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Short and Efficient Chemoenzymatic Syntheses of non-Natural (−)-Muscarine and (+)-*allo*-Muscarine from Cyano-Sugar Precursors Catalyzed by Immobilized *Burkholderia cepacia* Lipase

Alejandro Carnero,[a] Yogesh S. Sanghvi,[b] Vicente Gotor,[a] Susana Fernández,[a]* and Miguel Ferrero[a]*

[a] Departamento de Química Orgánica e Inorgánica and Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, Avenida Julián de Clavería 8, 33006-Oviedo (Asturias), Spain
M.F.: tel. +34 985 105 013, mferrero@uniovi.es. S.F.: tel. +34 985 102 984, fernandezgsusana@uniovi.es; FAX (for both): +34 985 103 446
[b] Rasayan Inc., 2802 Crystal Ridge Road, Encinitas, CA 92024-6615, USA

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Abstract. Enantiopure (2*R*)-configured non-natural (−)-muscarine and (+)-*allo*-muscarine were efficiently synthesized by a chemoenzymatic approach from an easily accessible cyano-sugar available on large-scale. The key enzymatic hydrolysis step was accomplished by immobilized *Burkholderia cepacia* lipase (also known as *Pseudomonas cepacia* lipase, PSL-IM). The PSL-IM mediated regioselective hydrolysis of the 5-*O*-toluoyl ester group in cyano-sugars α− and β−, furnished 3-*O*-toluoyl-α-cyano-sugar and 3-*O*-toluoyl-β-cyano-sugar, respectively, with total selectivity and in excellent yields. To demonstrate the industrial utility of this method, 3-*O*-toluoyl-β-cyano-sugar was synthesized on 10 g scale using PSL-IM and catalyst reused. A key feature of the synthesis route described herein is the simultaneous hydrogenolysis of a tosyl group, reduction of a nitrile and deprotection of a toluoyl group using $LiAlH_4$ in one-pot procedure. This study offers green protocol for synthesis of two muscarine derivatives in high yields employing a concise four-step strategy.

Keywords: biocatalysis; biotransformations; green chemistry; muscarine; synthesis design

Introduction

Functionalized tetrahydrofuran units are present in many naturally occurring molecules such as muscarin alkaloids.[1] The biological activity of these alkaloids have had remarkable influence on drug discovery efforts. Consequently, synthesis of muscarine derivatives have allured medicinal chemists for many years.[2] The toxic principle (+)-(2*S*,4*R*,5*S*)-muscarine (**1**, Figure 1) has generated much interest due to its potent and specific cholinergic activity.[3]

Muscarine alkaloids were first isolated from *Amanita muscaria*, a mushroom found in pinewoods[4] and few species of toadstools, most stereoisomers of compound **1**. Due to the three stereocenters in the molecule, eight stereoisomers are possible (Figure 1). Synthesis of all stereoisomers **2**-**8** have been reported.[5] Preparation of muscarine homologues continue to generate excitement due to their high level of activity.[6] A renewed interest in this class of compounds is due to its selectivity towards muscarinic receptors in the brain and the discovery of its activity in the treatment of

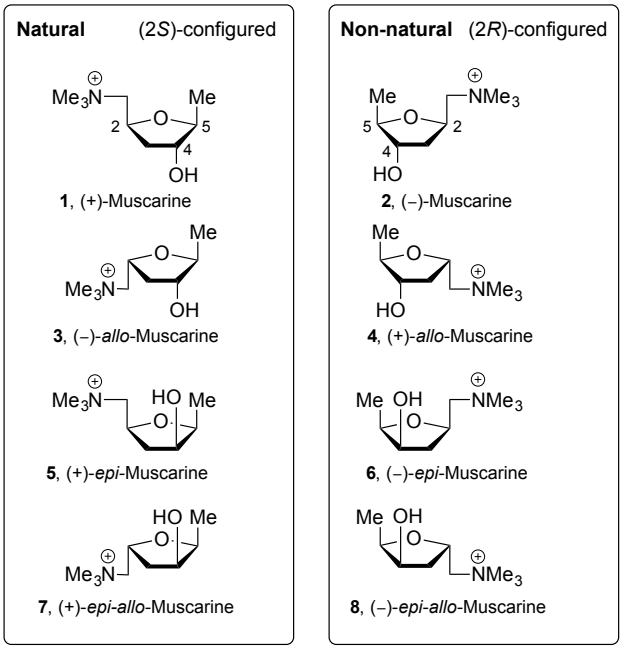


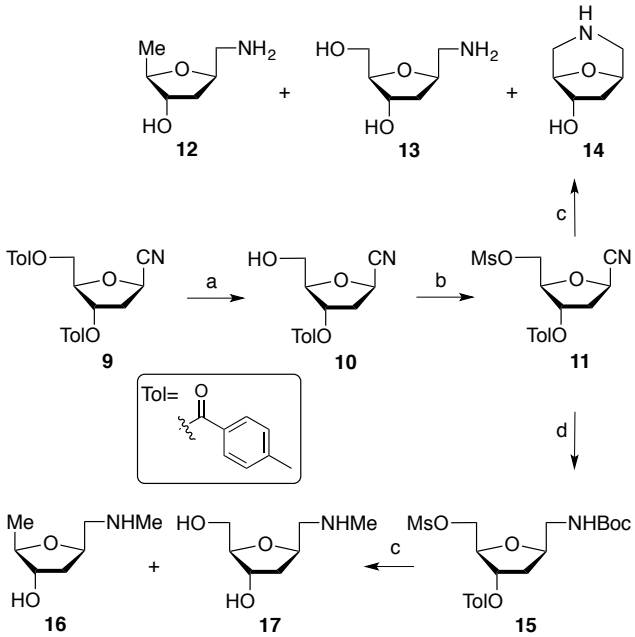
Figure 1. List of natural and non-natural diastereoisomers of muscarine alkaloids.

neurodegenerative diseases such as Parkinson's or Alzheimer's diseases and other cognitive disorders.[7]

Therefore, methods for selectively preparing enantiomerically pure muscarine alkaloids have received considerable attention. The published synthetic approaches are limited in versatility, specific for a single molecule, require multi-step synthesis and utilized expensive reagents and/or starting materials.[2,6a,8] The combination of growing demand and limited availability of muscarine derivatives from natural sources have led to syntheses from carbohydrate and non-carbohydrate starting materials. In view of this background and the biological relevance of muscarine alkaloids, herein we describe an efficient preparation of (−)-muscarine[9] and (+)-*allo*-muscarine[9b,d,f,10] for which just a few synthesis are available.

Results and Discussion

For the preparation of muscarine analogs **2** and **4**, we elected β-cyano-sugar **9** and α-cyano-sugar **19** as starting materials, respectively. The rationale behind the use of cyano-sugar is as follows: (i) cyano-sugar is easily synthesized from 2'-deoxyribose in few steps;[11] (ii) all chiral centers are predefined in 2-deoxyribose; and (iii) cyano-sugar is commercially available on large-scale.[12]



Scheme 1. Attempted syntheses of (−)-muscarine hydroxide through a mesylate. Reaction conditions: (a) Lipase PSL-IM, 0.15 M KH_2PO_4 (pH 7), 1,4-dioxane, 45 ºC, 14 h (94%); (b) MsCl, pyridine, DMAP, 0 °C to rt, 24 h (88%); (c) $LiAlH_4$, THF, reflux, 4 h; (d) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, Boc_2O, MeOH, 0 °C to rt, 16 h (55%).

Based on our prior experience[13] with regioselective enzymatic hydrolysis, we envisioned that the removal of toluoyl attached to the primary hydroxyl group would set the stage for subsequent transformations leading to muscarine derivatives. In order to accomplish this feast, *Candida antarctica* lipase A, *Candida antarctica* lipase B, *Pseudomonas cepacia* lipase,† *Thermomyces lanuginosus* lipase, and *Pseudomonas fluorescens* lipase were screened for selective hydrolysis. In the case of *Candida antarctica* lipase A and *Candida antarctica* lipase B, no reaction was observed. The other enzymes screened displayed total selectivity toward the hydrolysis of the ester at the C-5 position, but *Pseudomonas cepacia* lipase afforded the best hydrolysis rate in shortest reaction time. When 3,5-di-*O*-*p*-toluoyl protected compound **9** was treated with the enzyme preparation PSL-SD (2:1 w/w relative to **9**) in 0.15 M phosphate buffer (pH 7) containing 18% of 1,4 dioxane at 30 ºC, 45 ºC, and 60 ºC, ^{1}H-NMR spectra of the reaction mixture clearly indicated the formation of the 3-*O*-toluoyl ester **10** as the only product, due to selective hydrolysis of the 5-*O*-toluoyl ester (Figure 2). The increase of the temperature from 30 ºC to 45 ºC reduced the reaction time. Thus, after 14 h at 45 ºC the starting material was consumed and **10** was isolated in 95% yield.

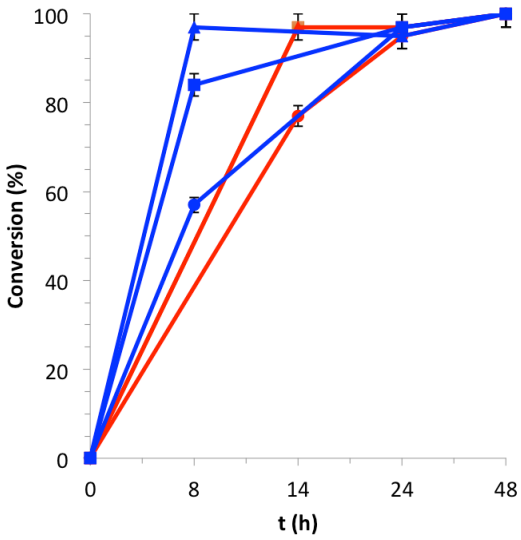


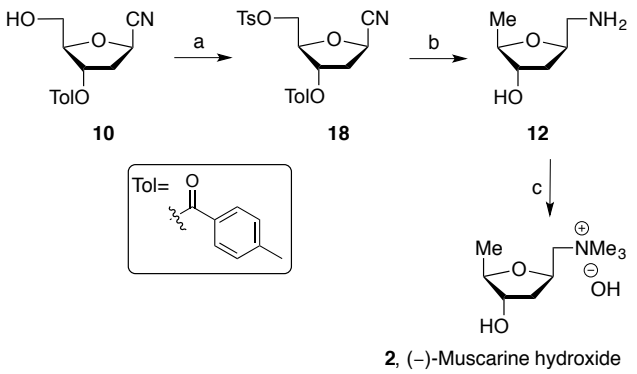
Figure 2. Hydrolysis reaction of **9** with lipases at different temperatures: PSL-SD 30 ºC (●), PSL-SD 45 ºC (■), PSL-SD 60 ºC (▲), PSL-IM 30 ºC (●), PSL-IM 45 ºC (■), and PSL-IM 60 ºC (▲).

Similarly, hydrolysis with immobilized *Pseudomonas cepacia* lipase (lipase PSL-IM) led exclusively to the formation of **10** in 24 h at 45 ºC with 94% isolated yield. We used the same ratio 2:1 (w/w) of lipase, but since it is immobilized fewer units are needed per gram basis, hence the longer reaction time. It is noteworthy that the excellent

† Note: *Burkholderia cepacia* is the most appropriate name, but most of the commercial suppliers use the old nomenclature *Pseudomonas cepacia* lipase or PSL-IM.

yields and purity of **10** were obtained without column chromatography. One advantage of the immobilized enzyme is the fact that it can be recycled to make the process more economical for industrial applications.

The reaction of **10** with methanesulfonyl choride in pyridine and DMAP afforded mesylate **11** in 88% yield. Subsequent treatment with lithium aluminium hydride in THF at reflux enabled simultaneous hydrogenolysis, reduction and cleavage of the toluoyl group. However, the desired compound **12** was obtained as an inseparable mixture with **13** and **14**. Formation of an alcohol (**13**) via S-O bond cleavage is often observed in the reduction of sulfonate esters, accounting for the low yields of desired product. It appears that the cyano group was reduced first, triggering the formation of a ring closed bicyclic structure **14**. In order to circumvent the formation of compound **14**, we opted for an alternate route where amino-group was protected with *tert*-butyloxycarbonyl group. The one-pot synthesis of *N*-Boc protected amine **15** was achieved by the reaction of **11** with di-*tert*-butyl dicarbonate and $NiCl_2 \cdot 6H_2O$-$NaBH_4$ in MeOH (55% yield).[14] Treatment of **15** with $LiAlH_4$ at reflux furnished compounds **16** and **17** as 2:1 mixture. These results prompted us to explore alternative synthetic strategy.



Scheme 2. Synthesis of (−)-muscarine hydroxide through a tosylate. Reaction conditions: (a) TsCl, pyridine, DMAP, 0 °C to rt, 24 h (85%); (b) $LiAlH_4$, THF, reflux, 4 h (84%); (c) i) MeI, 1,2,2,6,6-pentamethylpiperidine, EtOAc, 16 h, ii) NaOH 3 M (80%).

Gratifyingly, the use of tosylate instead the mesylate avoided formation of a mixture of products while improving the yield. Thus, tosylate derivative **18** was prepared from **10** in 85% yield by treatment with *p*-toluenesulfonyl chloride in pyridine and DMAP (Scheme 2). Next, compound **18** was treated with $LiAlH_4$ in THF at reflux to afford exclusively the desired derivative **12** in 84% yield. It is noteworthy that a single reaction allowed complete transformation of three groups simultaneously in excellent yield. Finally, compound **12** was converted to (–)-muscarine by treatment with MeI and 1,2,2,6,6-pentamethylpiperidine followed by ion-exchange with aqueous sodium hydroxide.

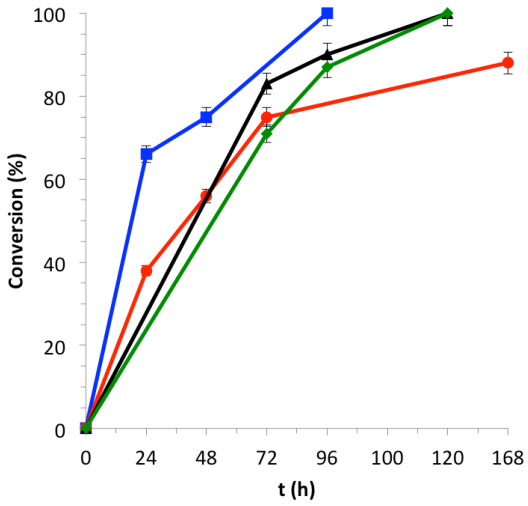
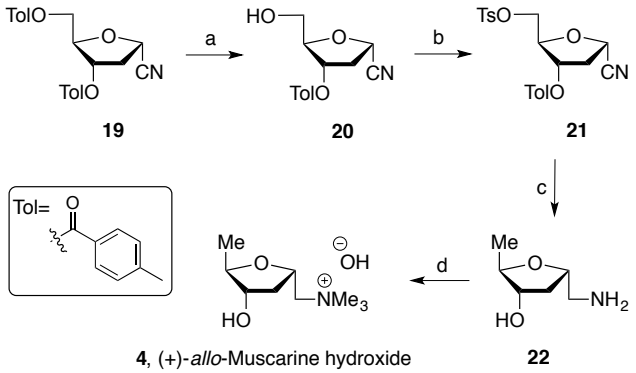


Figure 3. Hydrolysis reaction of **19** at 45 ºC with lipase PSL-SD and lipase PSL-IM at different ratios of enzyme: PSL-SD 2:1 (■), PSL-IM 2:1 (●), PSL-IM 4:1 (▲), and PSL-IM 6:1 (◆).

Next, the foregoing approach was utilized for the synthesis of (+)-*allo*-muscarine. Again the cyano-sugar **19** was accessible on large-scale.[12] Selective deprotection of the primary toluoyl group was accomplished by reaction of **19** with lipase PSL-SD at 45 ºC in 0.15 M phosphate buffer (pH 7), furnishing 3-*O*-toluoyl derivative **20** after 4 d in 90% yield. The work up of this reaction was simplified, since compound **20** was obtained in high purity which enabled isolation without column chromatography (Figure 3). Under the same reaction conditions, total selectivity was also observed with lipase PSL-IM, but the reaction was slower and a maximum of 88% conversion was achieved after 7 d.



Scheme 3. Synthesis of (+)-*allo*-muscarine hydroxide Reaction conditions: (a) Lipase PSL-IM, 0.15 M KH_2PO_4 (pH 7), 1,4-dioxane, 45 ºC, 5 d (90%); (b) TsCl, pyridine, DMAP, 0 °C to rt, 24 h (75%); (c) $LiAlH_4$, THF, reflux, 4 h (78%); (d) i) MeI, 1,2,2,6,6-pentamethylpiperidine, EtOAc, 16 h, ii) NaOH 3 M (76%).

To overcome this limitation, we evaluated the influence on the conversion using different ratios of

enzyme. In this case, 4:1 and 6:1 (w/w) ratios of lipase:**19** were employed. In 5 d transformation at 45 ºC and 4:1 ratio of enzyme furnished 3-*O*-toluoyl derivative **20** with excellent regioselectivity and conversion. Subsequently, tosylation of **20** generated **21** in 75% yield. Following same reaction conditions as in the case of **18**, treatment of tosylate **21** with $LiAlH_4$ resulted in the formation of the desired amino-alcohol **22** in a single step. Next, the amino group was quaternized to the corresponding (+)-*allo*-muscarine hydroxide using previous conditions (Scheme 3).

Scale-up studies. All of the above PSL-IM mediated hydrolysis experiments were carried out with 1:2 (w/w) enzyme ratio of **9**:PSL-IM or 1:4 (w/w) of **19**:PSL-IM to drive the reaction to completion. In order to show the industrial utility of the process, we have scaled-up the enzymatic conversion of β-cyano sugar **9** to compound **10**. The reaction was carried out with 10 g of **9** and reduced enzyme:substrate ratio of 1:1, making it cost-efficient. Thus, the reaction was complete in 24 h and work-up of the reaction provided crude 3-*O*-toluoylcyano sugar **10**, which was isolated in 91% yield via precipitation.

In order to reduce the cost of catalyst, we studied the possible reuse of immobilized lipase for multiple reactions (Table 1). Therefore, three sequential reactions were successfully carried out while maintaining same level of conversions and selectivity with a single lot of enzyme. During three reuse cycles same lot of enzyme was employed with 2 g of fresh substrate **9** or **19** in a substrate:enzyme ratio of 1:2 or 1:4, respectively, in two separate experiments (entries 1-3 and 4-6, Table 1). The reaction time for the third cycle was longer (entries 3 and 6, Table 1) perhaps due to loss of some immobilized enzyme.

Table 1. Enzyme reuse during regioselective hydrolysis of α- or β-cyano derivatives with PSL-IM on 2 g scale at 45 ºC.

Entry	Substrate	Cycle	t (h)	Product	Yield (%)[a]
1	**9**	1st	24	**10**	97
2	**9**	2nd	24	**10**	98
3	**9**	3rd	32	**10**	91
4	**19**	1st	120	**20**	97
5	**19**	2nd	120	**20**	89
6	**19**	3rd	132	**20**	91

[a] Percentages of isolated yields of 3-*O*-toluoyl cyano-sugars.

Conclusion

In summary, the chemoenzymatic strategy described herein provides an easy entry to non-natural muscarine derivatives using commercial biocatalysts, which have become an attractive and powerful tool in organic synthesis due to their high selectivity, mild reaction conditions and low environmental impact. The selective hydrolysis of toluoyl group with an enzyme provided much needed precision not available with traditional chemical approaches. The use of a predefined chiral auxillary permits rapid and affordable entry into the synthesis of modified muscarine analogs for discovery of potential therapeutics.[15] The key accomplishment of this route is the ability to synthesize two muscarine analogs in four easy steps and high overall yield. Yet another milestone of this route is the execution of three separate reactions in one-pot with a single reagent in high yield. The chemoenzymatic approach described herein was demonstrated on large-scale and constitutes a significant improvement over other methods reported in the literature for synthesis of non-natural muscarine.

Experimental Section

General

General. Lipase PS "Amano SD" (24,700 u/g) is a crude enzyme preparation. Lipase PS "Amano IM" (816 u/g) is the lipase PS "Amano SD" immobilized on diatomaceous earth. Both were purchased from Amano Enzyme. All other reagents were bought from Aldrich at highest commercial quality and used without further purification. All non-aqueous reactions were carried out under anhydrous conditions in dry, freshly destilled solvents. Reactions were monitored by TLC carried out on 0.25 mm Merck silica gel plates (60F-254) using UV light as visualizing agent and/or acidic aqueous permanganate or 3% of ninhydrin in ethanol. Flash chromatography was performed using silica gel 60 (230–400 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. ^{1}H, ^{13}C NMR, and DEPT were obtained using Bruker 300.13, 400.13 or 600.13 MHz for ^{1}H, and 75.5, 90.61 MHz or 150.90 for ^{13}C. The same spectrometers were used for the acquisition of ^{1}H-^{1}H homonuclear (COSY and NOESY) and ^{1}H-^{13}C heteronuclear (HSQC and HMBC) correlations. Optical rotations were recorded on a Jasco P-1010 polarimeter and values are reported as follows: $[\alpha]_\lambda^T$ (c: g/100 mL, solvent). High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under electron spray ionization (ESI) conditions.

Note on compound naming: the numbering of the muscarine and derivatives is the same as usual of that muscarine analogs (see Figure 1), but note that all other compounds have different numbering, related with ribose derivatives, in order to simplify the names and make it clearer.

(–)-Muscarine hydroxide (**2**).[16]

1,2,2,6,6-Pentamethylpiperidine (94.7 mg, 0.61 mmol) and MeI (187 μL, 3 mmol) were added to a solution of **12** (40 mg, 0.3 mmol) in EtOAc (1.3 mL). The reaction was stirred at rt during 16 h. The insoluble material was filtered and washed with EtOAc. The solid was poured in H_2O and aqueous 3 M NaOH was added until basic pH. After being stirred 15-20 min, the water was evaporated and the residue was taken up in acetonitrile. The solid was filtered and the filtrate evaporated. The resulting solid was taken up in Et_2O. The solid was filtered and washed with Et_2O to afford **2** in 80% yield as a hygroscopic white solid. $[\alpha]_D^{20}$ = −14.1 (*c* 1.0, EtOH); IR (KBr): ν 3424, 2970, 2919, 2850, 1275 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.23 (d, 3H, H_6, *J*= 6.4 Hz), 1.92 (m, 1H, H_3), 2.06 (ddd, 1H, H_3, *J*= 13.2, 6.0, 2.5 Hz), 3.29 (s, 9H, N*Me*$_3$), 3.51 (dd, 1H, H_7, *J*= 13.6, 9.9 Hz), 3.74 (dd, 1H, H_7, *J*= 13.6, 1.4 Hz), 3.97 (m, 2H, H_4+H_5), 4.63 (q, 1H, H_2, *J*= 9.7 Hz) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 20.2 (C_6), 39.6 (C_3), 54.97, 55.02, 55.07 (N*Me*$_3$), 71.5 (C_7), 73.1 (C_2), 76.6 (C_4), 85.3

(C_5) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_9H_{20}NO_2$ [(M+H)$^+$]: 174.1488, found: 174.1488.

(+)-*allo*-Muscarine hydroxide (**4**).[16]

1,2,2,6,6-Pentamethylpiperidine (74.5 mg, 0.48 mmol) and MeI (149.4 µL, 2.4 mmol) were added to a solution of **22** (32 mg, 0.24 mmol) in EtOAc (1.2 mL). The reaction was stirred at rt during 16 h. Next, solvent was evaporated and the insoluble material was washed with Et_2O. The solid was poured in H_2O and aqueous 3 M NaOH was added until basic pH. After being stirred 15-20 min, the water was evaporated and the residue was taken up in acetonitrile. The solid was filtered and the filtrate evaporated. The resulting solid was taken up in Et_2O. The solid was filtered and washed with Et_2O to afford **4** as a hygroscopic white solid in 76% yield. $[\alpha]_D^{20}$= +6,4 (*c* 1.0, EtOH); IR (KBr): ν 3397, 2970, 2923, 1266 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.19 (d, 3H, H_6, *J*= 6.3 Hz), 1.64 (m, 1H, H_3), 2.53 (ddd, 1H, H_3, *J*= 13.3, 7.9, 6.0 Hz), 3.24 (s, 9H, N*Me*$_3$), 3.49 (dd, 1H, H_7, *J*= 13.6, 1.7 Hz), 3.67 (dd, 1H, H_7, *J*= 13.6, 10.0 Hz), 3.99 (m, 2H, H_4+H_5), 4.69 (m, 1H, H_2) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 19.0 (C_6), 39.2 (C_3), 54.76, 54.81, 54.87 (N*Me*$_3$) 71.2 (C_7), 72.6 (C_2), 76.8 (C_4), 83.4 (C_5) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_9H_{20}NO_2$ [(M+H)$^+$]: 174.1488, found: 174.1490.

1β-Cyano-1,2-dideoxy-3-*O*-toluoyl-D-ribose (**10**).

To a solution of **9** (2 g, 5.3 mmol) in 1,4-dioxane (9 mL) was added 0.15 M KH_2PO_4 pH 7 (44 mL) and lipase PSL-IM (4 g). The mixture was allowed to react at 250 rpm and 45 ºC during 24 h. The enzyme was filtered off and washed with CH_2Cl_2, the solvents were distilled, and the residue was taken up in $NaHCO_3$ (aq) and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated to give **10** in 94% yield as a white solid. R_f (30% EtOAc/Hexane): 0.29; Mp: 88–90 ºC; IR (KBr): ν 3472, 2988, 2923, 2875, 1709, 1611, 1276, 1101 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 2.11 (br s, 1H, OH), 2.42 (s, 3H, *Me*-Tol), 2.59 (ddd, 1H, H_2, *J*= 13.8, 6.5, 1.8 Hz), 2.71 (ddd, 1H, H_2, *J*= 14.1, 9.3, 6.1 Hz), 3.87 (d, 2H, H_5, *J*= 3.7 Hz), 4.25 (q, 1H, H_4, *J*= 3.6 Hz), 4.87 (dd, 1H, H_1, *J*= 9.2, 6.6 Hz), 5.52 (d, 1H, H_3, *J*= 6.0 Hz), 7.25 (d, 2H, Horto-Me, *J*= 7.8 Hz), 7.88 (d, 2H, Horto-CO, *J*= 8.2 Hz) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.8 (*C*H$_3$), 38.3 (C_2), 62.6 (C_5), 66.0 (C_1), 75.7 (C_3), 86.8 (C_4), 118.3 (*C*N), 126.5 (Cipso-CO), 129.4 (2CHarom), 129.8 (2CHarom), 144.6 (Cipso-Me), 166.1 (*C*=O) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_{14}H_{15}NNaO_4$ [(M+Na)$^+$]: 284.0893, found: 284.0887.

Scale-up protocol for compound 10.

Similar procedure was followed, as described at small scale. Experiment was carried out with 10 g (26.5 mmol) of **9** dissolved in 45 mL of 1,4-dioxane, 220 mL of 0.15 M KH_2PO_4 pH 7 and 10 g of lipase PSL-IM.

1β-Cyano-1,2-dideoxy-5-*O*-methanesulfonyl-3-*O*-toluoyl-D-ribose (**11**).

Methanesulfonyl chloride (92.9 µL, 1.18 mmol) and DMAP (10 mg) was added to a solution of **10** (140 mg, 0.54 mmol) in anhydrous pyridine (1.1 mL) a 0 ºC. After being stirred at rt during 16 h, a new portion of MsCl (46 µL, 0.59 mmol) was added and stirred continue until 24 h. Next, the mixture was taken up in chilled H_2O and extracted with diethyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated to give a residue, which was purified by column chromatography (30% EtOAc/hexanes) to give **11** in 88% yield as a white solid. R_f (30% EtOAc/Hexane): 0.19; Mp: 78–80 ºC; IR (KBr): ν 3055, 2987, 2306, 1721, 1422, 1266 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 2.44 (s, 3H, *Me*-Tol), 2.69 (m, 2H, H_2), 3.14 (s, 3H, *Me*-Ms), 4.45 (m, 2H, H_4+ H_5), 4.53 (dd, 1H, H_5, *J*= 11.9, 4.3 Hz), 4.92 (dd, 1H, H_1, *J*= 8.6, 7.2 Hz), 5.54 (m, 1H, H_3), 7.27 (d, 2H, Horto-Me, *J*= 8.0 Hz), 7.90 (d, 2H, Horto-CO, *J*= 8.2 Hz) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.8 (*Me*-Tol), 37.77 (C_2), 37.81 (*Me*-Ms), 66.3 (C_1), 68.3 (C_5), 75.1 (C_3), 83.7 (C_4), 117.8 (*C*N), 126.1 (Cipso-CO), 129.4 (2CHarom), 129.8 (2CHarom), 144.9 (Cipso-Me), 165.9 (*C*=O) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_{15}H_{17}NNaO_6S$ [(M+Na)$^+$]: 362.0669, found: 362.0664.

1β-Aminomethyl-1,2,5-trideoxy-D-ribose (**12**).

From **18**. $LiAlH_4$ (132 mg, 3.47 mmol) was added to a solution of **18** (180 mg, 0.43 mmol) in anhydrous THF (2.8 mL). The reaction was stirred at reflux during 4 h. Excess of the reagent was decomposed by addition of EtOAc (8 mL) and MeOH (8 mL). The solvents were evaporated and the crude product was subjected to column chromatography (MeOH) affording **12** in 84% yield as a slightly yellowish viscous liquid. R_f (1% NH_3/MeOH): 0.35; IR (KBr): ν 3383, 3054, 2985, 1265 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.20 (d, 3H, Me, *J*= 6.4 Hz), 1.86 (dd, 2H, H_2, *J*= 7.4, 4.7 Hz), 2.64 (dd, 1H, H_6, *J*= 13.2, 6.7 Hz), 2.76 (dd, 1H, H_6, *J*= 13.1, 4.1 Hz), 3.80 (m, 1H, H_4), 3.89 (q, 1H, H_3, *J*= 4.9 Hz), 4.10 (m, 1H, H_1) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 19.9 (*C*H$_3$), 38.4 (C_2), 47.0 (C_6), 78.1 (C_3), 80.1 (C_1), 83.6 (C_4) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_6H_{14}NO_2$ [(M+H)$^+$]: 132.1019, found: 132.1023.

Mixture of compounds 12, 13, and 14.

From **11**. $LiAlH_4$ (30.4 mg, 0.8 mmol) was added to a solution of **11** (35 mg, 0.1 mmol) in anhydrous THF (0.7 mL). The reaction was stirred at reflux during 4 h. Excess of the reagent was decomposed by addition of EtOAc (2 mL) and MeOH (2 mL). The solvents were evaporated and the crude product was subjected to column chromatography (MeOH) affording **12** and **14** as a mixture, and **13**.

1β-Aminomethyl-1,2-dideoxy-D-ribose (**13**). Yellowish oil. R_f (1% NH_3/MeOH): 0.25; IR (NaCl): ν 3420, 2953, 1644 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.88 (m, 2H, H_2), 2.74 (dd, 1H, H_6, *J*= 13.2, 6.9 Hz), 2.92 (dd, 1H, H_6, *J*= 13.2, 3.5 Hz), 3.53 (dd, 1H, H_5, *J*= 11.7, 5.0 Hz), 3.61 (dd, 1H, H_5, *J*= 11.7, 4.1 Hz), 3.81 (q, 1H, H_4, *J*= 4.4 Hz), 4.10 (m, 2H, H_1+ H_3) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 38.9 (C_2), 45.9 (C_6), 63.8 (C_5), 73.9 (C_3), 79.1 (C_1), 88.8 (C_4) ppm; HRMS (ESI$^+$, *m/z*): cald for $C_6H_{14}NO_3$ [(M+H)$^+$]: 148.0968, found: 148.0974.

Selected signals for compound 14 from the mixture of 12 and 14. Yellowish oil. R_f (1% NH_3/MeOH): 0.38; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.89 (overlapped, 1H, H_2), 2.38 (dd, 1H, H_2, *J*= 13.5, 7.4 Hz), 2.50–3.10 (several signals, 2H_5 + 2H_6), 3.91, 4.39, 4.51 (H_1, H_3, H_4) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_6H_{12}NO_2$ [(M+H)$^+$]: 130.0862, found: 130.0862.

1β-*N*-(*tert*-Butyloxycarbonyl)aminomethyl-1,2-dideoxy-5-*O*-methanesulfonyl-3-*O*-toluoyl-D-ribose (**15**).

$NiCl_2 \cdot 6H_2O$ (5,7 mg, 0.024 mmol) and di-*tert*-butyldicarbonate (104.7 mg, 0.48 mmol) were added to a solution of **11** (80 mg, 0.24 mmol) in anhydrous MeOH (1.8 mL). Next, the mixture was cooled to 0 ºC and $NaBH_4$ (63.6 mg, 1.68 mmol) was added in small portions over 15 min. The reaction is allowed to reach room temperature in the bath and stirred overnight. Then, the mixture was filtered over celite®, the solvent evaporated and the crude product purified by column chromatography (30% EtOAc/hexanes) affording **15** in 55% yield as a white solid. R_f (30% EtOAc/Hexane): 0.35; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.45 (s, 9H, *Me*-Boc), 2.00 (m, 1H, H_2), 2.16 (m, 1H, H_2), 2.41 (s, 3H, *Me*-Tol), 3.15 (s, 3H, *Me*-Ms), 3.32 (m, 2H, H_6 overlapped with MeOH-d_4), 4.26 (m, 2H, H_1+H_4), 4.41 (d, 2H, H_5, *J*= 3.9 Hz), 5.36 (d, 1H, H_3, *J*= 6.4 Hz), 7.30 (d, 2H, Horto-Me, *J*= 8.2 Hz), 7.91 (d, 2H,

Horto-CO, *J*= 8.2 Hz) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 21.7 (*Me*-Tol), 28.7 (3*Me*-Boc), 36.4 (C_2), 37.5 (*Me*-Ms), 44.4 (C_6), 70.9 (C_5), 77.8 (C_3), 79.9 (C_1), 80.3 (C-^{t}Bu), 83.9 (C_4), 128.2 (Cipso-CO), 130.3 (2CHarom), 130.7 (2CHarom), 145.6 (Cipso-Me), 158.5 (NH-*C*=O), 167.6 (O-*C*=O) ppm.

1β-*N*-(Methyl)aminomethyl-1,2,5-trideoxy-D-ribose (**16**) and **1β-*N*-(Methyl)aminomethyl-1,2-dideoxy-D-ribose** (**17**).

$LiAlH_4$ (30.4 mg, 0.8 mmol) was added to a solution of **15** (45 mg, 0.1 mmol) in anhydrous THF (0.7 mL). The reaction was stirred at reflux during 4 h. Excess of the reagent was decomposed by addition of EtOAc (2 mL) and MeOH (2 mL). The solvents were evaporated and the crude product was subjected to column chromatography (MeOH) affording **16** and **17**.

1β-*N*-(Methyl)aminomethyl-1,2,5-trideoxy-D-ribose (**16**). Oil. R_f (1% NH_3/MeOH): 0.25; IR (NaCl): ν 3570, 3505, 2979, 2864, 1069 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.21 (d, 3H, H_5, *J*= 6.2 Hz), 1.89 (m, 1H, H_2), 1.98 (ddd, 1H, H_2, *J*= 13.2, 6.4, 3.1 Hz), 2.60 (s, 3H, *Me*-NH), 2.81 (dd, 1H, H_6, *J*= 12.5, 8.7 Hz), 3.02 (dd, 1H, H_6, *J*= 12.5, 3.1 Hz), 3.84 (m, 1H, H_4), 3.93 (m, 1H, H_3), 4.32 (m, 1H, H_1) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 19.9 (C_5), 34.7 (*Me*-NH), 39.0 (C_2), 55.6 (C_6), 75.5 (C_1), 77.8 (C_3), 84.2 (C_4) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_7H_{16}NO_2$ [(M+H)$^+$]: 146.1176, found: 146.1176.

1β-*N*-(Methyl)aminomethyl-1,2-dideoxy-D-ribose (**17**). Yellowish oil. R_f (2% NH_3/MeOH): 0.21; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.85 (m, 1H, H_2), 1.98 (ddd, 1H, H_2, *J*= 13.1, 6.1, 2.5 Hz), 2.62 (s, 3H, *Me*-NH), 2.89 (dd, 1H, H_6, *J*= 12.5, 8.5 Hz), 3.05 (dd, 1H, H_6, *J*= 12.5, 3.0 Hz), 3.54 (dd, 1H, H_5, *J*= 11.8, 4.9 Hz), 3.62 (dd, 1H, H_5, *J*= 11.8, 4.9 Hz), 3.84 (q, 1H, H_4, *J*= 4.3 Hz), 4.24 and 4.38 (2m, 2H, H_1, H_3) ppm.

1β-Cyano-1,2-dideoxy-5-*O*-toluenesulfonyl-3-*O*-toluoyl-D-ribose (**18**).

p-Toluenesulfonyl chloride (335 mg, 1.76 mmol) and DMAP (10 mg) was added to a solution of **10** (210 mg, 0.8 mmol) in anhydrous pyridine (1.6 mL) a 0 ºC. After being stirred at rt during 16 h, a fresh portion of *p*-TsCl (167 mg, 0.88 mmol) was added and stirred continue until 24 h. Next, the mixture was taken up in chilled H_2O and extracted with diethyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated to give a residue, which was purified by column chromatography (30% EtOAc/hexanes) to give **18** in 85% yield as a white solid. R_f (30% EtOAc/Hexane): 0.35; Mp: 132–134 ºC; IR (KBr): ν 3038, 2951, 2925, 2253, 1715, 1611, 1338, 1271, 1185 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 2.42 (s, 3H, *Me*-Tol), 2.45 (s, 3H, *Me*-Ts), 2.60 (m, 2H, H_2), 4.14 (m, 1H, H_5), 4.35 (m, 2H, H_4+ H_5), 4.80 (dd, 1H, H_1, *J*= 8.9, 7.1 Hz), 5.46 (m, 1H, H_3), 7.25 (d, 2H, Horto-Me-Tol, *J*= 8.7 Hz), 7.37 (d, 2H, Horto-Me-Ts, *J*= 8.1 Hz), 7.85 (2d, 4H, Harom) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.8 (*C*H_3), 21.9 (*C*H_3), 37.79 (C_2), 66.1 (C_1), 68.6 (C_5), 75.3 (C_3), 83.6 (C_4), 117.5 (*C*N), 126.1 (Cipso-CO), 128.2 (2CHarom), 129.4 (2CHarom), 129.8 (2CHarom), 130.2 (2CHarom), 132.2 (Cipso-SO_3), 144.8 (Cipso-Me-Tol), 145.5 (Cipso-Me-Ts), 165.9 (*C*=O) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_{21}H_{21}NNaO_6S$ [(M+Na)$^+$]: 438.0982, found: 438.0980.

1α-Cyano-1,2-dideoxy-3-*O*-toluoyl-D-ribose (**20**).

Similar procedure as that described for **10** starting from **19** using lipase PSL-IM [4:1 (w/w), ratio of lipase:**19**] yielded **20** (90%) after 5 d. The Erlenmeyer flask containing **19** and 1,4-dioxane was heated previously in an orbital shaker at 60 ºC during 5-10 min to improve the solubility of starting material. Yield a white solid. R_f (30% EtOAc/Hexane): 0.13; Mp: 90–92 ºC; IR (KBr): ν 3489, 3466, 3414, 2973, 2923, 2882, 2237, 1712, 1611, 1271, 1096 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 2.07 (s, 1H, OH), 2.43 (s, 3H, *Me*-Tol), 2.56 (d, 1H, H_2, *J*= 14.1 Hz), 2.68 (ddd, 1H, H_2, *J*= 14.2, 8.0, 6.3 Hz), 3.88 (d, 2H, H_5, *J*= 3.4 Hz), 4.41 (m, 1H, H_4), 5.05 (d, 1H, H_1, *J*= 7.0 Hz), 5.54 (d, 1H, H_3, *J*= 6.1 Hz), 7.28 (d, 2H, Horto-Me, *J*= 8.1 Hz), 8.00 (d, 2H, Horto-CO, *J*= 8.1 Hz) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.8 (*C*H_3), 38.3 (C_2), 62.5 (C_5), 66.7 (C_1), 75.1 (C_3), 87.0 (C_4), 118.7 (*C*N), 126.5 (Cipso-CO), 129.4 (2CHarom), 130.0 (2CHarom), 144.6 (Cipso-Me), 166.5 (*C*=O) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_{14}H_{15}NNaO_4$ [(M+Na)$^+$]: 284.0893, found: 284.0882.

General Protocol for Enzyme Reuse.

Similar procedure as that described for the preparation of β-**10** is followed except that after enzyme filtration, it was washed with 1,4-dioxane and dried under vacuum. No further manipulation was necessary to use the enzyme for the next run.

1α-Cyano-1,2-dideoxy-5-*O*-toluenesulfonyl-3-*O*-toluoyl-D-ribose (**21**).

Similar procedure as that described for **18** starting from **20** yielded **21** (75%) as a white solid. R_f (30% EtOAc/Hexane): 0.26; Mp: 156–158 ºC; IR (KBr): ν 2970, 2922, 2238, 1716, 1613, 1342, 1286, 1170 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 2.40 (s, 3H, *Me*-Tol), 2.45 (s, 3H, *Me*-Ts), 2.52 (d, 1H, H_2, *J*= 14.2 Hz), 2.61 (m, 1H, H_2), 4.25 (dd, 1H, H_5, *J*= 11.1, 2.8 Hz), 4.33 (dd, 1H, H_5, *J*= 11.1, 2.7 Hz), 4.44 (m, 1H, H_4), 4.93 (dd, 1H, H_1, *J*= 8.0, 1.3 Hz), 5.44 (d, 1H, H_3, *J*= 5.9 Hz), 7.25 (d, 2H, Horto-Me-Tol, *J*= 7.8 Hz), 7.36 (d, 2H, Horto-Me-Ts, *J*= 8.2 Hz), 7.79 (d, 2H, Horto-SO_3-Ts, *J*= 8.3 Hz), 7.95 (d, 2H, Horto-CO-Tol, *J*= 8.2 Hz) ppm; ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.8 (CH_3), 21.9 (CH_3), 37.9 (C_2), 66.9 (C_1), 69.0 (C_5), 74.9 (C_3), 84.1 (C_4), 118.4 (CN), 126.2 (Cipso-CO), 128.1 (2CHarom), 129.5 (2CHarom), 130.1 (2CHarom), 130.2 (2CHarom), 132.3 (Cipso-SO_3), 144.7 (Cipso-Me-Tol), 145.5 (Cipso-Me-Ts), 166.2 (*C*=O) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_{21}H_{21}NNaO_6S$ [(M+Na)$^+$]: 438.0982, found: 438.0982.

1α-Aminomethyl-1,2,5-trideoxy-D-ribose (**22**).

Similar procedure as that described for **12** starting from **21** yielded **22** (78%) as a slightly yellowish viscous liquid. R_f (1% NH_3/MeOH): 0.28; IR (NaCl): ν 3375, 3054, 2972, 2931, 1266 cm^{-1}; ^{1}H NMR (MeOH-d_4, 300.13 MHz): δ 1.16 (d, 3H, Me, *J*= 6.2 Hz), 1.60 (dt, 1H, H_2, *J*= 13.0, 5.7 Hz), 2.43 (m, 1H, H_2), 2.74 (d, 2H, H_6, *J*= 5.2 Hz), 3.89 (m, 2H, H_3+H_4), 4.11 (m, 1H, H_1) ppm; ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 18.9 (CH_3), 38.1 (C_2), 46.7 (C_6), 77.7 (C_1), 79.1, 82.4 (C_3+C_4) ppm; HRMS (ESI$^+$, *m/z*): calcd for $C_6H_{14}NO_2$ [(M+H)$^+$]: 132.1019, found 132.1017.

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[16] The chemical or enzymatic transformations described herein do not impact any change of chirality or compromise it. We did not furnish data comparison with reported compounds because the final products were isolated as hydroxide unlike the salt of other anions (Cl^-, Br^-, I^-, TsO^-, etc...) reported in the literature for natural products.

Short and Efficient Chemoenzymatic Syntheses of non-Natural (−)-Muscarine and (+)-*allo*-Muscarine from Cyano-Sugar Precursors Catalyzed by Immobilized *Burkholderia cepacia* Lipase

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Alejandro Carnero,[a] Yogesh S. Sanghvi,[b] Vicente Gotor,[a] Susana Fernández,[a]* and Miguel Ferrero[a]*