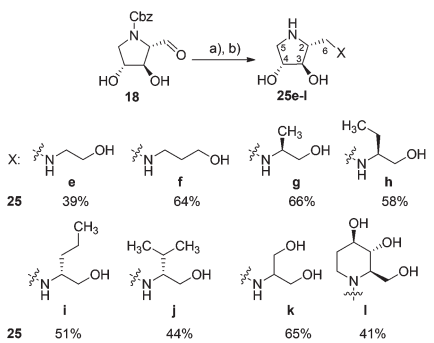
acid and O_2, gave the corresponding benzimidazole derivatives **23d** and **24d** in 52% and 41% isolated yields, respectively (Scheme 4).[36]

DAB aminoalcohol conjugates. In a similar line of thinking, DAB aminoalcohol conjugates were also considered. They can be regarded as analogues of 1,4-dideoxy-1,4-iminoalditols in which the corresponding polyhydroxyl chain is substituted by an aminoalcohol through a C–N bond.[37] Moreover, the introduction of different functionalities might increase the recognition sites of the molecule, improving its activity and selectivity.

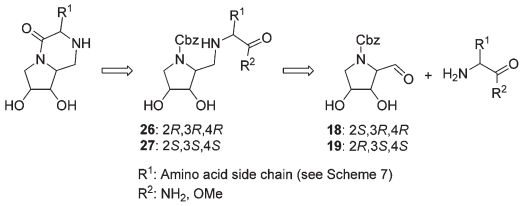
Following the devised strategy, aminoalcohols **e–k** provided the corresponding DAB conjugates (**25e–k**, Scheme 5) in 39–66% isolated yields. Secondary alcohols, *e.g.* D-fagomine (**l**), also reacted with the aldehyde affording the corresponding iminocyclitol conjugate (**25l**) in 41% isolated yield. It can be anticipated that an identical methodology can be followed to obtain the corresponding LAB conjugates.

The NMR analysis showed that for some conjugates a secondary product corresponding to the (2*S*,3*R*,4*R*)-2-(hydroxymethyl)pyrrolidine-3,4-diol 2-aminoalcohol derivative (see ESI†) was identified. This resulted from epimer formation at C2 that probably occurred during imine generation. The amount varied depending on the aminoalcohol employed, *e.g.* 25 : 75 2*S* : 2*R* **25e**, 1 : 9 2*S* : 2*R* **25f**, 17 : 83 2*S* : 2*R* **25g** and **25h**, though it was not observed for conjugates **25i–l**. Separation of epimer pairs was accomplished by cation exchange chromatography under conditions established in previous work (as an example see NMR spectra of purified compound **25g** in ESI†).[24]

Amino acid conjugates: preparation of iminocyclitol fused 2-oxopiperazine derivatives. By analogy to the general methodology described above, we envisaged that the aldehydes



Scheme 5 Synthesis and isolated yields of DAB aminoalcohol conjugates. (a) Aminoalcohol **e–l**, $NaBH_3CN$, AcOH; (b) H_2 (22 psi), Pd/C.

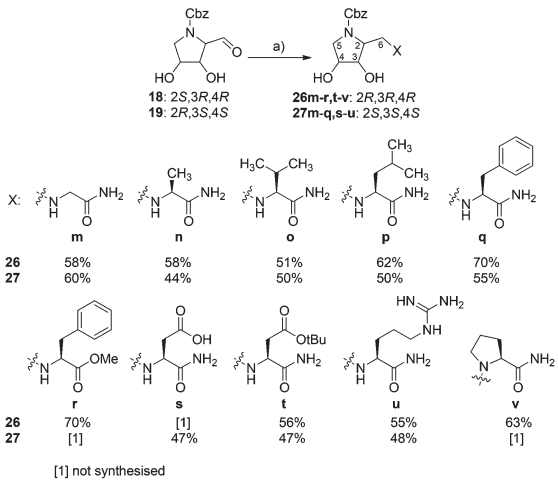


Scheme 6 Synthetic strategy towards iminocyclitol fused 2-oxopiperazine derivatives.

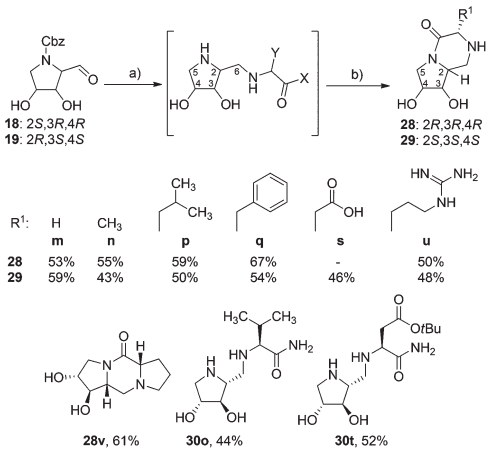
18 and **19** can react with 1 equiv. of an amino acid to furnish the 2-aminomethyl DAB or LAB derivatives **26** and **27**, respectively. Then, using ideas stemming from peptide chemistry, intramolecular lactamization can be forced, after Cbz removal from **26** and **27**, by analogy with the undesired diketopiperazine formation that occurs during solid phase peptide synthesis (Scheme 6).[26,38]

Given the approach adumbrated above, the reaction between aldehydes **18** or **19** and 1 equiv. of the selected amino acid derivatives (Scheme 7) furnished the corresponding DAB (**26m–r, t–v**) or LAB (**27m–q, s–u**) conjugates. Amino acid amides (*i.e.*, amide derivatives from amino acids) gave good isolated yields (51–70%) and were the derivatives of choice. Amino acid esters (*i.e.*, ester derivatives from amino acids) were the most obvious selection but it was discouraging to find out that only phenylalanine methyl ester (**r**) was readily able to form the corresponding **26r** derivative. Furthermore, the β-carboxylate of the aspartic acid amide (**s**) must be protected (**t**) to make the reaction proceed towards the product **26t** (or **27t**).

The next step entailed the deprotection of the Cbz group and the lactamization (*i.e.* intramolecular aminolysis) to furnish the corresponding iminocyclitol fused 2-oxopiperazine moiety (Scheme 8). Thus, the glycinamide conjugate **26m** gave complete lactamization readily after the removal of the Cbz group at room temperature. The alaninamide and prolinamide



Scheme 7 Synthesis and isolated yields of *N*-Cbz-DAB and -LAB amino acid amide and ester conjugates: (a) amino acids **m–v**, $NaBH_3CN$.



Scheme 8 Synthesis and isolated yields of iminocyclitol fused 2-oxopiperazine **28** and **29** and amino acid conjugate derivatives **30o**, **t**. (a) H_2 (22 psi), Pd/C; (b) cyclization at rt, 40 °C or 100 °C (see text).

conjugates **26n** and **26v**, respectively, needed 40 °C overnight to afford the complete reaction, whereas the rest required overnight heating at 100 °C. Attempted lactamization of the valinamide derivative **26o** failed under various reaction conditions, probably due to steric effects of the isopropyl group. The conjugate **26t** did not form the cyclic species under mild conditions, while a number of unidentified by-products appeared when it was heated at 100 °C. Removal of the β(O^tBu) group (**26s**) did not help the lactamization reaction, decomposing under the reflux conditions in water. The phenylalanine amide derivative **26q** furnished only 50% of the bicyclic product, whereas quantitative yields were obtained from the corresponding methyl ester derivative **26r**.

For the LAB derivatives, the cyclization was faster and Gly–NH_2 (**m**), Ala–NH_2 (**n**) and Leu–NH_2 (**p**) reacted at room temperature, readily after the removal of the Cbz protecting group. Derivatives from Phe–NH_2 (**q**), Asp–NH_2 (**s**) and Arg–NH_2 (**u**) partially cyclize at room temperature and the reaction was completed at 40 °C. The aspartic acid derivative, **27s**, gave the corresponding intramolecular product in quantitative yields whereas the corresponding O^tBu protected analogue (**27t**) did not react. Moreover, the Phe–NH_2 conjugate **27q** gave the corresponding 2-oxopiperazine–iminocyclitol derivative at 40 °C. Val (**o**) was the only derivative that did not perform the lactamization reaction.

These resulted in a new class of hexahydropyrrolo[1,2-*a*]-pyrazin-4(1*H*)-one derivatives (*i.e.* 2-oxopiperazine derivatives) that might be considered indolizidine analogues (Scheme 8).

2.2 Inhibitory activity

Commercial glycosidases. The DAB and LAB derivatives **23a–d**, **24a–d**, **25e–l**, **28**, **29** and **30** were screened as inhibitors against a panel of commercial glycosidases (Table 1) and rat intestinal disaccharidases, *i.e.* sucrase, lactase, threalase and maltase (Table 2). The inhibitory activity was compared with that of the parent compounds **1** and **2**, measured in our laboratory in previous work.[7] The inhibitory profiles of the new

Table 1 Activities, IC_{50} (μM) and K_i (μM) (in parentheses), of the compounds synthesized against commercial glycosidases[a]

Product	α-D-Glucosidase baker's yeast	α-D-Glucosidase rice	β-D-Glucosidase sweet almonds	β-D-Galactosidase bovine liver	α-L-Rhamnosidase *P. decumbens*	α-D-Mannosidase jack beans	α-L-Fucosidase bovine kidney
23a	n.i.	n.i.	370 ± 11 (215 ± 18) C	155 ± 19 (406 ± 128) NC, $\alpha = 1$	n.i.	n.i.	n.i.
24a	40 ± 6 (44 ± 13) NC, $\alpha = 1$	620 ± 29 (236 ± 61) C	n.i.	n.i.	15.6 ± 0.5 (38 ± 12) NC, $\alpha = 1$	n.i.	n.i.
23b	n.i.	n.i.	832 ± 105 (338 ± 116) C	n.i.	n.i.	n.i.	n.i.
24b	136 ± 13 (208 ± 102) NC, $\alpha = 1$	n.i.	38 ± 4 (41 ± 13) C	n.i.	274 ± 35 (699 ± 112) NC, $\alpha > 1$	n.i.	n.i.
23c	165 ± 44 (170 ± 95) C	n.i.	n.i.	263 ± 50 (308 ± 55) NC, $\alpha\alpha = 1$	n.i.	n.i.	n.i.
24c	247 ± 26 (100 ± 66) NC, $\alpha = 1$	n.i.	n.i.	n.i.	132 ± 31 (132 ± 27) C	n.i.	n.i.
24d	n.i.	n.i.	n.i.	n.i.	320 ± 130 (331 ± 19) NC, $\alpha = 1$	n.i.	n.i.
25e	273 ± 31 (358 ± 52) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
25f	158 ± 25 (153 ± 8) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
25h	n.i.	n.i.	260 ± 100 (734 ± 19) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.
25i	4.6 ± 1.6 (2.1 ± 0.8) C	342 ± 90 (144 ± 90) C	150 ± 71 (223 ± 9) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.
25j	110 ± 13 (96 ± 10) NC $\alpha > 1$	n.i.	460 ± 353 (561 ± 30) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.
25l	n.i.	466 ± 64 (186 ± 21) C	n.i.	n.i.	n.i.	n.i.	n.i.
28n	n.i.	76 ± 19 (39 ± 2) NC $\alpha = 1$	n.i.	1116 ± 28	n.i.	n.i.	n.i.
28p	9.2 ± 1.5 (7.1 ± 1.5) C	208 ± 23 (261 ± 29) C	285 ± 15 (394 ± 19) C	n.i.	n.i.	n.i.	n.i.
28u	n.i.	60 ± 19 (21 ± 7) C	n.i.	n.i.	n.i.	n.i.	n.i.
29m	401 ± 35 (323 ± 65) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
29n	239 ± 9 (323 ± 64) NC $\alpha > 1$	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
29p	340 ± 25 (337 ± 84) NC $\alpha > 1$	14 ± 2 (13 ± 2) C	n.i.	n.i.	n.i.	n.i.	n.i.
29q	50 ± 14 (76 ± 8) UC	148 ± 50 (87 ± 25) C	n.i.	691 ± 86	174 ± 12 (103 ± 27) NC $\alpha = 1$	n.i.	n.i.
29s	41 ± 8 (181 ± 24) UC	382 ± 41 (176 ± 4) C	n.i.	n.i.	n.i.	n.i.	n.i.
29u	140 ± 44 (124 ± 13) NC $\alpha = 1$	n.i.	n.i.	n.i.	709 ± 56	n.i.	n.i.

Table 1 *(Contd.)*

Product	α-D-Glucosidase baker's yeast	α-D-Glucosidase rice	β-D-Glucosidase sweet almonds	β-D-Galactosidase bovine liver	α-L-Rhamnosidase *P. decumbens*	α-D-Mannosidase jack beans	α-L-Fucosidase bovine kidney
30o	1.1 ± 0.1 (54 ± 20) NC $\alpha > 1$	448 ± 214 (190 ± 33) C	n.i.	n.i.	n.i.	n.i.	n.i.
30t	3.9 ± 3.1 (16.7 ± 7.8) C	685 ± 110 (451 ± 170) C	n.i.	n.i.	n.i.	n.i.	n.i.
1	0.33 ± 0.02 (0.17 ± 0.01) C	218 ± 3 (104 ± 75) C	276 ± 25 (100 ± 64) C	n.i.	n.i.	286 ± 27 (111 ± 60) NC, $\alpha > 1$	20 ± 1 (5 ± 1) C
2	1.8 ± 0.1 (0.8 ± 0.1) NC, $\alpha = 1$	0.05 ± 0.01 (0.040 ± 0.003) NC, $\alpha > 1$	685 ± 112 (1014 ± 81) NC, $\alpha > 1$	n.i.	56 ± 5 (98 ± 5) NC, $\alpha = 1$	n.i.	n.i.

[a] Data are means of triplicate experiments ± standard error of the mean (SE). C: competitive inhibition. NC: noncompetitive inhibition. UC: uncompetitive inhibition.[46] n.i.: no inhibition, *i.e.* $IC_{50} \geq 1$ mM.

Table 2 Activities, IC_{50} (μM), of the compounds synthesized against rat intestinal saccharidases[a]

Product	Sucrase (3.91 ± 0.05 UI)[b] ($n = 2$)	Lactase (0.85 ± 0.07 UI)[b] ($n = 2$)	Maltase (26.89 ± 1.50 UI)[b] ($n = 2$)
23a	318 ± 101	n.i.	n.i.
24a	538 ± 356	n.i.	n.i.
23c	739 ± 392	n.i.	n.i.
24c	321 ± 59	n.i.	n.i.
24d	173 ± 72	n.i.	n.i.
25i	99 ± 52	n.i.	77 ± 34
28n	57 ± 29	n.i.	119 ± 11
28p	320 ± 57	n.i.	304 ± 23
28u	77 ± 17	n.i.	151 ± 2
29p	19 ± 11	n.i.	56 ± 2
29q	174 ± 84	n.i.	263 ± 99
29s	342 ± 123	n.i.	n.i.
1	22 ± 12	140 ± 84	50 ± 36
2	0.29 ± 0.02	50 ± 36	0.2 ± 0.1

[a] The experiments were performed in triplicate for each set of saccharidases. Two sets of saccharidases from two different rats ($n = 2$) were used. IC_{50}s are expressed as μM ± standard error of the mean (SE). [b] 1 UI corresponds to 1 μmol of glucose formation per hour at 37 °C in phosphate buffer, pH 6.8. UI (μmol substrate h^{-1} mg^{-1} protein); n.i. no inhibition, $IC_{50} \geq 1$ mM.

derivatives differ considerably from those of **1** and **2** (Table 1). Compounds **23d**, **25g**, **25k**, **28m**, **28q** and **28v** (not included in Table 1) were not inhibitors (*i.e.* $IC_{50} \geq 1$ mM) of any assayed glycosidase.

α-D-Glucosidase from baker's yeast and rice: The aromatic conjugates **23a–c** and **24a–d** have much lower inhibitory properties against α-D-glucosidase from baker's yeast than the parent compounds DAB and LAB (Table 1). Only compound **24a** showed moderate non-competitive inhibition against this glucosidase. Among the rest of the derivatives, compounds **25i**, **28p**, and **30t** were the best competitive inhibitors of α-D-glucosidase from baker's yeast with comparable activities. Compound **30o** was the best non-competitive inhibitor of this glucosidase. Although the structure of the substituent differs considerably, all of them have a DAB configuration (see Schemes 6 and 8). The aromatic derivatives and aminoalcohol conjugates were mostly inactive against α-D-glucosidase from rice. Among the fused 2-oxopiperazine iminocyclitol derivatives, compound **29p** was the best inhibitor, while **28n** and **28u** showed inhibitory properties comparable to the parent compound DAB.

β-D-Glucosidase from sweet almonds: Compound **24b** was a moderate inhibitor of β-D-glucosidase from sweet almonds while the parent compound LAB and its enantiomer DAB were weak inhibitors of this glycosidase. The rest of the evaluated compounds had either weak activities or were completely inactive against this glycosidase.

β-D-Galactosidase from bovine liver: Aromatic derivatives **23a**, **23c** and **24c** were weak inhibitors while the parent DAB and LAB were inactive towards this glycosidase. It appears that the presence of an aromatic moiety is necessary for the inhibitory activity.[39,40] Indeed, among the 2-oxopiperazine iminocyclitol fused derivatives only **29q**, derived from phenyalanine, showed some activity. The rest of the derivatives were not inhibitors of this glucosidase.

α-L-Rhamnosidase from *Penicillium decumbens*: Among the aromatic substitutions only those with LAB configuration, **24a–d**, were moderately to weakly active against α-L-rhamnosidase; consequently, the orientation of the hydroxyl groups and the amine moiety is a strong factor that determines the right interaction with the glycosidase. It has been suggested that the α-L-rhamnosidase inhibition shown by some pyrrolidines can be rationalized in terms of stereochemical similarities with α-L-rhamnose.[40,41] Thus, it is apparent that the stereochemistry of **24a–d** (2*S*,3*S*,4*S*) matches that of the rhamnose moiety at C-3, C-4 and C-5. This would place the 2-aryl containing substituent at the same location as the 5-methyl group of rhamnose. The best inhibitor was compound **24a** whose structure is similar to those reported by Chapman *et al.* (*e.g.* (2*S*,3*S*,4*S*)-2-benzylpyrrolidine-3,4-diol).[40] Moreover, Kim *et al.* also proposed a role as aglycone for hydrophobic substituents at the C-2 position of pyrrolidines.[41] This is consistent

with the absence of inhibitory properties of the aminoalcohol and 2-oxopiperazine derivatives, where basically non-aromatic moieties are present.

α-D-Mannosidase from jack beans and α-L-fucosidase from bovine kidney: It appears that a *cis* arrangement of the hydroxyl groups at C-3 and C-4, which parallels that of the hydroxyl groups at C-2 and C-3 of α-D-mannose and other known pyrrolidine inhibitors like mannostatin A, is a strong structural requirement for the inhibition of α-D-mannosidase by pyrrolidine iminocyclitols.[19,42,43] The fact that the compounds synthesized do not match this C-3 and C-4 configuration is probably the reason for their inability to inhibit this glycosidase.

Similarly to α-D-mannosidase, α-L-fucosidase has also a strong stereochemical demanding active site.[43] Pyrrolidine derivatives with stereoconfiguration (3*S*,4*R*,5*S*) were reported as strong inhibitors of α-L-fucosidase,[43,44] whereas diastereomeric analogues were usually moderate to weak inhibitors.[45] The derivatives obtained in the present work have (3*R*,4*R*) or (3*S*,4*S*) configuration, therefore they showed no inhibition activities.

Intestinal disaccharide glycosidases. Concerning their inhibitory properties against intestinal disaccharide glycosidases, **23a**, **23c**, **24a**, **24c** and **24d** were weak inhibitors of sucrase while the parent compounds DAB and LAB were moderate to strong inhibitors of this glycosidase (Table 2). Moreover, the LAB derivatives **24a–d** lost the strong inhibitory activity against maltase. Among the aminoalcohol derivatives only compound **25i** showed inhibitory activity against sucrase and maltase. Concerning the iminocyclitol fused 2-oxopiperazine derivatives, it is remarkable the activity of **29p** against sucrase and maltase, which is comparable to the DAB compound, but with no inhibitory activity against lactase. This compound was also the best against α-D-glucosidase from rice. The rest of the compounds showed moderate to weak activity on both sucrase and maltase. Interestingly, no inhibitory activity was observed against lactase for the compounds under study.

Inhibitory activity against *Mycobacterium tuberculosis* H37Rv laboratory strain. It has been suggested that the iminocyclitol derivatives which are inhibitors of α-L-rhamnosidase from *P. decumbens* could have the ability to inhibit dTDP-L-rhamnose biosynthesis on *M. tuberculosis*, and therefore constitute potential chemotherapeutic agents for tuberculosis.[28] The inhibitors of α-L-rhamnosidase found in this work were assayed in mycobacterial systems and the Minimum Inhibitory Concentration values (MIC; *i.e.* the lowest drug concentration that prevented the development of colour akin to the bacteria growth) were determined (Table 3). The lowest MIC values were obtained for compounds **23d**, **24b** and **24d**, whereas no significant differences relative to **1** and **2** were observed for the other compounds. This might indicate that the products are not acting on the target rhamnosidase processing enzymes of the cell wall. The standard compound isoniazid (MIC: 0.25 μg mL^{-1}) was evaluated as a positive control in this assay and it was consistent with the reported values for this product.[47]

Table 3 Minimum inhibitory concentrations (mg mL^{-1}) against the H37Rv Pasteur *M. tuberculosis* strain

Product	MIC (mg mL^{-1})	Product	MIC (mg mL^{-1})
23a	2.5	**24a**	2.5
23b	1.3	**24b**	0.31
23c	1.3	**24c**	2.5
23d	0.63	**24d**	0.63
1	2.5	**2**	2.5

3. Conclusions

An efficient chemo-enzymatic strategy for the synthesis of 2-aminomethyl derivatives of DAB (**1**) and LAB (**2**) was developed. The straightforward chemo-enzymatic access to iminocyclitols **1** and **2**, the use of mild and selective oxidation conditions with IBX, followed by a reductive amination step, provides easy access to a number of new derivatives with aromatic amines, aminoalcohols or amino acids, the latter leading to the formation of piperazine–iminocyclitol fused compounds. An alternative method to generate the Cbz-iminosugar derivatives was tested that led to (2*S*,3*R*,4*R*)-2-(hydroxymethyl)pyrrolidine-3,4-diol. This may open new possibilities for obtaining novel stereocomplementary derivative epimers at C-2 to those from **1** and **2**.

The inhibitory properties of the new **1** and **2** derivatives against the panel of commercial glycosidases and rat intestinal disaccharidases differed considerably from those of the parent compounds DAB and LAB. The aromatic aminomethyl derivatives **23** and **24** are moderate to good inhibitors of α-L-rhamnosidase. Particularly **24a** has better inhibitory properties than its parent LAB. Aromatic derivatives **23a–d** and **24a–d** were found to be moderate inhibitors of the growth of *M. tuberculosis* H37Rv laboratory strain, the causative agent of tuberculosis. However, the inhibitory pattern could not be correlated with the activity observed against α-L-rhamnosidase. The aminoalcohol, amino acid and oxopiperazine derivatives are selective inhibitors of α-D-glucosidases, particularly **25i** and **30t** were selective towards α-D-glucosidase from baker's yeast. Concerning the intestinal disaccharide glycosidases it is remarkable that the derivatives are selective for sucrase and maltase and completely devoid of activity against rat intestinal lactase. Particularly **29p** has inhibitory activity against sucrase and maltase comparable to DAB, but it does not inhibit lactase.

Experimental

(2*R*,3*R*,4*R*)-*N*-Benzyloxycarbonyl-2-(hydroxymethyl)pyrrolidine-3,4-diol (**16**). FSA A165G (150 U, 150 mg, 1.06 U mg^{-1}) and dihydroxyacetone DHA (1.3 g, 14.5 mmol) were dissolved in 0.1 M triethanolamine buffer, pH 7.0 (135 mL). This solution was added to *N*-Cbz-glycinal (2.25 g, 11.6 mmol) dissolved in *N*,*N*-dimethylformamide (DMF, 15 mL) and the mixture was shaken at 25 °C. After 24 h (99% conversion) the crude was

diluted with H_2O up to a volume of 150 mL and loaded onto a glass column (5 cm diameter × 20 cm length, volume 400 mL) packed with the Amberlite™ XAD™ 16 (Rohm and Haas) stationary phase, which was previously equilibrated with H_2O. After loading, the column was washed with H_2O (800 mL, 2 column volumes). This step removed efficiently the enzyme, the excess of DHA and the salts. The aldol adduct was then eluted with 2 : 3 H_2O–EtOH (800 mL, 2 column volumes). The fractions containing the product were pooled and the solvent reduced to about 300 mL in vacuum. The aqueous residue was diluted with EtOH (200 mL). The solution was divided into two batches (250 mL) and each one was treated overnight with H_2 (50 psi) in the presence of Pd/C (0.4 g, 10% Pd). The catalyst was removed by filtration and EtOH–water was evaporated in vacuum. NMR data and physical properties matched those already published using the same methodology.[29,33] The aqueous solution (200 mL) was diluted with 1,4-dioxane (200 mL) and solid $NaHCO_3$ (1.9 g, 23.2 mmol) was added under stirring. After cooling to 0 °C, benzyl chloroformate (1.8 mL, 12.7 mmol) was added dropwise. After the addition, the reaction mixture was allowed to warm up to room temperature and it was stirred overnight. Dioxane was removed under vacuum, the aqueous residue was diluted with saturated $NaHCO_3$ solution (100 mL) and the product extracted with ethyl acetate (4 × 150 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuum. The crude material was purified by flash column chromatography on silica (from 100 : 0 to 9 : 1 EtOAc–MeOH) to yield the title compound (1.4 g, 46% yield). HPLC analysis: gradient elution from 10% to 70% B in 40 min; t_R = 14.5 min, 98% purity by HPLC. $[\alpha]_D^{22}$ = −28.0 (*c* 1.0 in MeOH) (lit.,[48] $[\alpha]_D^{22}$ = −28.9 (*c* 1.9 in MeOH); lit.,[5] $[\alpha]_D^{22}$ = −29.7 (*c* 0.3 in MeOH)). ^{1}H NMR (400 MHz, CD_3OD) δ 7.32 (m, 5H, arom), 5.13 (m, 2H, OC*H*$_2$Ph), 4.17 (s, 1H, H-3), 4.12 (s, 1H H-3-rotamer), 4.04 (br s, 1H, H-4), 3.82 (m, 4H, H-2-H-5-H-6-H-6′), 3.38 (m, 1H, H-5′) (complex spectrum due to the existence of rotamers as described also by Fleet and Smith[5]). ^{13}C NMR (101 MHz, CD_3OD) δ 157.3 (C═O), 157.1 (C═O rotamer), 138.0 (C-Ar), 137.9 (C-Ar rotamer), 129.5 (C-Ar), 129.1 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar), 128.8 (C-Ar), 79.4 (C-3), 78.7 (C-3 rotamer), 76.2 (C-4), 75.6 (C-4 rotamer), 68.6 (C-2), 68.3 (C-2 rotamer), 68.1 (*C*H$_2$Ph), 68.0 (*C*H$_2$Ph rotamer), 61.8 (C-6), 61.6 (C-6 rotamer), 54.8 (C-5), 54.4 (C-5 rotamer).

(2*S*,3*S*,4*S*)-*N*-Benzyloxycarbonyl-2-(hydroxymethyl)pyrrolidine-3,4-diol (**17**). RhuA (400 U, 32 mL of NH_4SO_4 suspension, 0.35 mg protein mL^{-1}, 12.5 U mL^{-1}) and dihydroxyacetone (DHA) (1.35 g, 15 mmol) were dissolved in sodium borate 0.25 M, pH 7.0 (160 mL). This solution was added to *N*-Cbz-glycinal (2.33 g, 12 mmol) dissolved in DMF (40 mL) and the mixture was shaken at 25 °C. After 24 h (90% conversion) the crude was diluted with H_2O up to a volume of 200 mL and loaded onto a glass column packed with Amberlite™ XAD™ 16 (Rohm and Haas) following an identical procedure to the one described above with minor modifications. The aldol adduct was then eluted with 2 : 3 H_2O–EtOH (1000 mL, 2.5 column volumes). The fractions containing the product were pooled and the solvent reduced to about 400 mL in vacuum. The aqueous residue was diluted with EtOH (400 mL). The solution was divided into two batches (400 mL) and each one was treated overnight with H_2 (50 psi) in the presence of Pd/C (0.6 g, 10% Pd). The catalyst was removed by filtration and EtOH–water was evaporated in vacuum. NMR data and physical properties matched those already published using the same methodology.[30] The aqueous solution (*ca.* 150 mL) was diluted with 1,4-dioxane (150 mL) and solid $NaHCO_3$ (1.8 g, 21.6 mmol) was added under stirring. After cooling to 0 °C, benzyl chloroformate (1.7 mL, 11.8 mmol) was added dropwise. After the addition, the reaction mixture was allowed to warm up to room temperature and it was stirred overnight. Dioxane was removed under vacuum, the aqueous residue was diluted with saturated $NaHCO_3$ solution (100 mL) and the product extracted with ethyl acetate (4 × 150 mL). The organic layer was dried over Na_2SO_4, and concentrated in vacuum. The crude material was purified by flash column chromatography on silica (from 100 : 0 to 9 : 1 EtOAc–MeOH) to yield the title compound (1.73 g, 53% yield, 98% purity by HPLC). $[\alpha]_D^{22}$ = +25.0 (*c* 1.2 in MeOH). HPLC retention time and NMR data were identical to those of DAB.

(2*S*,3*R*,4*R*)-2-(Hydroxymethyl)pyrrolidine-3,4-diol (also called 1,4-dideoxy-1,4-imino-L-xylitol) (**22**). The aldol adduct (500 mg, 1.77 mmol) obtained from the aldol addition of DHA to *N*-Cbz-glycinal catalysed by D-fructose-6-phosphate aldolase mutant A165G (see above) was dissolved in anhydrous CH_2Cl_2 (12 mL) and cooled down to 0 °C. A solution of $BF_3{\cdot}Et_2O$ (0.22 mL, 1.77 mmol, 1 equiv.) and subsequently Et_3SiH (0.57 mL, 3.54 mmol, 2 equiv.) were added under N_2 and the reaction mixture was left to reach room temperature under stirring. After 2 h the reaction was quenched to pH = 7 with saturated $NaHCO_3$ (5 mL). The aqueous layer was diluted with brine (5 mL), and the mixture extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (30 : 1 EtOAc–CH_3OH) to give the corresponding Cbz protected derivative **21** as an oil (320 mg, 68% yield). $[\alpha]_D^{22}$ = +20.0 (*c* 1.0 in MeOH). Purity 98% by HPLC. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.33 (m, 5H, arom), 5.14 (m, 2H, OC*H*$_2$Ph), 4.14 (m, 2H, H-3-H-4), 3.89 (m, 3H, H-2-H-6-H-6′), 3.65 (m, 1H, H-5), 3.40 (m, 1H, H-5′). ^{13}C NMR (101 MHz, CD_3OD) δ = 157.76 (C═O), 157.35 (C═O rotamer), 138.04 (C-Ar), 137.94 (C-Ar rotamer), 129.51 (C-Ar), 129.06 (2C-Ar), 128.86 (2C-Ar), 77.96 (C-3), 77.53 (C-3 rotamer), 75.21 (C-4), 74.86 (C-4 rotamer), 68.28 (CH_2Ph), 68.15 (CH_2Ph rotamer), 62.81 (C-2), 61.66 (C-2 rotamer), 60.85 (C-6), 60.27 (C-6 rotamer), 53.26 (C-5), 53.10 (C-5 rotamer). The presence of rotamers makes it difficult to obtain the relative stereochemistry. Moreover, some important signals were overlapped making it difficult to analyse the nOe experiments. Therefore it was decided to remove the Cbz group by treatment of a sample of the *N*-Cbz-1,4-dideoxy-1,4-imino-L-xylitol (40 mg, 0.14 mmol) with H_2 (22 psi) in the presence of Pd/C (20 mg). After removal of the Pd/C by filtration and lyophilisation, the title compound **22** (20 mg, 0.14 mmol) was obtained in quantitative yield. NMR and physical data matched those reported using other procedures.[49,50] $[\alpha]_D^{22}$ = −3.0 (*c* 1.0 in H_2O)

(lit.,[49] $[\alpha]_D^{22} = -4.0$ (*c* 0.10 in H_2O); lit.,[50] $[\alpha]_D^{22} = -4.4$ (*c* 0.04 in CH_3OH)). ^1H NMR (400 MHz, D_2O) δ = 4.25 (dt, *J* = 4.9, 1.9 Hz, 1H, H-4), 4.20 (dd, *J* = 4.0, 1.7 Hz, 1H, H-3), 3.88 (dd, *J* = 11.5, 6.0 Hz, 1H, H-6), 3.76 (dd, *J* = 11.5, 7.3 Hz, 1H, H-6′), 3.51 (ddd, *J* = 7.2, 6.1, 4.1 Hz, 1H, H-2), 3.41 (dd, *J* = 12.7, 5.0 Hz, 1H, H-5), 2.93 (dd, *J* = 12.8, 2.0 Hz, 1H, H-5′). ^{13}C NMR (101 MHz, D_2O) δ = 76.14 (C-4), 75.87 (C-3), 61.46 (C-2), 59.05 (C-6), 50.59 (C-5). HRMS (ESI-TOF): *m*/*z* $[M + H]^+$ for $C_5H_{12}NO_3^+$ calculated 134.0817; observed 134.0819.

Oxidation reaction. Typical example: Compound **16** (0.22 g, 0.82 mmol) was dissolved in AcOEt (50 mL) at 40 °C. To this solution, 2-iodoxybenzoic acid (IBX) (0.92 g, 3.3 mmol) was added and the reaction mixture was stirred under reflux. After 3.5 h the reaction mixture was cooled down to room temperature, the solid removed by filtration and the solvent evaporated under reduced pressure. This product was used in the next step without further purification and it was not characterized. *Reductive amination reactions*. Typical example: The crude from the oxidation reaction was dissolved in dry MeOH (50 mL) to have an aldehyde concentration of 0.016 M (0.8 mmol). To this solution, benzylamine (0.45 mL, 4.1 mmol) in glacial acetic acid (0.23 mL, 4.1 mmol) was added and stirred for 2 h at room temperature. Then, $NaBH_3CN$ (0.08 g, 1.23 mmol) was added and the reaction mixture was stirred overnight. The solvent was removed under reduced pressure, and the crude product was purified either by flash chromatography on silica or preparative HPLC. Purification by HPLC was performed as follows (general procedure). The crude was dissolved in MeOH and loaded onto a semi-preparative X-Terra Prep MS C-18, 10 μm, 19 × 250 mm column. The solvent system used was: solvent (A): aqueous trifluoroacetic acid (TFA) (0.1% (v/v)) and solvent (B): TFA (0.095% (v/v)) in 4 : 1 ACN–H_2O or plain MeOH. Salts and solvents were washed out with 100% A during 10 min. The product was eluted with a gradient of B (see below in each case). The flow rate was 10 mL min^{-1} and the products were detected at 215 nm. The fractions were analysed by HPLC. Fractions containing the product were pooled and lyophilized.

The Cbz-protected derivative (165 mg, 0.46 mmol) was dissolved in ethanol (100 mL) and treated with H_2 (22 psi) in the presence of Pd/C (10% Pd, 80 mg) for 24 h at room temperature. The catalyst was removed by filtration and the solvent evaporated under vacuum.

Purification. For amines, the crude was purified by preparative HPLC, see conditions in each case. For aminoalcohols, when necessary, the product was further purified by cation exchange chromatography on the CM-Sepharose CL-6B (Amersham Pharmacia) stationary phase in NH_4^+ form. The stationary phase was packed into a glass column (450–25 mm) to provide a final bed volume of 220 mL. The flow rate was 4 mL min^{-1}. The CM-Sepharose-NH_4^+ was washed initially with H_2O. An aqueous solution of the crude material at pH 5 was then loaded onto the column. Minor coloured impurities were washed away with H_2O (440 mL, 2 bed volumes). Products were eluted with aqueous NH_4OH (0.01 M) (400 mL). Pure fractions were pooled and lyophilized.

Formation of iminocyclitol fused 2-oxopiperazine derivatives

After the Cbz-group removal the product was left at room temperature without solvent or dissolved in water and heated at 40 or 100 °C depending on the derivative until the intramolecular aminolysis was completed (see text). Then the product was assayed without any further purification.

DAB and LAB aromatic 2-aminomethyl derivatives 23 (physical properties and characterization of compounds 24 can be found in ESI†). (2*R*,3*R*,4*R*)-3,4-Dihydroxy-2-((phenylamino)methyl)pyrrolidine (**23a**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((phenylamino)methyl)pyrrolidine (170 mg, 58% yield) was purified by flash chromatography on silica (3 : 2 AcOEt–hexane). HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 25.0 min. The title compound (100 mg, 66% yield) was prepared according to the general procedure described above. HPLC purification: gradient elution from 0 to 60% B in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 40 min; t_R = 13.3 min. $[\alpha]_D^{22}$ = +46.4 (*c* 1.4 in MeOH). ^1H NMR (500 MHz, CD_3OD) δ = 7.15 (dd, *J* = 8.5, 7.4 Hz, 2H), 6.92–6.52 (m, 3H), 4.27–4.18 (m, 1H), 4.11 (d, *J* = 0.8 Hz, 1H), 3.72 (ddd, *J* = 9.4, 5.4, 1.5 Hz, 1H), 3.58 (dd, *J* = 14.0, 5.5 Hz, 1H), 3.53 (dd, *J* = 14.0, 9.6 Hz, 1H), 3.48 (dd, *J* = 12.0, 3.7 Hz, 1H), 3.34 (d, *J* = 11.9 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 148.8, 130.2, 119.0, 114.1, 78.5, 76.2, 67.2, 52.2, 45.0. HRMS (ESI-TOF): *m*/*z* $[M + H]^+$ for $C_{11}H_{17}N_2O_2^+$ calculated 209.1285; observed 209.1276.

(2*R*,3*R*,4*R*)-2-((Benzylamino)methyl)-3,4-dihydroxypyrrolidine (**23b**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-((benzylamino)methyl)-3,4-dihydroxypyrrolidine (165 mg, 56% yield) was purified by flash chromatography on silica (9 : 1 AcOEt–MeOH). HPLC analysis: gradient elution from 2% to 62% B in 40 min; t_R = 10.6 min. The title compound (81 mg, 78% yield) was prepared according to the general procedure described above. HPLC purification: gradient elution from 0 to 60% B in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 25.0 min. $[\alpha]_D^{22}$ = +14.3 (*c* 1.0 in MeOH). ^1H NMR (500 MHz, CD_3OD) δ = 7.55–7.44 (m, 5H), 4.28 (s, 2H), 4.26–4.23 (m, 1H), 4.21 (s, 1H), 3.82 (t, *J* = 6.1 Hz, 1H), 3.58 (dd, *J* = 12.2, 5.0 Hz, 3H), 3.41 (d, *J* = 12.0 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 132.8, 131.0, 130.6, 130.2, 79.3, 75.9, 64.9, 53.1, 53.1, 48.5. HRMS (ESI-TOF): *m*/*z* $[M + H]^+$ for $C_{12}H_{19}N_2O_2^+$ calculated 223.1441; observed 223.1431.

(2*R*,3*R*,4*R*)-3,4-Dihydroxy-2-((quinolin-3-ylamino)methyl)-pyrrolidine (**23c**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((quinolin-3-ylamino)methyl)pyrrolidine (167 mg, 55% yield) was purified by flash chromatography on silica (19 : 1 AcOEt–MeOH). HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 22.6 min. The title compound (110 mg, 70% yield) was prepared according to the general procedure described above. HPLC purification: gradient elution from 0 to 50% B in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 40 min; t_R = 11.2 min. $[\alpha]_D^{22}$ = +22.6 (*c* 0.5 in MeOH). ^1H NMR (400 MHz, CD_3OD) δ = 8.70 (d, *J* = 2.8 Hz, 1H), 7.98 (ddd, *J* = 5.4, 4.9, 2.8 Hz, 3H), 7.74–7.68 (m, 2H), 4.31–4.27 (m, 1H), 4.20 (d, *J* = 1.1 Hz, 1H),

3.81 (dd, J = 13.4, 3.4 Hz, 3H), 3.56 (dd, J = 12.0, 3.6 Hz, 1H), 3.41 (d, J = 12.0 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 143.77, 138.36, 135.37, 131.65, 130.61, 130.10, 128.14, 122.92, 119.63, 78.62, 76.30, 66.67, 52.78, 44.54. HRMS (ESI-TOF): m/z $[M + H]^+$ for $C_{14}H_{18}N_3O_2^+$ calculated 260.1393; observed 260.1381.

(2*R*,3*R*,4*R*)-3,4-Dihydroxy-2-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)pyrrolidine (**23d**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)-pyrrolidine (184 mg, 67% yield) was prepared as follows. The aldehyde **7** dissolved in MeOH (see above in the general procedure) was treated with the amine (5 equiv. mol^{-1} aldehyde) in glacial acetic acid (5 equiv. mol^{-1} aldehyde) and stirred for 5 h at room temperature. The precursor was purified by flash chromatography on silica (7 : 3 AcOEt–hexane). HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 22.2 min. The title compound (136 mg, 78% yield) was prepared by removing the Cbz group by catalytic hydrogenation according to the general procedure described above. HPLC purification: gradient elution from 0 to 60% B in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 40 min; t_R = 12.0 min. $[\alpha]_D^{22}$ = −2.4 (c 1 in MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 7.70 (d, J = 8.3 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 7.42–7.36 (m, 1H), 7.36–7.30 (m, 1H), 5.03 (d, J = 3.1 Hz, 1H), 4.53 (t, J = 2.5 Hz, 1H), 4.37 (dt, J = 4.7, 2.4 Hz, 1H), 3.95 (s, 3H), 3.75 (dd, J = 12.0, 4.6 Hz, 1H), 3.59 (dd, J = 11.9, 2.3 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 149.2, 137.2, 125.2, 124.6, 119.4, 111.6, 80.8, 76.6, 62.3, 52.8, 30.9. HRMS (ESI-TOF): m/z $[M + H]^+$ for $C_{12}H_{16}N_3O_2^+$ calculated 234.1237; observed 234.1235.

(2*R*,3*R*,4*R*)-2-(Aminomethyl)-1-(quinolin-3-yl)pyrrolidine-3,4-diol (**23e**). The title compound (7 mg, 5% yield) was obtained after treatment of the precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((quinolin-3-ylamino)methyl)pyrrolidine with H_2 in the presence of Pd/C. HPLC purification: gradient elution from 0 to 60% B in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 40 min; t_R = 10.9 min. $[\alpha]_D^{22}$ = −28.0 (c 0.5 in MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.84 (d, J = 2.9 Hz, 1H), 8.13 (d, J = 2.8 Hz, 1H), 8.08–8.02 (m, 2H), 7.80–7.70 (m, 2H), 4.37 (d, J = 3.8 Hz, 1H), 4.35 (s, 1H), 4.15 (dd, J = 7.1, 1.8 Hz, 1H), 3.88–3.74 (m, 2H), 3.67 (dd, J = 13.8, 7.2 Hz, 1H), 3.37 (dd, J = 13.9, 2.0 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ = 143.5, 135.6, 134.95, 131.5, 130.7, 130.7, 128.4, 122.7, 122.4, 80.7, 75.3, 66.6, 57.8, 39.5. HRMS (ESI-TOF): m/z $[M + H]^+$ for $C_{14}H_{17}N_3O_2^+$ calculated 260.1399; observed 260.1389.

DAB 2-aminomethyl alcohol derivatives 25. (2*R*,3*R*,4*R*)-2-(((2-Hydroxyethyl)amino)methyl)pyrrolidine-3,4-diol (**25e**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-(((2-hydroxyethyl)amino)methyl)pyrrolidine (95 mg, 40% yield) was purified by HPLC: gradient elution 0% MeOH for 5 min, then from 0% to 70% MeOH in 35 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 16.7 min. The title compound (64 mg, 98% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +4.7 (c 1.0 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ = 4.21 (m, 1H), 3.97 (t, ^3J(H,H) = 3.5 Hz, 1H), 3.76 (t, ^3J(H,H) = 5.3 Hz, 2H), 3.45 (m, 1H), 3.37 (dd, ^3J(H,H) = 12.3, 5.2 Hz, 1H), 3.24 (d, ^3J(H,H) = 4.9 Hz, 1H), 3.15 (dd, ^3J(H,H) = 13.2, 9.1 Hz, 1H), 3.06 (m, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 78.9, 75.6, 62.1, 57.7, 50.7, 49.8, 48.9. HRMS (ESI-TOF): m/z calcd for $C_7H_{17}N_2O_3^+$ 177.1239 $[M + H]^+$; found 177.1226.

(2*R*,3*R*,4*R*)-2-(((3-Hydroxypropyl)amino)methyl)pyrrolidine-3,4-diol (**25f**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-(((3-hydroxypropyl)amino)methyl)pyrrolidine (172 mg, 70% yield) was purified by HPLC: gradient elution from 0% to 60% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 17.0 min. The title compound (103 mg, 91% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +6.7 (c 0.9 in MeOH). ^{1}H NMR (500 MHz, D_2O) δ = 4.21 (dt, ^3J(H,H) = 5.5, 3.4 Hz, 1H), 3.96 (t, ^3J(H,H) = 3.8 Hz, 1H), 3.68 (t, ^3J(H,H) = 6.1 Hz, 2H), 3.42 (dt, ^3J(H,H) = 8.9, 4.2 Hz, 1H), 3.38–3.30 (m, 2H), 3.22 (dd, ^3J(H,H) = 13.1, 8.7 Hz, 1H), 3.14 (m, 2H), 3.02 (dd, ^3J(H,H) = 12.3, 3.3 Hz, 1H), 1.89 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ = 78.78, 75.31, 61.03, 58.85, 50.64, 48.68, 46.05, 27.92. HRMS (ESI-TOF): m/z calcd for $C_8H_{19}N_2O_3^+$ 191.1395 $[M + H^+]$; found 191.1375.

(2*R*,3*R*,4*R*)-2-((((*S*)-1-Hydroxypropan-2-yl)amino)methyl)pyrrolidine-3,4-diol (**25g**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((((*S*)-1-hydroxypropan-2-yl)amino)methyl)-pyrrolidine (175 mg, 72% yield) was purified by HPLC: gradient elution from 0% to 60% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 18.0 min. The title compound (100 mg, 92% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +14.0 (c 1.5 in MeOH). ^{1}H NMR (500 MHz, D_2O) δ = 4.39 (dt, ^3J(H,H) = 4.1, 1.9 Hz, 1H), 4.30 (t, ^3J(H,H) = 2.4 Hz, 1H), 3.94 (td, ^3J(H,H) = 6.4, 2.9 Hz, 1H), 3.87 (dd, ^3J(H,H) = 12.7, 3.6 Hz, 1H), 3.66 (m, 4H), 3.53 (ddd, ^3J(H,H) = 10.2, 6.6, 3.4 Hz, 1H), 3.48 (d, ^3J(H,H) = 12.7 Hz, 1H), 1.33 (d, ^3J(H,H) = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 77.55, 73.70, 62.03, 60.93, 56.70, 51.24, 44.53, 12.63. HRMS (ESI-TOF): m/z calcd for $C_8H_{19}N_2O_3^+$ 191.1395 $[M + H^+]$; found 191.1375.

(2*R*,3*R*,4*R*)-2-((((*S*)-1-Hydroxybutan-2-yl)amino)methyl)pyrrolidine-3,4-diol (**25h**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((((*S*)-1-hydroxybutan-2-yl)amino)methyl)-pyrrolidine (122 mg, 64% yield) was purified by HPLC: gradient elution from 10% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 19.9 min. The title compound (64 mg, 91% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +16.1 (c 1.0 in MeOH). ^{1}H NMR (500 MHz, D_2O) δ = 4.23 (m, 1H), 3.98 (t, ^3J(H,H) = 3.5 Hz, 1H), 3.79 (dd, ^3J(H,H) = 12.5, 3.7 Hz, 1H), 3.64 (m, 1H), 3.44 (dt, ^3J(H,H) = 8.9, 4.6 Hz, 1H), 3.38 (dd, ^3J(H,H) = 12.3, 5.3 Hz, 1H), 3.26 (dd, ^3J(H,H) = 13.2, 5.0 Hz, 1H), 3.17 (dd, ^3J(H,H) = 13.2, 8.4 Hz, 1H), 3.02 (m, 2H), 1.62 (m, 2H), 0.93 (t, ^3J(H,H) = 7.5 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 78.8, 75.5, 61.8, 60.6, 59.2, 50.4, 46.0, 21.0, 9.1. HRMS (ESI-TOF): m/z calcd for $C_9H_{21}N_2O_3^+$ 205.1552 $[M + H]^+$; found 205.1547.

(2*R*,3*R*,4*R*)-2-((((*R*)-1-Hydroxypentan-2-yl)amino)methyl)-pyrrolidine-3,4-diol (**25i**). The precursor (2*R*,3*R*,4*R*)-*N*-

benzyloxycarbonyl-3,4-dihydroxy-2-((((*R*)-1-hydroxypentan-2-yl)amino)methyl)pyrrolidine (112 mg, 57 yield) was purified by HPLC: gradient elution from 10% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 22.0 min. The title compound (59 mg, 89% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +1.05 (*c* 0.9 in MeOH). ^{1}H NMR (500 MHz, D_2O) δ = 4.31 (m, 1H), 4.16 (s, 1H), 3.85 (m, 1H), 3.70 (m, 2H), 3.53 (m, 2H), 3.43 (dd, ^3J(H,H) = 13.5, 8.3 Hz, 1H), 3.29 (m, 2H), 1.62 (dd, ^3J(H,H) = 15.2, 7.5 Hz, 2H), 1.37 (m, 2H), 0.90 (t, ^3J(H,H) = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ = 78.0, 74.4, 62.0, 59.9, 58.6, 50.9, 45.1, 29.2, 18.1, 12.9. HRMS (ESI-TOF): *m*/*z* calcd for $C_{10}H_{23}N_2O_3^+$ 219.1709 [M + H$^+$]; found 219.1691.

(2*R*,3*R*,4*R*)-2-((((*R*)-1-Hydroxy-3-methylbutan-2-yl)amino)methyl)-pyrrolidine-3,4-diol (**25j**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((((*R*)-1-hydroxy-3-methylbutan-2-yl)amino)methyl)pyrrolidine (94 mg, 47% yield) was purified by HPLC: gradient elution from 10% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 21.6 min. The title compound (50 mg, 94% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +18.3 (*c* 0.9 in MeOH). ^{1}H NMR (500 MHz, D_2O) δ = 4.27 (dt, ^3J(H,H) = 5.1, 2.7 Hz, 1H), 4.08 (t, ^3J(H,H) = 2.8 Hz, 1H), 3.83 (dd, ^3J(H,H) = 12.5, 3.9 Hz, 1H), 3.69 (dd, ^3J(H,H) = 12.5, 6.8 Hz, 1H), 3.62 (dt, ^3J(H,H) = 8.2, 4.2 Hz, 1H), 3.48 (dd, ^3J(H,H) = 12.5, 5.0 Hz, 1H), 3.40 (dd, ^3J(H,H) = 13.5, 4.8 Hz, 1H), 3.31 (dd, ^3J(H,H) = 13.5, 8.5 Hz, 1H), 3.20 (dd, ^3J(H,H) = 12.5, 2.4 Hz, 1H), 2.95 (m, 1H), 2.00 (dq, ^3J(H,H) = 13.4, 6.8 Hz, 1H), 0.96 (dd, ^3J(H,H) = 25.3, 6.9 Hz, 6H). ^{13}C NMR (101 MHz, D_2O) δ = 78.2, 74.8, 64.8, 62.4, 58.1, 50.6, 46.2, 27.1, 18.1, 17.0. HRMS (ESI-TOF): *m*/*z* calcd for $C_{10}H_{23}N_2O_3^+$ 219.1709 [M + H$^+$]; found 219.1691.

(2*R*,3*R*,4*R*)-2-(((1,3-Dihydroxypropan-2-yl)amino)methyl)-pyrrolidine-3,4-diol (**25k**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((1,3-dihydroxypropan-2-yl)amino)methyl)-3,4-dihydroxypyrrolidine (126 mg, 66 yield) was purified by HPLC: gradient elution from 10% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 17.1 min. The title compound (78 mg, 99% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +15.2 (*c* 1.2 in MeOH). ^{1}H NMR (500 MHz, D_2O) δ = 4.29 (dt, ^3J(H,H) = 5.0, 2.6 Hz, 1H), 4.08 (t, ^3J(H,H) = 2.7 Hz, 1H), 3.70 (dd, ^3J(H,H) = 11.8, 4.4 Hz, 2H), 3.59 (m, 3H), 3.52 (dd, ^3J(H,H) = 12.6, 4.8 Hz, 1H), 3.30 (d, ^3J(H,H) = 2.4 Hz, 1H), 3.25 (dd, ^3J(H,H) = 13.4, 5.0 Hz, 1H), 3.12 (dd, ^3J(H,H) = 13.4, 8.9 Hz, 1H), 2.93 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ = 77.5, 74.5, 64.3, 59.9, 59.8, 50.2, 45.9. HRMS (ESI-TOF): *m*/*z* calcd for $C_8H_{19}N_2O_4^+$ 207.1316 [M + H$^+$]; found 207.1333.

(2*R*,3*R*,4*R*)-1-(((2*R*,3*R*,4*R*)-3,4-Dihydroxypyrrolidin-2-yl)methyl)-2-(hydroxymethyl)piperidine-3,4-diol (**25l**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((2*R*,3*R*,4*R*)-3,4-dihydroxy-2-(hydroxymethyl)piperidin-1-yl)methyl)-3,4-dihydroxypyrrolidine (102 mg, 52% yield) was purified by HPLC: gradient elution from 0% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 17.3 min. The title compound (60 mg, 79% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +35.4 (*c* 0.9 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ = 4.30 (s, 1H), 4.09 (s, 1H), 3.94 (dd, *J*(H,H) = 12.3, 3.5 Hz, 1H), 3.70 (m, 2H), 3.51 (m, 2H), 3.32 (d, ^3J(H,H) = 12.9 Hz, 1H), 3.20 (t, ^3J(H,H) = 9.4 Hz, 1H), 3.11 (dd, ^3J(H,H) = 14.7, 5.4 Hz, 1H), 2.98 (m, 2H), 2.57 (m, 2H), 1.85 (dd, ^3J(H,H) = 13.2, 4.6 Hz, 1H), 1.62 (ddd, ^3J(H,H) = 25.0, 12.7, 4.0 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ = 77.0, 74.8, 73.0, 71.4, 65.2, 64.2, 58.3, 50.3, 48.9, 48.2, 29.1. HRMS (ESI-TOF): *m*/*z* calcd for $C_{11}H_{23}N_2O_5^+$ 263.1607 [M + H$^+$]; found 263.1592.

DAB and LAB fused 2-oxopiperazine derivatives and amino acid conjugates 28, 29, and 30. (7*R*,8*R*,8a*R*)-7,8-Dihydroxyhexahydropyrrolo[1,2-*a*]pyrazin-4(1*H*)-one (**28m**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-((glycineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26m**) (140 mg, 58% yield) was purified by HPLC: gradient elution 0% MeOH for 5 min, then from 0% to 70% MeOH in 35 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 16.9 min. ^{1}H NMR (400 MHz, D_2O) δ 7.47 (m, 5H, arom), 5.22 (m, 2H, OCH_2Ph), 4.26 (broad s, 1H, H-4), 4.13 (m, 2H, H-2-3), 3.88 (m, 3H, H-5-7-7′), 3.48 (m, 3H H-5′-6-6′). ^{13}C NMR (101 MHz, D_2O) δ 168.2 ($CONH_2$), 157.9 (COBn), 156.7 (COBn-rotamer), 135.8 (C-Ar), 135.4 (C-Ar-rotamer), 128.8 (C-Ar), 128.8 (C-Ar), 128.5 (C-Ar), 128.5 (C-Ar), 127.9 (C-Ar), 78.3 (C-3 rotamer), 77.6 (C-3), 73.6 (C-4), 73.1 (C-4 rotamer), 68.4 (CH_2Ph), 68.0 (CH_2Ph rotamer), 62.1 (C-2), 61.9 (C-2 rotamer), 53.0 (C-5 rotamer), 52.4 (C-5), 49.6 (C-6), 48.7 (C-6 rotamer), 48.0 (C-7). The title compound (78 mg, 92% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +2.3 (*c* 1.4 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.33 (dd, ^3J(H,H) = 13.8, 7.0 Hz, 1H), 3.96 (t, ^3J(H,H) = 6.7 Hz, 1H), 3.83 (m, 5H), 3.49 (m, 1H), 3.15 (t, ^3J(H,H) = 11.3 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 164.3, 77.6, 72.7, 57.9, 48.8, 44.4, 43.5. HRMS-ESI: *m*/*z* calcd for $C_7H_{13}N_2O_3$ 173.0926 [M + H$^+$]; found: 173.0923.

(3*S*,7*R*,8*R*,8a*R*)-7,8-Dihydroxy-3-methylhexahydropyrrolo[1,2-*a*]pyrazin-4(1*H*)-one (**28n**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-alanineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26n**) (110 mg, 58% yield) was purified by HPLC: gradient elution 0% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 13.5 min. ^{1}H NMR (400 MHz, D_2O) δ 7.47 (m, 5H, Ar), 5.21 (m, 2H, OCH_2Ph), 4.25 (br s, 1H, H-4), 4.18 (s, 1H, H-3 rotamer), 4.12 (br s, 2H, H-3-H-2), 4.05 (m, 2H, H-2-rotamer-H-7), 3.91 (m, 1H, H-7-rotamer), 3.84 (td, *J* = 12.2, 5.0 Hz, 2H, H-5;H-5-rotamer), 3.52 (d, *J* = 12.5 Hz, 1H, H-5′), 3.48 (d, *J* = 12.6 Hz, 1H, H-5′ rotamer), 3.44 (br s, 2H, H-6-H-6 rotamer), 3.36 (br s, 2H, H-6′-H-6′ rotamer), 1.53 (d, *J* = 7.0 Hz, 3H, H-8), 1.43 (d, *J* = 7.0 Hz, 3H, H-8-rotamer). ^{13}C NMR (101 MHz, D_2O) δ 172.2 ($CONH_2$), 157.8 (COBn), 156.6 (COBn-rotamer), 135.9 (C-Ar), 135.5 (C-Ar-rotamer), 128.9 (C-Ar), 128.8 (C-Ar), 128.5 (C-Ar), 128.5 (C-Ar), 127.9 (C-Ar), 78.1 (C-3-rotamer), 77.4 (C-3), 73.6 (C-4), 73.1 (C-4-rotamer), 68.3 (CH_2Ph-rotamer), 68.1 (CH_2Ph), 62.2 (C-2), 62.0 (C-2-rotamer), 56.2 (C-7-rotamer), 56.2 (C-7), 52.9 (C-5), 52.3 (C-5-rotamer), 47.6 (C-6), 46.9 (C-6-rotamer), 15.2 (C-8). The title compound (63 mg, 95% yield) was prepared according to the general procedure described above.

$[\alpha]_D^{22}$ = +22.2 (*c* 1.2 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.34 (dd, ^3J(H,H) = 13.7, 6.4 Hz, 1H), 4.22 (q, ^3J(H,H) = 7.3 Hz, 1H), 4.04 (m, 1H), 3.85 (m, 3H), 3.51 (dd, ^3J(H,H) = 12.6, 6.3 Hz, 1H), 3.40 (m, 1H), 1.61 (d, ^3J(H,H) = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 166.1, 77.5, 72.8, 57.3, 51.2, 49.2, 40.5, 14.2. HRMS-ESI: *m*/*z* calcd for $C_8H_{15}N_2O_3$ 187.1083 [M + H^+]; found: 187.1089.

(3*S*,7*R*,8*R*,8a*R*)-7,8-Dihydroxy-3-isobutylhexahydropyrrolo-[1,2-*a*]pyrazin-4(1*H*)-one (**28p**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-leucineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26p**) (132 mg, 62% yield) was purified by HPLC: gradient elution 0% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 18.3 min. ^{1}H NMR (400 MHz, D_2O) δ 7.46 (m, 5H, Ar), 5.20 (m, 2H, OCH_2Ph), 4.24 (m, 1H, H-4), 4.18 (s, 1H, H-3-rotamer), 4.10 (br s, 2H, H-2-H-3), 4.03 (br s, 1H, H-2-rotamer), 3.94 (t, *J* = 7.0 Hz, 1H, H-7), 3.82 (m, 2H, H-5-H-7-rotamer), 3.50 (m, 2H, H-5′-H-6), 3.36 (m, 2H, H-6′-H-6-rotamer), 3.20 (dd, *J* = 13.1, 6.6 Hz, 1H, H-6′-rotamer), 1.62 (m, 3H, H-8-H8′-H-9), 0.94 (m, 6H, CH_3). ^{13}C NMR (101 MHz, D_2O) δ 171.3 ($CONH_2$), 158.0 (COBn), 156.6 (COBn-rotamer), 135.8 (C-Ar), 135.3 (C-Ar-rotamer), 128.9 (C-Ar), 128.8 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 127.9 (C-Ar), 78.3 (C-3 rotamer), 77.5 (C-3), 73.4 (C-4), 73.0 (C-4 rotamer), 68.4 (CH_2Ph rotamer), 68.1 (CH_2Ph), 62.2 (C-2), 62.1 (C-2 rotamer), 59.8 (C-7 rotamer), 59.6 (C-7), 53.0 (C-5 rotamer), 52.3 (C-5), 48.5 (C-6), 47.4 (C-6 rotamer), 38.9 (C-8), 38.7 (C-8 rotamer), 24.0 (C-9 rotamer), 24.0 (C-9), 21.8 (C-10 rotamer), 21.5 (C-10), 21.3 (C-11), 21.0 (C-11 rotamer). The title compound (94 mg, 96% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −9.0 (*c* 1.0 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.32 (dd, ^3J(H,H) = 13.3, 6.1 Hz, 1H), 4.00 (m, 2H), 3.81 (m, 1H), 3.50 (dd, ^3J(H,H) = 12.7, 5.9 Hz, 1H), 3.30 (m, 1H), 1.81 (m, 1H), 0.98 (t, ^3J(H,H) = 5.5 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 166.6, 77.7, 72.9, 57.7, 53.6, 49.3, 41.1, 37.9, 24.3, 21.7, 20.6. HRMS-ESI: *m*/*z* calcd for $C_{11}H_{21}N_2O_3$ 229.1552 [M + H^+]; found: 229.1538.

(3*S*,7*R*,8*R*,8a*R*)-3-Benzyl-7,8-dihydroxyhexahydropyrrolo[1,2-*a*]-pyrazin-4(1*H*)-one (**28q**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-phenylalanineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26q**) (165 mg, 70% yield) was purified by HPLC: 0% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 20.0 min. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.33 (m, 10H, Ar), 5.06 (q, *J* = 12.3 Hz, 2H, OCH_2Ph), 4.15 (t, *J* = 7.0 Hz, 1H, H-7), 4.05 (br s, 1H, H-4), 3.93 (br s, 2H, H-2-H-3), 3.70 (dd, *J* = 11.6, 4.3 Hz, 1H, H-5), 3.43 (m, 3H, H-5′-H-6-H-6′), 3.19 (ddd, *J* = 38.2, 14.1, 7.0 Hz, 2H, CH_2Ph). ^{13}C NMR (101 MHz, CD_3OD) δ = 170.6 ($CONH_2$), 159.0 (COBn), 137.5 (C-Ar), 135.0 (C-Ar), 130.6 (2C-Ar), 130.11 (2C-Ar), 129.6 (C-Ar), 129.3 (C-Ar), 129.1 (C-Ar), 128.9 (C-Ar), 79.4 (C-3), 75.11 (C-4), 68.9 (OCH_2Ph), 64.3 (C-2), 63.4 (C-7), 54.2 (C-5), 51.2 (C-6), 37.9 (CH_2Ph). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-3,4-dihydroxy-2-((((*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl)amino)methyl)pyrrolidine (**26r**) (230 mg, 70% yield) was purified by HPLC: gradient elution 10% to 80% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 23.5 min. ^{1}H NMR (400 MHz, CD_3OD) δ = 7.30 (m, 10H, Ar), 5.12 (m, 2H, CH_2Ph), 4.29 (t, *J* = 6.6 Hz, 1H, H-7), 4.04 (br s, 1H, H-4), 3.93 (m, 2H, H-2-H-3), 3.77 (s, 3H, OCH_3), 3.73 (dd, *J* = 11.6, 4.5 Hz, 1H, H-5), 3.48 (dd, *J* = 13.0, 2.8 Hz, 1H, H-6), 3.43 (d, *J* = 12.0 Hz, 1H, H-5′), 3.36 (m, 1H, H-6′), 3.22 (d, *J* = 6.6 Hz, 2H, H-8-H-8′). ^{13}C NMR (101 MHz, CD_3OD) δ = 173.87 ($CONH_2$), 158.95 (COBn), 137.62 (C-Ar), 135.15 (C-Ar), 130.44 (2C-Ar), 130.14 (2C-Ar), 129.60 (2C-Ar), 129.32 (2C-Ar), 129.06 (2C-Ar), 79.54 (C-3), 75.32 (C-4), 68.89 (CH_2Ph), 64.86 (C-2), 62.83 (C-7), 54.50 (C-5), 53.53 (OCH_3), 50.85 (C-6), 37.20 (C-8). The title compound (100 mg, 95% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −11.4 (*c* 1.2 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 7.37 (m, 5H), 4.41 (dd, ^3J(H,H) = 7.5, 5.5 Hz, 1H), 4.28 (dd, ^3J(H,H) = 12.3, 6.1 Hz, 1H), 3.80 (m, 3H), 3.65 (m, 1H), 3.52 (dd, ^3J(H,H) = 12.8, 5.8 Hz, 1H), 3.43 (dd, ^3J(H,H) = 15.1, 5.3 Hz, 1H), 3.31 (dd, ^3J(H,H) = 15.1, 7.7 Hz, 1H), 2.91 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 164.6, 134.0, 129.3, 129.2, 128.1, 77.7, 72.8, 57.2, 55.8, 49.3, 41.5, 34.5. HRMS-ESI: *m*/*z* calcd for $C_{14}H_{19}N_2O_3$ 263.1396 [M + H^+]; found: 263.1377.

1-(3-((3*S*,7*R*,8*R*,8a*R*)-7,8-Dihydroxy-4-oxooctahydropyrrolo-[1,2-*a*]pyrazin-3-yl)propyl)guanidine (**28u**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-arginineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26u**) (129 mg, 55% yield) was purified by HPLC: gradient elution 0% MeOH for 5 min, then from 0% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 12.4 min. ^{1}H NMR (400 MHz, D_2O) δ 7.45 (m, 5H, Ar), 5.20 (m, 2H, OCH_2Ph), 4.25 (m, 1H, H-4), 4.18 (s, 1H, H-3 rotamer), 4.10 (br s, 2H, H-2-H-3), 4.06 (br s, 1H, H-2 rotamer), 3.97 (t, *J* = 6.3 Hz, 1H, H-7), 3.85 (m, 2H, H-5-H-7 rotamer), 3.44 (m, 3H, H-5′-H-6-H-6′), 3.19 (m, 2H, H-10-H-10′), 1.93 (m, 1H, H-8), 1.64 (m, 3H, H-8′-H-9-H-9′). ^{13}C NMR (101 MHz, D_2O) δ 170.4 ($CONH_2$), 158.1 (COBn), 156.7 (C=NH), 135.9 (C-Ar), 135.4 (C-Ar rotamer), 128.9 (C-Ar), 128.8 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 127.8 (C-Ar), 78.2 (C-3 rotamer), 77.4 (C-3), 73.4 (C-4), 73.0 (C-4 rotamer), 68.3 (CH_2Ph rotamer), 68.1 (CH_2Ph), 62.2 (C-2), 62.0 (C-2 rotamer), 60.4 (C-7 rotamer), 60.2 (C-7), 53.0 (C-5 rotamer), 52.3 (C-5), 48.6 (C-6), 47.6 (C-6 rotamer), 40.2 (C-10), 26.8 (C-8), 26.7 (C-8 rotamer), 23.3 (C-9 rotamer), 23.3 (C-9). The title compound (100 mg, 92% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −2.0 (*c* 1.0 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.32 (dd, ^3J(H,H) = 12.6, 6.5 Hz, 1H), 4.03 (dd, ^3J(H,H) = 11.9, 5.7 Hz, 2H), 3.82 (m, 3H), 3.53 (dd, ^3J(H,H) = 12.7, 5.7 Hz, 1H), 3.26 (m, 3H), 2.14 (m, 1H), 1.91 (m, 1H), 1.78 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 165.8, 156.7, 77.7, 72.9, 57.8, 54.7, 49.3, 41.6, 40.4, 26.4, 24.8. HRMS-ESI: *m*/*z* calcd for $C_{11}H_{22}N_5O_3$ 272.1723 [M + H^+]; found: 272.1726.

(1*R*,2*R*,5a*S*,10a*R*)-1,2-Dihydroxyoctahydrodipyrrolo[1,2-*a*:1′,2′-*d*]pyrazin-5(1*H*)-one (**28v**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-prolineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26v**) (128 mg, 63% yield) was purified by HPLC: gradient elution 0% to 70% MeOH in 40 min. HPLC analysis: gradient elution from 2% to 62% B in 30 min; t_R = 18.0 min. Assigned the major rotamer: ^{1}H NMR (500 MHz, CD_3OD) δ 7.44 (m, 5H, Ar), 5.23 (m, 2H, OCH_2Ph), 4.28 (s, 1H, H-7), 4.16 (m, 2H, H-3-

H-4), 4.00 (t, J = 5.6 Hz, 1H, H-2), 3.87 (br s, 1H, H-10), 3.75 (m, 2H, H-5-H-6), 3.53 (m, 2H, H-5′-H-6′), 3.38 (m, 1H, H-10′), 2.59 (m, 1H, H-8), 2.23 (m, 1H, H-9), 2.12 (m, 2H, H-8′-H-9′). ^{13}C NMR (101 MHz, CD_3OD) δ 171.0 ($CONH_2$), 159.8 (COBn), 137.8 (C-Ar), 130.1 (C-Ar), 129.9 (C-Ar), 129.6 (C-Ar), 129.3 (C-Ar), 129.1 (C-Ar), 78.8 (C-3), 76.0 (C-4), 70.5 (C-7) 68.7 (OCH_2Ph), 63.7 (C-2), 59.5 (C-6), 55.4 (C-10), 54.1 (C-5), 30.3 (C-8), 24.2 (C-9). The title compound (90 mg, 97% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −19.0 (c 1 in MeOH) of the mixture. (major) ^{1}H NMR (400 MHz, D_2O) δ 4.38 (m, 1H), 4.33 (dd, ^3J(H,H) = 13.7, 6.9 Hz, 1H), 3.99 (m, 1H), 3.93 (dd, ^3J(H,H) = 12.1, 2.7 Hz, 1H), 3.86 (m, 1H), 3.85 (m, 1H), 3.83 (m, 1H), 3.47 (dd, ^3J(H,H) = 12.6, 6.8 Hz, 1H), 3.39 (m, 1H), 3.23 (t, ^3J(H,H) = 11.4 Hz, 1H), 2.55 (m, 1H), 2.24 (m, 1H), 2.20 (m, 1H), 2.09 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 165.7, 77.0, 72.6, 62.1, 58.2, 56.9, 50.6, 48.7, 27.4, 21.8. (minor) ^{1}H NMR (400 MHz, D_2O) δ 4.40 (m, 1H), 4.22 (m, 1H), 4.07 (m, 1H), 4.04 (m, 1H), 3.83 (m, 1H), 3.58 (m, 1H), 3.52 (d, ^3J(H,H) = 14.0 Hz, 1H), 3.47 (m, 1H), 3.33 (m, 1H), 3.32 (m, 1H), 2.53 (m, 1H), 2.10 (m, 1H), 2.11 (m, 1H), 1.98 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 168.2, 74.1, 68.1, 61.0, 52.7, 51.8, 51.6, 48.5, 29.5, 23.6. HRMS-ESI: m/z calcd for $C_{10}H_{17}N_2O_3$ 213.1239 [M + H$^+$]; found: 213.1226.

(2*R*,3*R*,4*R*)-2-(((*S*)-Valineamidyl)methyl)-3,4-dihydroxypyrrolidine (**30o**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-valineamidyl)methyl)-3,4-dihydroxypyrrolidine (**26o**) (104 mg, 51% yield) was purified by HPLC: gradient elution: 0% MeOH for 5 min, then from 10% to 80% MeOH in 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 16.5 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (m, 5H, Ar), 5.19 (s, 2H, OCH_2Ph), 4.09 (br s, 1H, H-4), 3.99 (m, 2H, H-2-H-3), 3.75 (dd, J = 11.5, 4.3 Hz, 1H, H-5), 3.68 (d, J = 5.8 Hz, 1H, H-7), 3.49 (m, 2H, H-5′-H-6), 3.32 (m, 1H, H-6′), 2.20 (td, J = 13.5, 6.8 Hz, 1H, H-8), 1.07 (m, 6H, $2CH_3$). ^{13}C NMR (101 MHz, CD_3OD) δ 170.1 ($CONH_2$), 159.3 (COBn), 137.5 (C-Ar), 129.6 (2C-Ar), 129.3 (C-Ar), 129.1 (2C-Ar), 79.5 (C-3), 75.0 (C-4), 69.1 (OCH_2Ph), 68.0 (C-7), 64.4 (C-2), 54.3 (C-5), 51.8 (C-6), 31.1 (C-8), 18.6 (CH_3), 18.5 (CH_3). The title compound (65 mg, 87% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +8.0 (c 1.0 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.35 (dt, ^3J(H,H) = 4.4, 2.2 Hz, 1H), 4.14 (t, ^3J(H,H) = 2.3 Hz, 1H), 3.60 (m, 2H), 3.41 (dd, ^3J(H,H) = 12.6, 1.7 Hz, 1H), 3.05 (m, 2H), 2.97 (dd, ^3J(H,H) = 13.3, 8.5 Hz, 1H), 1.93 (tt, ^3J(H,H) = 13.6, 6.8 Hz, 1H), 0.98 (dd, ^3J(H,H) = 8.7, 6.9 Hz, 6H). ^{13}C NMR (101 MHz, D_2O) δ 179.0, 77.1, 74.5, 67.9, 65.6, 50.3, 47.3, 31.0, 18.4, 18.1. HRMS-ESI: m/z calcd for $C_{10}H_{22}N_3O_3$ 232.1661 [M + H$^+$]; found: 232.1642.

(2*R*,3*R*,4*R*)-2-(((*S*)-Aspartic(β*tert*-butyl)amidyl)methyl)-3,4-dihydroxypyrrolidine (**30t**). The precursor (2*R*,3*R*,4*R*)-*N*-benzyloxycarbonyl-2-(((*S*)-aspartic(β^{t}Bu)amidyl)methyl)-3,4-dihydroxypyrrolidine (**26t**) (138 mg, 56% yield) was purified by HPLC: gradient elution 10% to 80% MeOH in 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 20.8 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (m, 5H, Ar), 5.18 (m, 2H, OCH_2Ph), 4.07 (m, 2H, H-4-H-7), 3.99 (br s, 2H, H-2-H-3), 3.76 (dd, J = 11.6, 4.5 Hz, 1H, H-5), 3.42 (m, 3H, H-5′-H-6-H-6′), 2.87 (m, 2H, H-8-H-8′), 1.48 (s, 9H, $3CH_3$). ^{13}C NMR (101 MHz, CD_3OD) δ 170.2 ($CONH_2$), 170.1 (COO^tBu), 158.9 (COBn), 137.5 (C-Ar), 129.6 (C-Ar), 129.6 (C-Ar), 129.4 (C-Ar), 129.3 (C-Ar), 129.0 (C-Ar), 84.1 (C-CH_3), 79.4 (C-3), 75.3 (C-4), 68.8 (OCH_2Ph), 64.4 (C-2), 58.4 (C-7), 54.3 (C-5), 51.0 (C-6), 36.3 (C-8), 28.2 ($3CH_3$). The title compound (95 mg, 93% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +16.6 (c 0.9 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.36 (dt, ^3J(H,H) = 4.4, 2.3 Hz, 1H), 4.14 (t, ^3J(H,H) = 2.3 Hz, 1H), 3.62 (m, 3H), 3.42 (dd, ^3J(H,H) = 12.6, 1.8 Hz, 1H), 3.13 (dd, ^3J(H,H) = 13.3, 5.4 Hz, 1H), 3.05 (dd, ^3J(H,H) = 13.3, 9.0 Hz, 1H), 2.74 (d, ^3J(H,H) = 6.3 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, D_2O) δ 177.7, 172.3, 83.5, 77.0, 74.5, 65.4, 57.7, 50.3, 46.4, 37.8, 27.3. HRMS-ESI: m/z calcd for $C_{13}H_{26}N_3O_5$ 304.1872 [M + H$^+$]; found: 304.1869.

(7*S*,8*S*,8a*S*)-7,8-Dihydroxyhexahydropyrrolo[1,2-*a*]pyrazin-4-(1*H*)-one (**29m**). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-((glycineamidyl)methyl)-3,4-dihydroxypyrrolidine (**27m**) (147 mg, 60% yield) was purified by HPLC: gradient elution 0% MeOH for 5 min, then from 0% to 70% MeOH in 35 min. HPLC analysis: identical to that **26m**. ^{1}H and ^{13}C NMR matched those listed above for compound **26m**. The title compound (100 mg, 99% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −2.5 (c 2.0 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.31 (dd, ^3J(H,H) = 14.3, 7.1 Hz, 1H), 3.94 (t, ^3J(H,H) = 6.2 Hz, 1H), 3.83 (m, 4H), 3.47 (dd, ^3J(H,H) = 12.5, 6.9 Hz, 1H), 3.18 (m, 1H). ^{13}C NMR (101 MHz, D_2O) δ 163.3, 77.5, 72.6, 57.4, 48.8, 43.9, 43.3. HRMS-ESI: m/z calcd for $C_7H_{13}N_2O_3$ 173.0926 [M + H$^+$]; found: 173.0930.

(3*S*,7*S*,8*S*,8a*S*)-7,8-Dihydroxy-3-methylhexahydropyrrolo[1,2-*a*]-pyrazin-4(1*H*)-one (**29n**). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-(((*S*)-alanineamidyl)methyl)-3,4-dihydroxypyrrolidine (**27n**) (50 mg, 44% yield) was purified by HPLC: gradient elution 0% to 70% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 13.4 min. ^{1}H NMR (400 MHz, D_2O) δ 7.30 (m, 5H, Ar), 5.05 (d, J = 5.7 Hz, 2H, OCH_2Ph), 4.11 (m, 1H, H-4), 4.08 (m, 1H, H-4-rotamer), 4.02 (s, 1H, H-3-rotamer), 3.93 (m, 3H, H-2-H-3-H-7), 3.77 (q, J = 7.0 Hz, 1H, H-7-rotamer), 3.68 (dt, J = 11.0, 5.3 Hz, 1H, H-5), 3.36 (m, 2H, H-5′-H-6), 3.15 (ddd, J = 25.4, 13.1, 7.1 Hz, 1H, H-6′), 1.41 (d, J = 7.1 Hz, 3H, CH_3), 1.24 (d, J = 7.1 Hz, 3H, CH_3 rotamer). ^{13}C NMR (101 MHz, D_2O) δ 172.0 ($CONH_2$), 171.8 ($CONH_2$ rotamer), 158.0 (COBn), 156.5 (COBn-rotamer), 135.8 (C-Ar), 135.3 (C-Ar-rotamer), 128.9 (C-Ar), 128.7 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 127.9 (C-Ar), 78.2 (C-3-rotamer), 77.6 (C-3), 73.5 (C-4-rotamer), 73.0 (C-4), 68.4 (OCH_2Ph-rotamer), 68.1 (CH_2Ph), 62.1 (C-2), 62.1 (C-2-rotamer), 56.4 (C-7-rotamer), 56.2 (C-7), 53.1 (C-5-rotamer), 52.3 (C-5), 48.3 (C-6), 47.4 (C-6-rotamer), 15.4 (C-8), 15.2 (C-8-rotamer). The title compound (45 mg, 99% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +26.0 (c 1.0 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.34 (dd, ^3J(H,H) = 14.2, 7.3 Hz, 1H), 4.09 (q, ^3J(H,H) = 7.2 Hz, 1H), 3.91 (m, 3H), 3.82 (dd, ^3J(H,H) = 12.7, 8.0 Hz, 1H), 3.50 (dd, ^3J(H,H) = 12.7, 6.9 Hz, 1H), 3.31 (m, 1H), 1.59 (d, ^3J(H,H) = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 165.9, 77.5, 72.8, 57.4, 52.6, 49.1, 43.3,

14.4. HRMS-ESI: *m*/*z* calcd for $C_8H_{15}N_2O_3$ 187.1083 [M + H^+]; found: 187.1085.

(3*S*,7*S*,8*S*,8a*S*)-7,8-Dihydroxy-3-isobutylhexahydropyrrolo[1,2-*a*]-pyrazin-4(1*H*)-one (**29p**). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-(((*S*)-leucineamidyl)methyl)-3,4-dihydroxypyrrolidine (**27p**) (45 mg, 50% yield) was purified by HPLC: gradient elution 0% to 70% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 18.5 min. ^{1}H NMR (400 MHz, D_2O) δ 7.47 (d, *J* = 13.8 Hz, 5H, Ar), 5.21 (m, 2H, OCH_2Ph), 4.26 (m, 1H, H-4), 4.15 (m, 2H, H-2-H-3), 4.10 (br s, 1H, H-3-rotamer), 4.02 (m, 1H, H-7), 3.84 (m, 2H, H-5-H-7-rotamer), 3.47 (m, 2H, H-5′-H-6), 3.32 (m, 1H, H-6′), 1.75 (m, 3H, H-8-H-8′-H-9), 1.55 (m, 2H, H-8-rotamer-H-9-rotamer), 0.94 (m, 6H, CH_3). ^{13}C NMR (101 MHz, D_2O) δ 171.08 ($CONH_2$), 158.19 (COBn), 156.45 (COBn-rotamer), 135.74 (C-Ar), 131.96 (C-Ar-rotamer), 129.03 (C-Ar), 128.95 (C-Ar), 128.74 (C-Ar), 128.54 (C-Ar), 127.97 (C-Ar), 78.27 (C-3), 77.51 (C-3 rotamer), 73.42 (C-4), 72.93 (C-4 rotamer), 68.53 (CH_2Ph), 68.18 (CH_2Ph rotamer), 62.01 (C-2), 59.80 (C-7), 59.42 (C-7 rotamer), 53.07 (C-5), 52.35 (C-5 rotamer), 48.92 (C-6), 48.26 (C-6 rotamer), 38.91 (C-8), 38.62 (C-8 rotamer), 23.96 (C-9), 21.92 (C-10), 21.86 (C-10 rotamer), 21.12 (C-11), 20.92 (C-11 rotamer). The title compound (20 mg, 99% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −3.8 (*c* 0.9 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.33 (dd, ^3J(H,H) = 14.3, 7.2 Hz, 1H), 4.06 (dd, ^3J(H,H) = 10.0, 3.5 Hz, 1H), 3.96 (t, ^3J(H,H) = 7.5 Hz, 1H), 3.88 (m, 2H), 3.80 (dd, ^3J(H,H) = 12.5, 7.8 Hz, 1H), 3.52 (dd, ^3J(H,H) = 12.6, 6.8 Hz, 1H), 3.29 (t, ^3J(H,H) = 11.6 Hz, 1H), 1.96 (m, 1H), 1.79 (m, 2H), 0.98 (t, ^3J(H,H) = 5.7 Hz, 6H). ^{13}C NMR (101 MHz, D_2O) δ 165.7, 77.4, 72.6, 57.1, 54.8, 49.2, 43.3, 38.6, 23.7, 22.1, 19.9. HRMS-ESI: *m*/*z* calcd for $C_{11}H_{21}N_2O_3$ 229.1552 [M + H^+]; found: 229.1545.

(3*S*,7*S*,8*S*,8a*S*)-3-Benzyl-7,8-dihydroxyhexahydropyrrolo[1,2-*a*]-pyrazin-4(1*H*)-one (**29q**). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-(((*S*)-phenylalanineamidyl)methyl)-3,4-dihydroxypyrrolidine (**27q**) (54 mg, 55% yield) was purified by HPLC: gradient elution: 0% to 70% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 19.4 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.32 (m, 10H, Ar), 5.20 (m, 2H, OCH_2Ph), 4.23 (dd, *J* = 7.4, 6.1 Hz, 1H, H-7), 4.06 (br s, 1H, H-4), 4.00 (m, 1H, H-2), 3.95 (s, 1H, H-3), 3.73 (dd, *J* = 11.7, 4.3 Hz, 1H, H-5), 3.40 (m, 3H, H-5′-H-6-H-6′), 3.21 (ddd, *J* = 21.5, 13.9, 6.8 Hz, 2H, CH_2Ph). ^{13}C NMR (101 MHz, CD_3OD) δ 170.3 ($CONH_2$), 159.1 (COBn), 137.6 (C-Ar), 135.0 (C-Ar), 130.6 (2C-Ar), 130.0 (2C-Ar), 129.6 (C-Ar), 129.3 (C-Ar), 129.2 (C-Ar), 128.9 (C-Ar), 79.4 (C-3), 75.3 (C-4), 68.9 (OCH_2Ph), 64.3 (C-2), 62.8 (C-7), 54.5 (C-5), 50.7 (C-6), 37.8 (C-8). The title compound (34 mg, 99% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = −5.7 (*c* 1.1 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 7.42 (m, 5H), 4.34 (m, 2H), 3.95 (m, 1H), 3.84 (m, 3H), 3.61 (dd, ^3J(H,H) = 14.8, 4.2 Hz, 1H), 3.56 (dd, ^3J(H,H) = 12.7, 6.9 Hz, 1H), 3.26 (t, ^3J(H,H) = 12.1 Hz, 1H), 3.14 (dd, ^3J(H,H) = 14.9, 10.1 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 164.2, 133.9, 129.3, 129.2, 128.1, 77.4, 72.7, 58.0, 57.1, 49.2, 43.5, 35.0. HRMS-ESI: *m*/*z* calcd for $C_{14}H_{19}N_2O_3$ 263.1396 [M + H^+]; found: 263.1399.

2-((3*S*,7*S*,8*S*,8a*S*)-7,8-Dihydroxy-4-oxooctahydropyrrolo[1,2-*a*]-pyrazin-3-yl)acetic acid (**29s**). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-(((*S*)-aspartic(β^tBu)amidyl)methyl)-3,4-dihydroxypyrrolidine (**27t**) (64 mg, 47% yield) was purified by HPLC: gradient elution 0% to 70% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 20.6 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (m, 5H, Ar), 5.19 (s, 2H, OCH_2Ph), 4.19 (m, 1H, H-7), 4.09 (br s, 1H, H-4), 4.02 (br s, 2H H-2-H-3), 3.75 (dd, *J* = 11.6, 4.1 Hz, 1H, H-5), 3.47 (m, 3H, H-5′-H-6-H-6′), 3.00 (t, *J* = 4.8 Hz, 2H, H-8-H-8′), 1.48 (s, 9H, CH_3). ^{13}C NMR (101 MHz, CD_3OD) δ 170.0 (2C-$CONH_2$-COO^tBu), 159.0 (COBn), 137.6 (C-Ar), 129.7 (C-Ar), 129.6 (C-Ar), 129.4 (C-Ar), 129.2 (C-Ar), 129.1 (C-Ar), 84.1 (*C*-CH_3), 79.5 (C-3), 75.2 (C-4), 68.9 (OCH_2Ph), 64.4 (C-2), 58.4 (C-7), 54.4 (C-5), 51.8 (C-6), 36.7 (C-8), 28.2 ($3CH_3$). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-(((*S*)-asparticamidyl)methyl)-3,4-dihydroxypyrrolidine (**27s**) (55 mg, 47% yield) was prepared by removing the *t*Bu ester of the β-carboxylate group. *Deprotection of the tBu group*: **27t** (60 mg, 0.13 mmol) was dissolved in trifluoroacetic acid (TFA) (7 mL) and left to stand at room temperature for 6 h. After that time, no starting material was detected by HPLC. The derivative was purified by HPLC using a gradient elution: 0% to 70% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 13.3 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (m, 5H, Ar), 5.20 (m, 2H, OCH_2Ph), 4.21 (m, 1H, H-7), 4.09 (br s, 1H, H-4), 4.03 (br s, 2H, H-2-H-3), 3.75 (dd, *J* = 11.7, 4.3 Hz, 1H, H-5), 3.56 (dd, *J* = 13.0, 2.6 Hz, 1H, H-6), 3.45 (m, 2H, H-5′-H-6′), 3.05 (dd, *J* = 9.2, 5.8 Hz, 2H, H-8-H-8′). ^{13}C NMR (101 MHz, CD_3OD) δ 172.5 ($CONH_2$), 170.2 (COOH), 158.9 (COBn), 137.6 (C-Ar), 129.6 (2C-Ar), 129.3 (C-Ar), 129.1 (2C-Ar), 79.6 (C-3), 75.3 (C-4), 68.9 (OCH_2Ph), 64.5 (C-2), 58.5 (C-7), 54.5 (C-5), 51.6 (C-6), 35.5 ($CONH_2$). The title compound (32 mg, 99% yield) was then prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +46.6 (*c* 0.8 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.35 (dd, ^3J(H,H) = 14.3, 7.1 Hz, 1H), 4.29 (m, 1H), 3.95 (m, 3H), 3.84 (dd, ^3J(H,H) = 12.6, 7.9 Hz, 1H), 3.48 (dd, ^3J(H,H) = 12.6, 6.8 Hz, 1H), 3.39 (t, ^3J(H,H) = 11.8 Hz, 1H), 3.23 (dd, ^3J(H,H) = 18.4, 5.5 Hz, 1H), 3.07 (dd, ^3J(H,H) = 18.4, 3.7 Hz, 1H). ^{13}C NMR (101 MHz, D_2O) δ 173.8, 164.3, 77.4, 72.7, 57.2, 53.1, 49.1, 43.8, 33.6. HRMS-ESI: *m*/*z* calcd for $C_9H_{15}N_2O_5$ 231.0981 [M + H^+]; found: 231.0995.

1-(3-((3*S*,7*S*,8*S*,8a*S*)-7,8-Dihydroxy-4-oxooctahydropyrrolo-[1,2-*a*]pyrazin-3-yl)propyl)guanidine (**29u**). The precursor (2*S*,3*S*,4*S*)-*N*-benzyloxycarbonyl-2-(((*S*)-arginineamidyl)methyl)-3,4-dihydroxypyrrolidine (**27u**) (49 mg, 48% yield) was purified by HPLC: gradient elution 0% to 60% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 12.2 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.36 (m, 5H, Ar), 5.15 (m, 2H, OCH_2Ph), 4.10 (d, *J* = 3.6 Hz, 1H, H-4), 4.03 (br s, 3H, H-2-H-3-H-7), 3.75 (dd, *J* = 11.7, 4.3 Hz, 1H, H-5), 3.49 (m, 2H, H-5′-H-6), 3.35 (m, 1H, H-6′), 3.23 (t, *J* = 5.8 Hz, 2H, H-10-H-10′), 1.98 (m, 2H, H-8-H-8′), 1.66 (m, 2H, H-9-H-9′). ^{13}C NMR (101 MHz, CD_3OD) δ 170.7 ($CONH_2$), 159.2 (COBn), 158.7 (C═NH), 137.6 (C-Ar), 129.7 (C-Ar), 129.6 (C-Ar), 129.4 (C-Ar), 129.2 (C-Ar), 129.0 (C-Ar), 79.4 (C-3), 75.3 (C-4), 68.9 (OCH_2Ph), 64.4 (C-2), 61.1 (C-7), 54.5 (C-5), 50.9 (C-6), 41.7 (C-10), 28.8

(C-8), 25.0 (C-9). The title compound (60 mg, 99% yield) was prepared according to the general procedure described above. $[\alpha]_D^{22}$ = +16.9 (*c* 1.2 in MeOH). ^{1}H NMR (400 MHz, D_2O) δ 4.33 (dd, ^3J(H,H) = 14.4, 7.1 Hz, 1H), 4.00 (dd, ^3J(H,H) = 8.3, 4.4 Hz, 1H), 3.96 (m, 1H), 3.88 (m, 2H), 3.81 (dd, ^3J(H,H) = 12.6, 7.8 Hz, 1H), 3.52 (dd, ^3J(H,H) = 12.6, 6.9 Hz, 1H), 3.26 (m, 3H), 2.18 (ddd, ^3J(H,H) = 15.6, 10.2, 4.8 Hz, 1H), 1.91 (m, 1H), 1.77 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 166.8, 156.7, 77.5, 72.7, 58.4, 56.3, 49.1, 43.7, 40.3, 27.2, 24.2. HRMS-ESI: *m*/*z* calcd for $C_{11}H_{22}N_5O_3$ 272.1723 [M + H$^+$]; found: 272.1716.

(2*S*,3*S*,4*S*)-*N*-Benzyloxycarbonyl-2-(((*S*)-valineamidyl)methyl)-3,4-dihydroxypyrrolidine (**27o**). The title compound (60 mg, 50% yield) was prepared according to the general procedure described above and purified by HPLC: gradient elution 0% to 70% B during 40 min. HPLC analysis: gradient elution from 10% to 70% B in 30 min; t_R = 16.4 min. ^{1}H NMR (400 MHz, CD_3OD) δ 7.37 (m, 5H, Ar), 5.18 (m, 2H, OCH_2Ph), 4.08 (br s, 1H, H-4), 4.05 (d, *J* = 7.0 Hz, 1H, H-2), 4.00 (br s, 1H, H-3), 3.83 (d, *J* = 4.5 Hz, 1H, H-7), 3.75 (dd, *J* = 11.7, 4.3 Hz, 1H, H-5), 3.48 (d, *J* = 11.8 Hz, 1H, H-5′), 3.43 (d, *J* = 2.2 Hz, 1H, H-6), 3.35 (m, 1H, H-6′), 2.26 (m, 1H, H-8), 1.10 (m, 6H, $2CH_3$). ^{13}C NMR (101 MHz, CD_3OD) δ 169.7 ($CONH_2$), 159.3 (COBn), 137.6 (C-Ar), 129.6 (2C-Ar), 129.3 (C-Ar), 129.1 (2C-Ar), 79.5 (C-3), 75.3 (C-4), 69.0 (OCH_2Ph), 66.8 (C-7), 64.4 (C-2), 54.5 (C-5), 51.4 (C-6), 31.1 (C-8), 18.8 (CH_3), 17.8 (CH_3). This compound could not be converted to the corresponding bicyclic (3*S*,7*S*,8*S*,8a*S*)-7,8-dihydroxy-3-isopropylhexahydropyrrolo[1,2-*a*]pyrazin-4(1*H*)-one. No further characterization was conducted.

Inhibitory activity assays

Enzymatic inhibition assays on commercial glycosidases, the kinetics of the inhibition, preparation of gut mucosal suspensions, inhibition assays on rat intestinal disaccharidases and MIC determination against the H37Rv Pasteur *Mycobacterium tuberculosis* strain using the REMA plate method were performed as described in previous work.[7,24] The experimental details are given in the ESI.†

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