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Synthesis and Fucosidase Inhibitory Study of Unnatural Pyrrolidine Alkaloid 4-*epi*-(+)-Codonopsinine

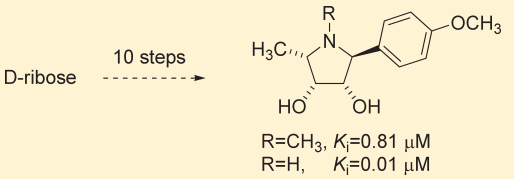
Alexis Kotland,[†] Fabien Accadbled,[†] Koen Robeyns,[‡] and Jean-Bernard Behr*[,†]

[†]Institut de Chimie Moléculaire de Reims (ICMR), UFR Sciences, CNRS, BP 1039, 51687 Reims Cedex 2, France

[‡]Institute of Condensed Matter and Nanosciences (IMCN), Université Catholique de Louvain (UCL), Bâtiment Lavoisier, place Louis Pasteur 1, 1348 Louvain-la-Neuve, Belgium.

Supporting Information

ABSTRACT: The total synthesis of enantiopure 4-*epi*-(+)-codonopsinine was achieved in 10 steps starting from D-ribose as a chiral building block. The key step involved a highly stereoselective nucleophilic addition of a Grignard reagent to a protected ribosylamine. Synthesis of the *N*-desmethyl derivative and its *p*-tolyl analogue was also accomplished, and the compounds were assayed against α-fucosidase.



Natural polyhydroxylated pyrrolidines are secondary metabolites that have been isolated from a great variety of plants and microorganisms.[1] On a structural point of view, these five-membered-ring alkaloids can be regarded as sugar mimics in which the ring oxygen has been replaced by nitrogen. This alteration, although slight, greatly impacts the biological properties since iminosugars bind much more efficiently to glycosidases than the parent carbohydrate substrates, thus acting as potent inhibitors.[2] Since glycosidases are involved in a number of pathological events, interest in iminosugars has increased over the past decades. The inhibition of α-L-fucosidase, an enzyme responsible for the trimming of fucosides located on the cell surface, was recently envisioned as a therapeutic option for the treatment of inflammation, cancer or viral infection.[3] Pyrrolidines such as **1**[4] or **2**[5] (Figure 1), which feature the fucose substitution pattern, are potent inhibitors of fucosidase (K_i = 5 nM and 8 nM, respectively).

Interestingly, a new class of polyhydroxypyrrolidines was discovered, which incorporates an aryl moiety at C2 (Figure 1). (−)-Codonopsinine and its analogue (−)-codonopsine, both isolated from *Codonopsis clematidae*, were found to exhibit antibiotic activity and hypotensive activity.[6] Their hydroxylated congeners radicamine A and radicamine B were isolated from *Lobelia chinensis* Lour, a herb used in Chinese folk medicine.[7] Due to their intriguing molecular structures and promising biological activities, the synthesis of new 2-aryl polyhydroxylated pyrrolidines with structural diversities is of interest. Plagiaring Nature, we wished to explore the potential of new *fuco*-configurated codonopsinine analogues and test their ability to inhibit fucosidase. Thus, we present herein the synthesis, X-ray characterization, and biological evaluation of 4-*epi*-(+)-codonopsinine **3** as well as the syntheses and anti-fucosidase activities of the *N*-desmethyl and *p*-tolyl analogues, **4** and **5**.

The biological potential of *fuco*-pyrrolidines that incorporate a hydrophobic substituent at C2 has been recently exemplified by us and others.[8] The biological evaluation of such compounds suggest that the aryl moiety might be a major player in enzyme recognition and that a 2(*S*) configuration induces the best interactions in the fucosidase active site. Among the numerous methods devoted to the preparation of five-membered iminocyclitols,[9] the stereoselective addition of an organometallic reagent to a glycosylamine is straightforward and occurs usually with high selectivity.[9a] As depicted in Figure 2, target compounds **3**−**5** could be synthesized by such a method, using a protected 5-deoxyribosylamine as the starting material.[10] Indeed, D-ribose features the adequate hydroxyl distribution to reach the targeted L-*fuco* configuration. Furthermore, the 5-hydroxy group of D-ribose could be easily removed to lead, after inversion of configuration, to the required 5(*S*)-methyl substituent.

Combining different methods from literature, 5-deoxyribose **8** was prepared in four steps and 59% overall yield (Scheme 1). First, acetonation of D-ribose was achieved by treatment with acetone in the presence of sulfuric acid.[11] The crude product was tosylated selectively at the primary hydroxyl by addition of one equivalent of TsCl in pyridine for 16 h at room temperature.[12] Under these conditions, sulfonyl ester **6** was obtained as a colorless oil in 80% yield after purification by flash chromatography over silica gel (Et_2O/petroleum ether, 7:3). Treatment of **6** with sodium iodide in dioxane/DMF at 80 °C for 3 h yielded 5-iodoribose derivative **7** (82%, white solid). Reductive dehalogenation was accomplished by hydrogenation at 1 atm over Pd/C in the presence of NEt_3.[13] Purification by silica gel chromatography (Et_2O/petroleum ether, 5:5) yielded deoxyribose **8** as a colorless oil.

The corresponding *p*-methoxybenzylglycosylamine **9** formed quantitatively after stirring hemiacetal **8** with commercial

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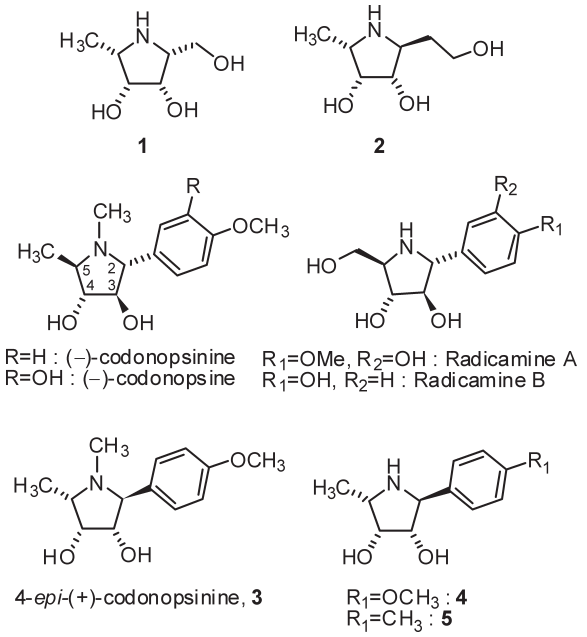


Figure 1. Structures of polyhydroxypyrrolidines.

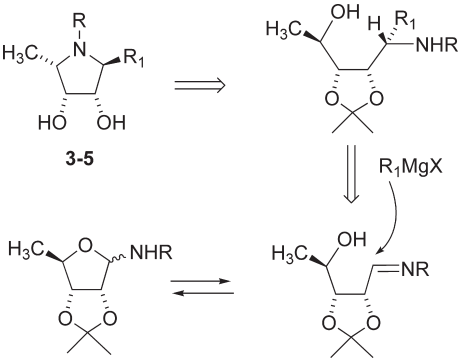
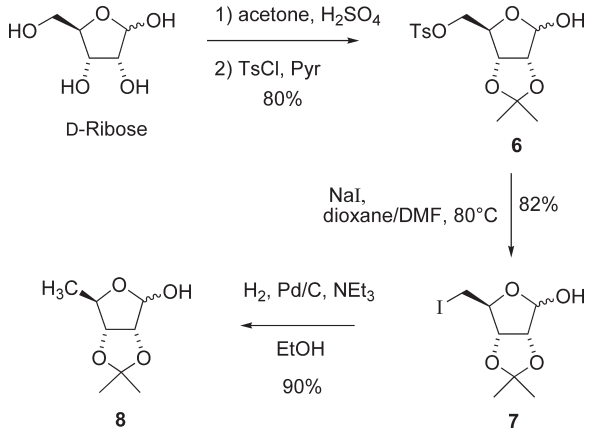


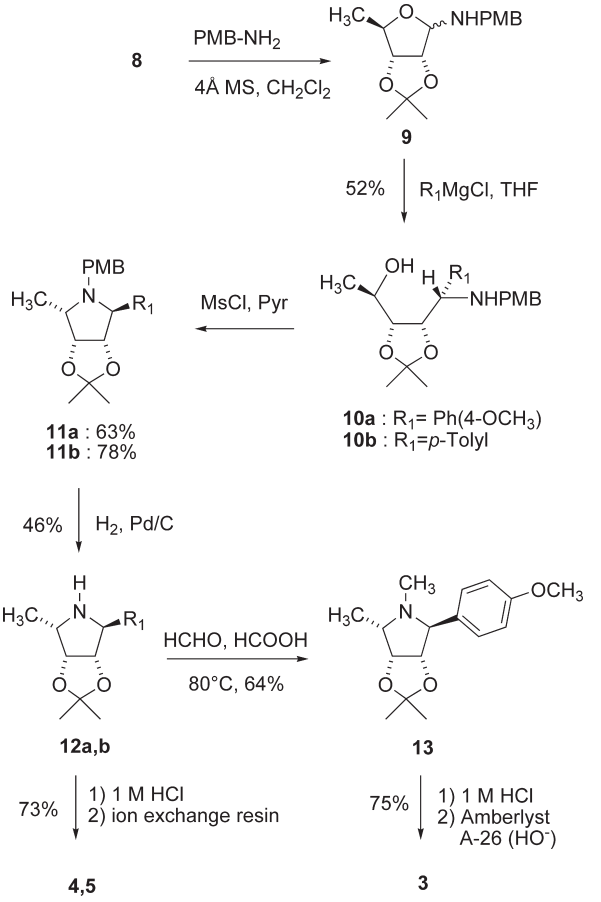
Figure 2. Retrosynthetic pathway for the synthesis of **3**−**5**.

Scheme 1. Synthesis of Deoxyribose 8



p-methoxybenzylamine for 24 h at room temperature in the presence of 4Å molecular sieves (Scheme 2). The excess amine was removed by evaporation under reduced pressure, and

Scheme 2. Synthesis of Iminosugars 3−**5**



glycosylamine **9**, which is very sensitive to hydrolysis, was used as such in the next step.

Organometallics usually add to protected aldoses[14] or glycosylamines[9] in a straightforward manner to yield the corresponding alditols or aminoalditols. The reaction requires an excess of reagent (one equivalent being consumed by neutralization of the acidic proton from the substrate) and occurs usually with high selectivity. Interestingly, the stereochemical outcome of the reaction is identical in both the aldose or aldimine series. Moreover, the nature of the protecting groups deeply impacts the diastereoisomeric ratio. In the ribose series, a 2,3-*O*-isopropylidene give rise mainly to 1,2-*anti* product,[15] whereas OBn protecting groups afford the *syn* diastereoisomer as the major adduct.[16]

In our case, addition of paramethoxyphenylmagnesium bromide to **9** was performed in THF at room temperature, and consumption of the starting material occurred over 4 h. The ^{1}H and ^{13}C NMR spectra of the crude reaction mixture definitely disclosed formation of a single addition product. Purification by silica gel chromatography (AcOEt/petroleum ether, 3:7) yielded **10a** as a colorless oil in 52% yield. The modest yield of the reaction might be attributed to the high tendency of glycosylamines to hydrolyze back to the corresponding hemiacetal, giving rise to quantitative amounts of nonisolated side products. The configuration of the newly formed stereocenter was established by NOE experiments on subsequent pyrrolidine **11a** and was firmly ascertained on the basis of X-ray crystallographic analysis of final compound **3**. As anticipated, the nucleophilic addition proceeded with *anti* selectivity providing aminoalcohol **10a** with

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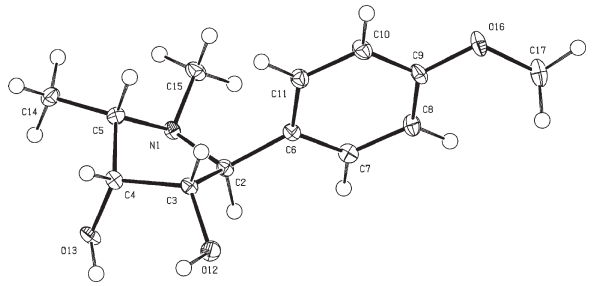

Figure 3. X-ray structure of 4-*epi*-(+)-codonopsinine **3**.

the 1(*S*) configuration. Mesylation was then carried out under our standard conditions (MsCl, pyridine, room temperature)[16] affording pyrrolidine **11a** in 63% isolated yield *via* an *in situ* nucleophilic displacement of the intermediary sulfonic ester. This sequence brought about inversion of configuration at C-4 of starting aminoalcohol, affording the expected 5(*S*) configuration in the pyrrolidine framework.

The removal of the *N*-paramethoxybenzyl group proved somewhat tricky and afforded **12a** in only 46% yield. *N*-methylation was then performed under the acidic Eschweiler−Clarke conditions (HCOOH/HCHO) in the aim of deprotecting simultaneously the isopropylidene acetal.[17] However, the acetonide was resistant, and a mixture of fully deprotected **3** (24%) and its precursor **13** (40%) were obtained in 64% global yield, which were separated by column chromatography. Subsequent treatment of **13** with 1 M hydrochloric acid was necessary to obtain 4-*epi*-codonopsinine **3** (75% yield). After neutralization (Amberlyst A-26, OH^- form), pyrrolidine **3** was purified by silica gel chromatography ($AcOEt/CH_2Cl_2$/MeOH, 4:4:2) and was crystallized from chloroform for X-ray analysis. The structure (see Figure 3) shows the compound in a ^3E conformation with atom C-3 out of the plane of the other four atoms. The planar aromatic ring is twisted relative to the pyrrolidine ring by ∼90°. The absolute configuration at C-2 and C-5 could be firmly assigned as 2(*S*),5(*S*).

The *N*-desmethyl derivative **4** was prepared in 73% yield by acidic treatment of pyrrolidine **12a** and was purified by ion exchange chromatography (Dowex 50WX8, elution with 0.8 M NH_4OH). The *p*-tolyl iminosugar **5** was prepared following a similar reaction sequence. Here again, addition of *p*-tolylmagnesium bromide to glycosylamine **9** was completely selective and afforded aminoalcohol **10b** in 52% yield. Subsequent cyclization (MsCl in pyridine, 78% yield), hydrogenolysis (H_2, Pd/C, 46% yield) and acetonide deprotection (aq. HCl, 73% yield) were performed under the conditions stated for **10a** to afford pyrrolidine **5** as a colorless oil.

New aryl-iminosugars **3**−**5** were assayed for their inhibitory activity toward α-L-fucosidase from bovine kidney (Table 1). A strong inhibition occurred with **3**−**5** at 100 μM concentration (Table 1). IC_{50}'s and K_i's were determined from Dixon plots, by assaying at least five dilutions. Iminosugars **3**−**5** were highly potent inhibitors of fucosidase, the *N*-desmethyl derivatives **4** and **5** being significantly more active (IC_{50} = 58 nM and 54 nM, respectively) than *epi*-codonopsinine **3** (IC_{50} = 5.9 μM). All three compounds dispayed a competitive inhibition pattern according to Lineweaver−Burk plots, with inhibition constants around 10 nM for **4** and **5** (Table 1). Title compound 4-*epi*-(+)-codonopsinine displayed an inhibition constant K_i = 0.8 μM. As observed by others, the *N*-Me supplementary substituent induces

Table 1. Inhibition of α-L-Fucosidase by Iminosugars 3−5

compound	% inhibition at 0.1 mM	IC_{50} (μM)	K_i (μM)
3	89	5.9	0.81
4	96	0.058	0.0095
5	99	0.054	0.010

a drop in affinity by 2 orders of magnitude.[18] However, the aryl moiety seems not to be detrimental for binding, and compounds **4** and **5** are among the most potent inhibitors of fucosidase described to date.

In summary, a short and stereoselective synthesis of 4-*epi*-codonopsinine **3** was developed, starting from D-ribose. The key step, i.e. the addition of a Grignard reagent to a protected ribosylamine, yielded only the *anti* isomer. The structure of **3** was firmly ascertained on the basis of X-ray crystallographic analysis. Two derivatives, i.e. the *N*-desmethyl compound **4** and its *p*-tolyl analogue **5**, were also prepared, and the compounds exhibited very strong inhibition toward α-L-fucosidase, with K_i's in the nanomolar range. Due to their unique hydrophobic character, these aryl-polyhydroxypyrrolidines could be a response to the poor cellular bioavailability of the most common fucosidase inhibitors with therapeutic potential like that of **1** or **2**.

EXPERIMENTAL SECTION

General Details. All reactions were performed under argon. The solvents were dried and distilled prior to use. Grignard reagents were purchased from standard commercial sources. Silica gel F254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic solution of phosphomolybdic acid or *p*-anisaldehyde or an aqueous solution of $KMnO_4$ (2%)/Na_2CO_3 (4%), followed by heating. Flash column chromatography was performed over silica gel M 9385 (40−63 μm) Kieselgel 60. NMR spectra were recorded on a 250 MHz (250 MHz for ^1H, 62.5 MHz for ^{13}C). Chemical shifts are expressed in parts per million (ppm) using TMS as internal standard. Coupling constants are in hertz, and splitting pattern abbreviations are br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded with an IR^{TM} plus MIDAC spectrophotometer and are expressed in cm^{-1}. Optical rotations were determined at 20 °C with a Perkin-Elmer model 241 polarimeter in the specified solvents. High resolution mass spectra (HRMS) were performed on Q-TOF Micro micromass positive ESI (CV = 30 V).

General Method for the Synthesis of Aminoalcohols 10a,b. Arylmagnesium bromide (10 mL of a commercial 1M solution, 10 mmol) was added to a stirred solution of glycosylamine **9** (0.746 g, 2.55 mmol) in THF (5 mL) at 0 °C, and the resulting mixture was left to react at rt for 7 h. Saturated NH_4Cl was then added, and the solution was extracted with Et_2O (3 × 20 mL) . The combined organic layers were dried ($MgSO_4$) and evaporated, and the crude aminoalcohol was purified by FC (EtOAc/petroleum ether, 3:7) to give pure **10** as a colorless oil (0.522 g, 52%, for **10a**).

*(1S,2S,3R,4R)-N-(4-Methoxybenzyl)-1-[4-methoxyphenyl]-2,3-O-isopropylidene-pentan-2,3-diol (**10a**):* as a colorless oil: R_f = 0.28 (PE/EtOAc, 7:3); $[\alpha]^{20}_D$ = −12.9 (*c* 1.16, $CHCl_3$); IR (film) ν_{max} 2990, 2935, 2837, 1612, 1516, 1251 (cm^{-1}); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.22 (3 H, s), 1.24 (3 H, s), 1.35 (3 H, d, *J* = 5.9 Hz), 3.49 (2 H, s), 3.70−3.93 (8 H, m), 4.03 (1 H, dd, *J* = 5.2, 9.4 Hz), 4.26 (1 H, dd, *J* = 5.2, 10.1 Hz), 6.81 (2 H, d, *J* = 8.4 Hz), 6.95 (2 H, d, *J* = 8.4 Hz), 7.12 (2 H, d, *J* = 8.4 Hz), 7.18 (2 H, d, *J* = 8.4 Hz); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 20.7 (CH_3), 25.6 (CH_3), 28.1 (CH_3), 50.2 (CH_2), 55.4 (CH_3), 55.5 (CH_3), 61.3 (CH), 65.0 (CH), 80.0 (CH), 83.3 (CH), 108.4 (C), 114.2 (Ar−CH), 114.4 (Ar−CH), 128.9 (Ar−CH), 130.3 (Ar−CH), 132.7

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(Ar−C), 159.1 (Ar−C), 159.3 (Ar−C); ESI-HRMS: calcd for $C_{23}H_{32}NO_5$ ($[M + H]^+$) 402.2280; found 402.2276.

*(1S,2S,3R,4R)-N-(4-Methoxybenzyl)-1-tolyl-2,3-O-isopropylidene-pentan-2,3-diol (**10b**)*: as a colorless oil: R_f = 0.30 (PE/EtOAc, 7:3); $[\alpha]^{20}_D$ = −9.1 (*c* 1, $CHCl_3$); IR (film) ν_{max} 2989, 2933, 2862, 1613, 1515, 1250 (cm^{-1}); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.12 (3 H, s), 1.18 (3 H, s), 1.30 (3 H, d, *J* = 5.9 Hz), 2.39 (3 H, s), 3.48 (2 H, bs), 3.70−3.90 (5 H, m), 4.02 (1 H, dd, *J* = 5.4, 9.2 Hz), 4.30 (1 H, dd, *J* = 5.4, 10.1 Hz), 6.80 (2 H, d, *J* = 8.6 Hz), 7.10−7.30 (6 H, m); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 20.8 (CH_3), 21.6 (CH_3), 25.7 (CH_3), 28.3 (CH_3), 50.4 (CH_2), 55.7 (CH_3), 61.8 (CH), 65.2 (CH), 80.2 (CH), 83.5 (CH), 108.7 (C), 114.4 (Ar−CH), 127.9 (Ar−CH), 129.9 (Ar−CH), 130.5 (Ar−CH), 137.7 (Ar−C), 137.8 (Ar−C), 159.5 (Ar−C); ESI-HRMS: calcd for $C_{23}H_{32}NO_4$ ($[M + H]^+$) 386.2331; found 386.2349.

General Method for the Synthesis of Iminosugars 11a,b. Methanesulfonyl chloride (87 μL, 2 equiv) was added to a stirred solution of aminoalcohol **10** (0.418 g, 1.04 mmol) in pyridine (3.6 mL) at 0 °C, and the resulting mixture was left to react at rt for 16 h. The reaction was quenched with water and the solution was extracted twice with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), evaporated and the crude pyrrolidine was purified by FC (Et_2O/petroleum ether, 3:7) to give pure **11** as a colorless oil (0.251 g, 63% for **11a**).

*(2S,3S,4R,5S)-N-(4-Methoxybenzyl)-2-[4-methoxyphenyl]-3,4-O-isopropylidene-5-methylpyrrolidine-3,4-diol (**11a**)*: as a colorless oil: R_f = 0.40 (PE/EtOAc, 7:3); $[\alpha]^{20}_D$ = +56.8 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 2978, 2935, 2835, 1612, 1513, 1379, 1247 (cm^{-1}); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.09 (3 H, d, *J* = 6.5 Hz), 1.36 (3 H, s), 1.60 (3 H, s), 3.19 (1 H, d, *J* = 13.9 Hz), 3.22−3.30 (1 H, m), 3.57 (1 H, d, *J* = 13.9 Hz), 3.70 (6 H, s), 3.95 (1 H, d, *J* = 2.3 Hz), 4.59 (1 H, dd, *J* = 2.3, 6.7 Hz), 4.78 (1 H, t, *J* = 6.7 Hz), 6.75−6.85 (4 H, m), 7.07 (2 H, d, *J* = 8.5 Hz), 7.20 (2 H, d, *J* = 8.5 Hz); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 10.6 (CH_3), 25.2 (CH_3), 26.3 (CH_3), 49.6 (CH_2), 55.2 (CH_3), 55.2 (CH_3), 57.1 (CH), 69.1 (CH), 80.9 (CH), 86.3 (CH), 112.5 (C), 113.5 (Ar−CH), 113.7 (Ar−CH), 129.2 (Ar−CH), 129.5 (Ar−CH), 131.5 (Ar−C), 131.7 (Ar−C), 158.3 (Ar−C), 158.9 (Ar−C); ESI-HRMS: calcd for $C_{23}H_{30}NO_4$ ($[M + H]^+$) 384.2175; found 384.2187.

*(2S,3S,4R,5S)-N-(4-Methoxybenzyl)-2-tolyl-3,4-O-isopropylidene-5-methylpyrrolidine-3,4-diol (**11b**)*. Following the same procedure, **11b** was obtained as a colorless oil (78%): R_f = 0.60 (PE/EtOAc, 7:3); $[\alpha]^{20}_D$ = +63.8 (*c* 1.16, $CHCl_3$); IR (film) ν_{max} 2977, 2933, 2834, 1611, 1512, 1246 (cm^{-1}); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.01 (3 H, d, *J* = 6.3 Hz), 1.26 (3 H, s), 1.51 (3 H, s), 2.25 (3 H, s), 3.11 (1 H, d, *J* = 13.9 Hz), 3.20−3.24 (1 H, m), 3.49 (1 H, d, *J* = 13.9 Hz), 3.70 (3 H, s), 3.90 (1 H, d, *J* = 2.1 Hz), 4.51 (1 H, dd, *J* = 2.1, 6.3 Hz), 4.68 (1 H, t, *J* = 6.1 Hz), 6.78 (2 H, d, *J* = 8.4 Hz), 6.95−7.20 (6 H, m); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 12.1 (CH_3), 22.5 (CH_3), 26.5 (CH_3), 27.6 (CH_3), 51.0 (CH_2), 56.6 (CH_3), 58.5 (CH), 70.8 (CH), 82.3 (CH), 87.7 (CH), 113.7 (C), 114.9 (Ar−CH), 129.7 (Ar−CH), 130.4 (Ar−CH), 130.6 (Ar−CH), 133.0 (Ar−C), 138.0 (Ar−C), 138.4 (Ar−C), 159.7 (Ar−C); ESI-HRMS: calcd for $C_{23}H_{30}NO_3$ ($[M + H]^+$) 368.2226; found 368.2220.

General Methods for Pyrrolidine Deprotection. Procedure A: removal of *N*-4-methoxybenzyl group was achieved by hydrogenation of pyrrolidine **11** (0.16 mmol) over Pd/C (50 mg) in 3 mL MeOH for 48 h. The resulting suspension was filtered over a pad of Celite washed several times with MeOH. Evaporation of the solvent gave a colorless oil, which was purified by silica gel chromatography (Et_2O/petroleum ether, 6:4) to give pure **12a,b** in 46% yield.

Procedure B: acetonide hydrolysis was performed by stirring a solution of **12** or **13** (0.1 mmol) in 1 *M* HCl (0.5 mL) for 24 h at rt. After evaporation, the crude product was applied to a column of Dowex 50WX8. The resin was washed with distilled water and then eluted with 0.8 M NH_4OH to give pure pyrrolidines **4** or **5** in 73% yield. Alternately, the crude hydrochloride obtained after acidic treatment could be neutralized with Amberlite A-25 (OH^-) to give pyrrolidine **3** as the free base in almost pure form (75%).

*(2S,3S,4R,5S)-2-[4-Methoxyphenyl]-5-methylpyrrolidine-3,4-diol (**4**)*: as a colorless oil from **11a** following deprotection procedures A and B: $[\alpha]^{20}_D$ = −56.1 (*c* 0.2, CH_3OH); ^1H NMR (D_2O, 250 MHz) δ 1.29 (3 H, d, *J* = 6.9 Hz), 3.70−3.82 (4 H, m), 4.17 (1 H, t, *J* = 3.8 Hz), 4.30 (1 H, d, *J* = 9.6 Hz), 4.49 (1 H, dd, *J* = 3.8, 9.6 Hz), 7.02 (2 H, d, *J* = 8.4 Hz), 7.40 (2 H, d, *J* = 8.4 Hz); ^{13}C NMR (62.5 MHz; D_2O) δ 10.9 (CH_3), 54.6 (CH_3), 55.9 (CH), 61.9 (CH), 71.3 (CH), 75.5 (CH), 114.0 (Ar−CH), 125.3 (Ar−C), 128.7 (Ar−CH), 159.0 (Ar−C); ESI-HRMS: calcd for $C_{12}H_{18}NO_3$ ($[M + H]^+$) 224.1287; found 224.1282.

*(2S,3S,4R,5S)-2-Tolyl-5-methylpyrrolidine-3,4-diol (**5**)*: as a colorless oil from **11b** following deprotection procedures A and B: $[\alpha]^{20}_D$ = −48.6 (*c* 0.37, CH_3OH); ^1H NMR (D_2O, 250 MHz) δ 1.12 (3 H, d, *J* = 6.9 Hz), 2.29 (3 H, s), 3.48−3.54 (1 H, m), 4.00−4.08 (2 H, m), 4.24 (1 H, dd, *J* = 4.0, 9.0 Hz), 7.21 (2 H, d, *J* = 8.1 Hz), 7.28 (2 H, d, *J* = 8.1 Hz); ^{13}C NMR (62.5 MHz; D_2O) δ 16.2 (CH_3), 22.4 (CH_3), 57.1 (CH), 66.1 (CH), 76.4 (CH), 81.8 (CH), 129.5 (Ar−CH), 131.0 (Ar−C), 131.7 (Ar−CH), 140.3 (Ar−C); ESI-HRMS: calcd for $C_{12}H_{18}NO_2$ ($[M + H]^+$) 208.1338; found 208.1344.

Procedure for *N*-Methylation. *(2S,3S,4R,5S)-N-Methyl-2-[4-methoxy-phenyl]-3,4-O-isopropylidene-5-methyl-pyrrolidine-3,4-diol (**13**)*. Pyrrolidine **12a** (84 mg, 0.32 mmol) was dissolved in formic acid (0.7 mL) and formaldehyde (1.4 mL of a 37% solution in water). The resulting mixture was heated to 80 °C until complete consumption of the starting compound. Then, an aq solution of K_2CO_3 (20 mL) was added, and the resulting solution was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic fractions were dried over anhydrous $MgSO_4$, filtered, and concentrated to yield a crude, yellow residue. Purification by flash chromatography (Et_2O/petroleum ether: 6:4 then Et_2O) afforded **13** (36 mg, 40%) and fully deprotected **3** (18 mg, 24%). Compound **13**: R_f = 0.34 (PE/Et_2O, 4:6); $[\alpha]^{20}_D$ = +30.0 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 2976, 2934, 2836, 1611, 1512, 1249 (cm^{-1}); ^1H NMR ($CDCl_3$, 250 MHz) δ 1.03 (3 H, d, *J* = 6.5 Hz), 1.25 (3 H, s), 1.51 (3 H, s), 1.98 (3 H, s), 3.03 (1 H, quin), 3.70 (3 H, s), 3.90 (1 H, bs), 4.55 (1 H, d, *J* = 6.7 Hz), 4.72 (1 H, t, *J* = 6.7 Hz), 6.75 (2 H, d, *J* = 8.6 Hz), 6.99 (2 H, d, *J* = 8.6 Hz); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 11.2 (CH_3), 24.7 (CH_3), 26.1 (CH_3), 35.1 (CH_3), 55.2 (CH_3), 60.6 (CH), 71.6 (CH), 81.2 (CH), 85.6 (CH), 111.9 (C), 113.7 (2 × Ar−CH), 129.5 (2 × Ar−CH), 131.5 (Ar−C), 158.3 (Ar−C); ESI-HRMS: calcd for $C_{16}H_{24}NO_3$ ($[M + H]^+$) 278.1756; found 278.1763.

Deprotection of **13** (36 mg, 0.13 mmol) following procedure B gave **3** (23 mg, 75%) as colorless crystals: mp = 81 °C; R_f = 0.28 (CH_2Cl_2/EtOAc/MeOH, 7:4:2); $[\alpha]^{20}_D$ = +7 (*c* 0.34, CH_3OH); ^1H NMR (D_2O, 250 MHz) δ 1.09 (3 H, d, *J* = 6.7 Hz), 2.10 (3 H, s), 3.00 (2 H, bs), 3.38−3.45 (1 H, m), 3.56 (1 H, d, *J* = 4.4 Hz), 3.81 (3 H, s), 4.00 (1 H, dd, *J* = 4.4, 6.2 Hz), 4.25 (1 H, t, *J* = 6.2 Hz), 6.88 (2 H, d, *J* = 8.4 Hz), 7.20 (2 H, d, *J* = 8.4 Hz); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 8.8 (CH_3), 34.8 (CH_3), 55.3 (CH_3), 61.1 (CH), 71.7 (CH), 73.5 (CH), 78.4 (CH), 113.9 (Ar−CH), 129.0 (Ar−CH), 132.9 (Ar−C), 159.0 (Ar−C); ESI-HRMS: calcd for $C_{13}H_{20}NO_3$ ($[M + H]^+$) 238.1443; found 238.1441.

■ ASSOCIATED CONTENT

Supporting Information. Copies of NMR spectra (^1H-, ^{13}C) for all new compounds, crystallographic data for **3**, Dixon plots for fucosidase inhibitory assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
jb.behr@univ-reims.fr

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